American Journal of Microbiological Research, 2020, Vol. 8, No. 3, 103-109
Available online at http://pubs.sciepub.com/ajmr/8/3/4
Published by Science and Education Publishing
DOI:10.12691/ajmr-8-3-4

The Impact of Secondary Metabolites Produced by *Streptomyces* on Uro-pathogens

Suhndh Ahmed Mohmmed Musa*

Department of Botany, Faculty of Science, University of Khartoum
*Corresponding author: suhndh87@gmail.com

Received July 10, 2020; Revised August 12, 2020; Accepted August 21, 2020

Abstract Soil *streptomyces* are known as a rich source to produce secondary metabolites. This study was conducted to examine the effect of the *Streptomyces* sp. extracts against the uro-pathogen isolates from urinary tract infections in pregnancy from Kassala state. Cultural, morphological and physiological characterization of 15 microorganisms isolates from four soil samples indicated that all isolates belonged to the *Streptomyces* genus. All *Streptomyces* isolates produced chitinase enzyme except isolate SU11. After 72 hours all of the isolates were produced L-asparaginase enzyme. Ethyl acetate extracts of Antimicrobial were used for secondary screening against uro-pathogen. The high activity has been observed against Gram-positive bacteria with a percentage of 73.3% when compared to gram-negative bacteria. Whilst 26.7% has a high activity against Gram-negative comparing with Gram-positive bacteria, and different activity was shown against candida spp.

Keywords: *Streptomyces, Chitinase, L-asparaginase, Antimicrobial, Uro-pathogen*

Cite This Article: Suhndh Ahmed Mohmmed Musa, "The Impact of Secondary Metabolites Produced by *Streptomyces* on Uro-pathogens." *American Journal of Microbiological Research*, vol. 8, no. 3 (2020): 103-109. doi: 10.12691/ajmr-8-3-4.

1. Introduction

Antimicrobial resistance is a worldwide health problem [1]. In the past new drug discovery come from natural sources [2]. Actinomycetes is big group of microorganisms that are widely distributed in insect microbiomes, freshwater, marine and terrestrial environments [3,4]. These are not only an antibiotic producer but also an important source of bioactive compounds [5,6]. *Streptomyces*, an Actinomycetes member, produces secondary metabolites and enzymes commercially important in medical and agricultural applications combating problems caused by pathogenic bacteria and/or fungi [7,8]. Some *Streptomyces* spp. produces antibiotics during sporulation [9]. Their ability to produce different antibiotics attracted researchers [10]. L-asparaginase produced by microorganisms has been widely used as an effective therapeutic agent against acute lymphoblastic leukemia, lymphosarcoma as well as in the food industry [11,12]. Some *Streptomyces* spp are known to produce L-asparaginase [13]. Microorganisms like *Serratia* and Actinomycetes (principally *Streptomyces*) are good producers of chitinases [8,14,15]. It inhibits fungal growth and controls its pathogenicity in plants [16], but it is not fully effective in all cases [17].

According to Hamza *et al* soil microbiology was not given due attention in Sudan, despite the high variability in soil types and the environment [18].

The aims of this study isolation and identification of *Streptomyces* bacteria from different localities in Kassala state, detection of their ability to produce secondary metabolites(Antimicrobial and Enzyme), and application of these products to investigate their ability to inhibit the uro-pathogens isolated from pregnant women urine in Kassala state.

2. Material and Methods

2.1. Soil Samples Collection and Pre-treatment

Soil samples were collected from different locations in Kassala state. The samples were taken from a depth of 5-10 cm after removing approximately 2-3cm of the earth's surface and were then mixed with 1% CaCO3 and left to dry for in open air 3-5 days.

2.2. Isolation of *Streptomyces* spp.

Isolation of *Streptomyces* spp was performed by the soil dilution plate technique [19]. In this method usually (10^{-3}-10^{-5}) 0.1 ml aliquot was pipetted from each dilution and spread onto Starch Casein Agar (SCA). The inoculated Petri-dishes were incubated at 27°C for 3-5 days.

2.3. Characterization of *Streptomyces* spp Isolates

Cultural, morphological and physiological features of the presumptive *Streptomyces* spp isolates were

determined as described by International *Streptomyces* Project (ISP) documented by Shirling and Gottlieb [20]; Locci [21] and Bergey's Manual for Determinative Bacteriology [22].

Colony characteristics followed by [23,24].

2.4. Morphological Characterization of *Streptomyces* spp

A- Aerial mass and reverse color

Aerial mass and reverse colors were detected according to Shirling and Gottlieb by using ISP2 [20].

B- Diffusible soluble pigments

Streptomyces cultures were detected by the method of Shirling and Gottlieb on the ISP7 medium [20].

2.5. Microscopic Characteristics

Gram staining method followed by Collins *et al*. [25]. Spore chain morphology required culturing *Streptomyces* isolates on ISP-2 agar medium using cover slip culture technique as described by [26].

2.6. Physiological Characterization

A- Melanoid Pigments

The production of melanoid pigments by *Streptomyces* cultures was detected by the method of Shirling and Gottlieb on the ISP7 medium [20].

B- Carbohydrate Utilization

Carbohydrate utilization test was performed by using 10 % (w/v) of the carbon sources. The carbon sources were: L- arabinose, D- xylose, meso- inositol, D- mannitol, D- fructose, rhamnose, raffinose, xylose and sucrose. [20].

C- NaCl Tolerance

Sodium chloride tolerance was detected according to [27]. *Streptomyces* isolates were cultured in yeast extract agar medium. After sterilization, gradient concentrations of NaCl (0, 2.5, 5.7,5 and 10%) were aseptically added.

2.7. Screening of *Streptomyces* Isolates for Enzyme Production

A- Chitinase production

The isolates were cultured in the chitin agar (CCA) medium [28]. After incubation, 0.1% Congo red solution was fed over the plates, the appearance of clearance zone around the colonies after seven days of incubation was noted and measured.

B- L-Asprginase production

The isolated cultures were re-cultured in the ADS medium with phenol red. The cultures were incubated at 37°C at pH 6.5, change of the medium colour to pink indicates the production of L-Asprginase enzyme [29].

2.8. Antimicrobial Activities

A- Preliminary screening of the *Streptomyces* isolates for antibiotic production

Antibiotic activities of the *Streptomyces* isolates were tested in vitro against uro-pathogene (bacteria and fungi). Antibacterial activities of pure *Streptomyces* isolates were performed by the cross- streak method [30].

B- Screening for secondary metabolites

- Culture and extraction of bioactive compounds

Screening for the secondary metabolites was done by using *Streptomyces* Antibiotic Activity broth medium. Method described by [31]. Ethyl - acetate extraction method was used [32]. The solvent layers were collected in tubes and evaporated to dryness at 40oC [33]. The dried extracts were used for antibiotic screening.

- Biological activities of the extracts

Inhibitory activities of the *Streptomyces* extracts were examined against uro-pathogenies (*E. coli*, *Vibrio cholera*, *Neisseria*, *Listeria*, *Staphylococcus* and *Candida* sp.).

3. Result

3.1. Isolation and Cultural Characterization of *Streptomyces* spp

Fifteen strains were isolated from 4 soil samples on Starch Casein KNO3 agar medium, colony characteristics (color, Shape, Size, margin, surface, Elevation, texture and opacity) were study (Table 1) and (Figure 1). In all strain the surface was powdery, with raised elevation, the texture was dry and the opacity was opaque consequently these were not included in the table. The strains were purified, given codes prefixed with SU1 to SU 15.

Table 1. Soil type and Cultural characteristics of *Streptomyces* isolate

Isolat No.	Soil type	Color	Shape	Size	Margin
SU1	Sandy	pale brown	Irregular	Medium	Erose
SU2	Clay	pale brown		Medium	
SU3	Clay	White		Small	
SU4	Clay	off white		Small	
SU5	Sandy	pale brown		Small	
SU6	Sandy	Brown		Medium	
SU7	Clay	Grey		Small	
SU8	Clay	White	Circular	Small	Entire
SU9	Sandy	pale brown	Irregular	Medium	Erose
SU10	Sandy	Grey	Circular	Medium	Entire
SU11	Clay	Grey		Small	
SU12	Clay	Brown	Irregular	Medium	Erose
SU13	Sandy	White	Circular	Small	
SU14	Clay	off white	Circular	Small	Entire
SU15	Sandy	pale grey	Irregular		Erose

Figure 1. Cultural characteristics of *Streptomyces* isolates

3.2. Morphological Characterization of *streptomyces* spp

3.2.1. Aerial Mass and Reverse Color

Different colure was recorded by different isolate White, Pink, Cream and White, Pink and White, Gray and Gray and White in (Aerial mycelium) while Yellow, Cream, Brown and Gray in (Reverse color) (Table 2, Figure 2).

3.2.2. Diffusible Soluble Pigments

The ability to produce soluble pigments other than Melanoids was noted as producers (+) and not producers (-), 46.7% produced and 53.3 % was not produced. (Table 2, Figure 3).

3.3. Microscopic Characteristics

All the isolates were Gram-positive bacteria with extensive branching (Table 2, Figure 4).

3.4. Physiological characterization

3.4.1. Melanoid Pigments

The ability to produce Melanoids other than soluble pigments was noted as producers (+) and not producers (-), 46.7% were positive and 53.3 % were negative (Table 2, Figure 3).

Table 2. Morphological, some of Physiological and Microscopic characteristics of *Sstreptomyces* isolates.

Isolate code	Aerial mycelium	Reverse colour	Soluble pigment	Melanoied pigment	Spore chain morphology
SU1	White	Yellow	+ve	-ve	Open loops primitive spirals hooks
SU2	Pink	Yellow	+ve	-ve	
SU3	Cream& White	Cream	+ve	-ve	Flexous
SU4	White	Cream	+ve	-ve	
SU5	Pink	Brown	+ve	-ve	Open loops primitive spirals hooks
SU6	Pink& White	Yellow	+ve	-ve	Biverticillate no spirals
SU7	Gray	Brown	-ve	+ve	Open loops primitive spirals hooks
SU8	White	Cream	+ve	-ve	Biverticillate
SU9	White & pink	Yellow	-ve	+ve	Monovreticillate
SU10	Gray	Yellow	-ve	+ve	Flexous
SU11	Gray& White	Gray	-ve	+ve	Monovreticillate with spirals
SU12	White and pink	Yellow	-ve	+ve	Biverticillate
SU13	Gray& White	Gray	-ve	+ve	Flexous
SU14	Gray	Brown	-ve	-ve	
SU15	White	Cream	-ve	+ve	Fascicled

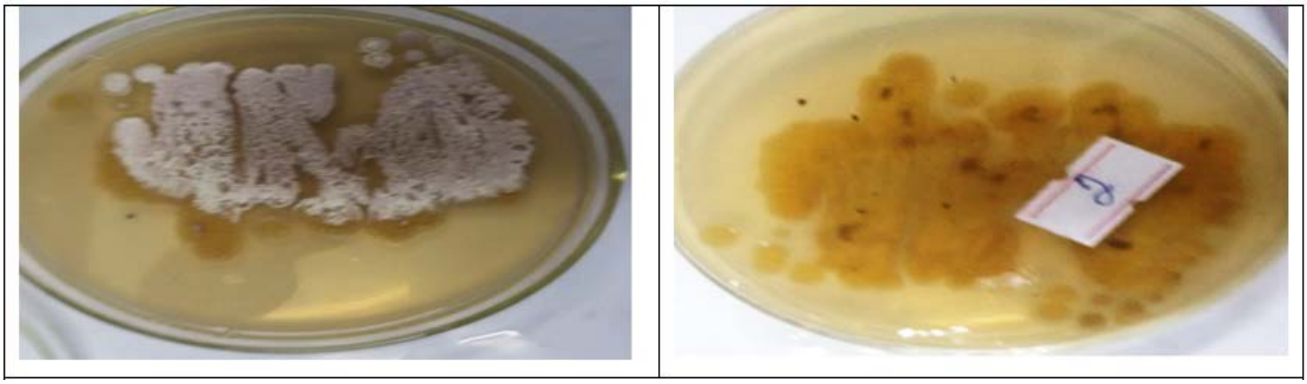

Figure 2. Aerial mass colour (forward side) and reverse colour (rearward side) of *Streptomyces* isolate SU2

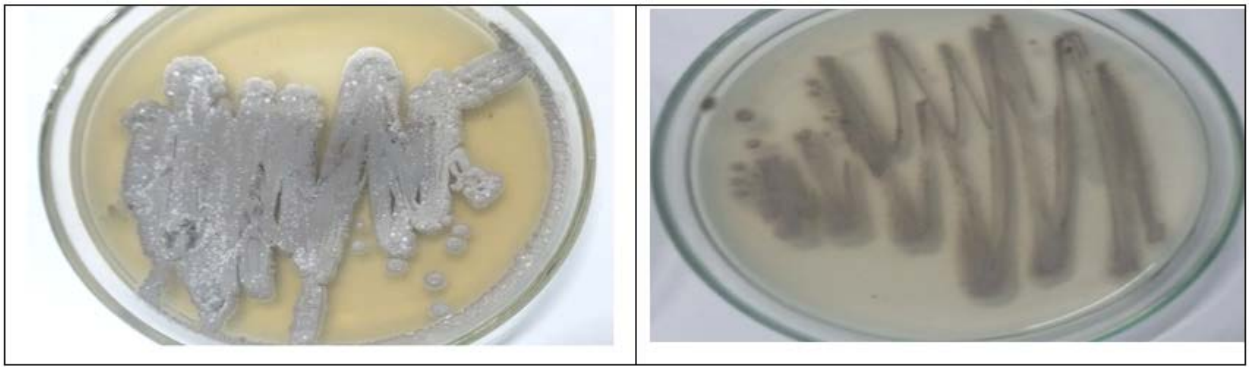

Figure 3. Melanoid and soluble pigments of *Streptomyces* isolate SU6

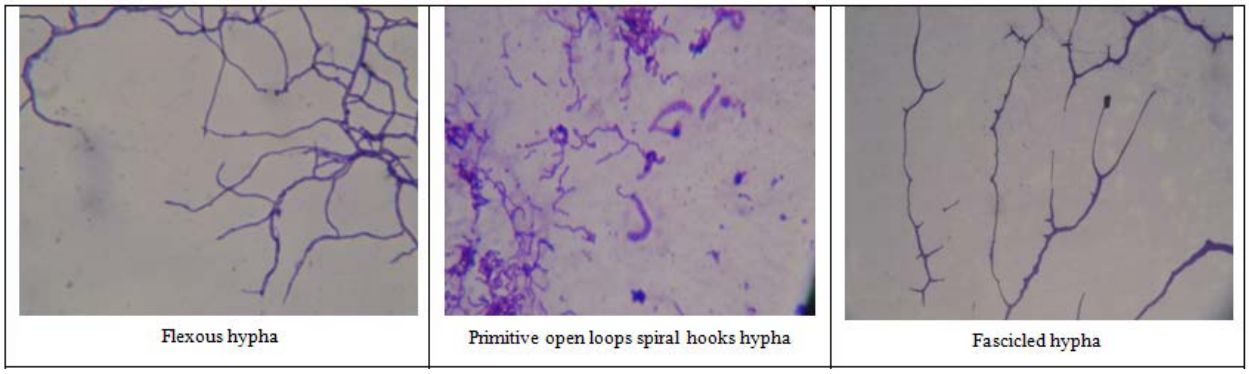

Figure 4. Different shapes of *Streptomyces* hyphae

Table 3. *Streptomyces* isolates growth in different carbohydrate source

Sugar	SU No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Inositol	++	+	++	++	-	+	++	+	+	++	+	+	++	+	++
Glucose	+	++	++	+	+	+	++	-	+	+	++	-	++	++	++
Xylose	++	++	++	++	+	+	++	++	+	+	+	-	++	++	+
Arabinose	-	+	+	++	++	+	++	+	+	+	+	+	++	+	++
Mannitol	+	+	++	++	+	+	++	+	+	+	+	+	++	++	++
Sucrose	+	++	++	++	+	++	++	+	+	+	+	+	++	++	++
Fructose	+	++	+	++	+	+	++	-	+	+	++	+	++	++	+
Rhamnose	+	+	+	++	+	++	++	-	+	+	++	-	++	++	++
Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

3.4.2. Carbohydrate Utilization

Table 3 shows that all tested isolates were able to grow on media supplemented with (Mannitol, Sucrose and Raffinose) when used as a carbohydrate source.

In the table cells with (-) indicated growth not better than negative control; the (+) mean growth is better just like negative control but not like positive control and in cells with (**++**) the growth better like a positive control.

3.4.3. NaCl Tolerance for *Streptomyces* Isolates

The NaCl tolerance test for the 15 *Streptomyces* isolates revealed that all concentrations 2.5, 5, 7.5 and 10% as well as the control, supported either low, medium or high growth with no constant pattern isolates wise or NaCl concentration wise.

3.5. Chitinase Enzyme Production

All *Streptomyces* the isolates produced chitinase except isolate SU11. High production is shown by isolate SU1 (Table 4 and Figure 5).

3.6. L-asparaginase Enzyme Production

The L-Asparaginase production was recorded over a period of 72 hrs. After 24h only isolated SU1 and SU2 produced the enzyme. After 48h more isolates produce the enzymes and after 72h all isolates were able to produce the enzyme (Table 4 and Figure 5).

3.7 Screening of Antimicrobial

All of the *Streptomyces* isolates were subjected to primary screening for antimicrobial production by using cross a streak method (Figure 6) against uro-pathogens. These isolates which demonstrated antimicrobial activities against uro-pathogens were again subjected to secondary screening disk method. High inhibitión zone was shown against Gram- negative bacteria by *Streptomyces* isolates SU (1, 2, 4, 5, 7, 9, 10, 12, 13, 14, 15) and against Gram- positive bacteria by *Streptomyces* isolates SU (3, 6, 8, 11). Various in inhibition zones results showed against all *candida* sp. (Figure 6).

Table 4. Chitinase and L Asparagine Production by *Streptomyces* isolates

Isolate code	Chitinase Production	L Asparagine Production		
	Diameter of Zones	24h	48h	72h
SU 1	23 mm	-	-	+
SU 2	5 mm	-	+	++++
SU 3	16 mm	-	++	++++
SU 4	11 mm	-	++	++++
SU 5	18 mm	-	-	+++
SU 6	16 mm	-	+	++++
SU 7	8 mm	-	-	+
SU 8	8 mm	-	+	++
SU 9	3 mm	-	+	++++
SU 10	9 mm	-	+++	++++
SU 11	0 mm	-	+	++
SU 12	8 mm	-	+	++++
SU 13	18 mm	+	++++	++++
SU 14	2 mm	-	+++	++++
SU 15	17 mm	+	++++	++++

No production (-), low production (+), medium production (++), high production (+++), and Very high production (++++).

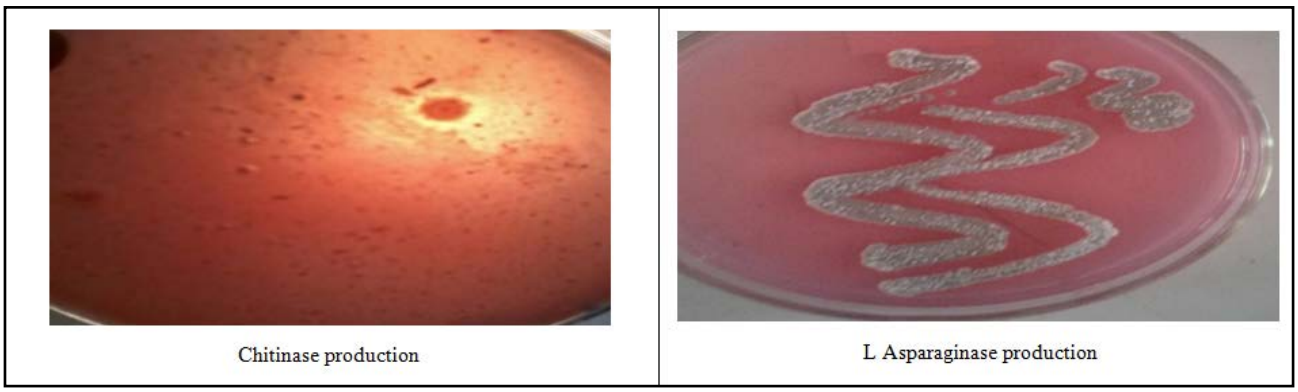

Figure 5. Enzymes produce by *Streptomyces*

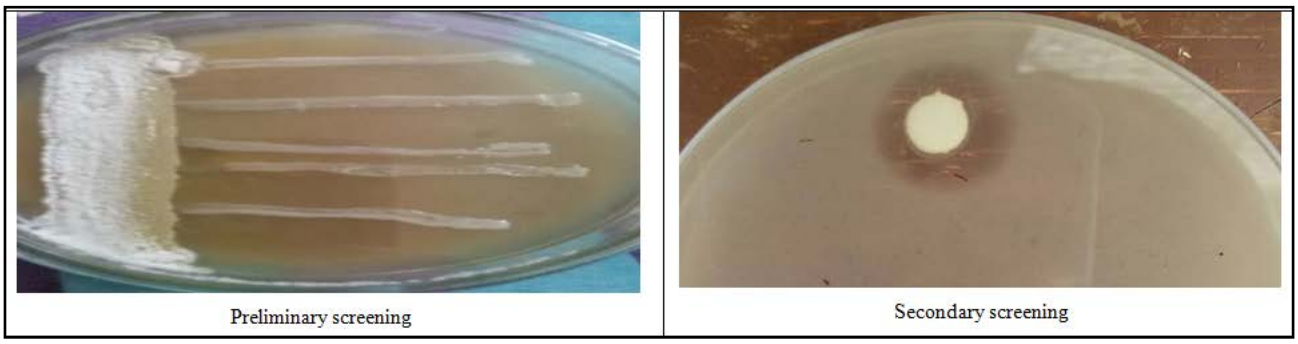

Figure 6. Inhibición zone by *streptomyces* aginst uro-pathogen

4. Discussion

Soil is an ecological niche of many organisms living together, Williams *et al*. [34] reported that the soil is a natural habitat for *Streptomyces* sp., and wide species of *Streptomyces* was isolated from it [35,36]. In this study, morphological and physiological characterization indicated that 15 isolates from soil type from Kassala belonged to the *Streptomyces* genus. These isolates were similar in surface, elevation, texture and opacity and differ markedly in colour, shape, size and margin. All isolates which had pale brown color had the same Cultural characteristic except for isolate SU5 which was observed. Different aerial mass and reverse colors of *Streptomyces* isolates were recorded According to the Bergey's manual of systematic bacteriology [21].

The present study detected 46.7% positive and 53.3% negative for melanoid and soluble pigments. White series producing melanoid pigments were found during this study but not by Taddei e*t al*. [37] who recorded melanoid pigments in other series. Microscopically the current S*treptomyces* isolates showed branched hyphae and were Gram-positive confirming Claessen *et al*. findings [38]. Utilization of carbon sources plays an important role in species differentiation by *Streptomyces* sp. [39]. During this study, all the isolates were able to grow in L-arabinose, D-xylose, meso-inositol, D-glucose, D-mannitol, D-fructose, rhamnose, raffinose and sucrose. Similar results obtained in *Streptomyces* sp. strain 3B which failed to assimilate meso-inositol and D-mannitol as a carbon source [40]. NaCl tolerance test was proved that the optimum growth at zero concentration, while another

study reported that the optimum of NaCl, for most *Streptomyces* strains, was (5-10 % NaCl) [41,42]. In this study *Streptomyces* were produced enzyme and anti-microbial, other study verification it was able to produce secondary metabolites, like antibacterial, antifungal, anti-cancer, and anti-HIV [43,44,45].

In this work, L-aspreginase and chitinase were produced by the isolates of *Streptomyces*. This confirmed earlier findings. Production of L-asparaginase by *Streptomyces karnatakensis* and *Streptomyces venezuelae* was reported by [46]. On the other hand, Chitinase production was detected by Kolla *et al.* from *Streptomyces* sp. ANU 6277 and Meriem *et al.* from *Streptomyces griseorubens* C9 isolates [47,48]. L-asparagine was increased through time, [49] reported that the production began after 24 h and gets to the top rung after 72 h of incubation and Amena *et al*. demonstrated that the production of L-Asparaginase increase through time at constant pH and temperature [50]. Different zones of chitin hydrolysis were formed by all *Streptomycetes* isolates except for one isolate (SU11), high concentration produced by most strains [51].

Preliminary screening of antibacterial activity indicated that all the isolates they have antibacterial activity against one or more isolates of uro-pathogen. After that, all of it were subjected to secondary screening. Ethyl acetate extracted were used, the extraction by ethyl acetate of secondary metabolites showed clear activity against pathogenic bacteria than other solvents [52]. Secondary screening showed different activities from that of primary screening, similar results reported by [53]. Variability in activities obtained in primary and secondary screening test may be attributed to the use of different media (solid, liquid media) or improved techniques which may lead to production of different secondary metabolites, also some compounds may be lost during the organic solvent [54].

Some *Streptomyces* extract activity against Gram- positive bacteria were high compare with Gram negative bacteria, while some *Streptomyces* extract activity against Gram-negative bacteria were high compare with Gram-positive bacteria. [55,56,57,58] reported that the inhibition zone of *Streptomyces* was relatively larger against Gram-positive bacteria compared with Gram-negative bacteria. Some *Streptomyces* extracts have an inhibition zone against *candida* sp., [59,60,61] reported that different strains of *Streptomyces* showed various degrees of antifungal activity against *A. niger* and *C. albicans*. *Streptomyces* isolate SU 13 and SU 15 high produce Chitinase and L Asparagine enzyme but it differed in antimicrobial activity. When isolate su11 doesn't produce Chitinase put have antimicrobial activity against fungi, Inbar and Chet explain that chitinases do in fungal cell walls [62].

References

[1] Singer, R. S., Finch, R., Wegener, H. C., Bywater, R., Walters, J., and Lipsitch, M. (2003). Antibiotic resistance—the interplay between antibiotic use in animals and human beings. The Lancet infectious diseases, 3(1), 47-51.

[2] Bevan, P., Ryder, H., and Shaw, I. (1995). Identifying small-molecule lead compounds: the screening approach to drug discovery. Trends in Biotechnology, 13(3), 115-121.

[3] Mabrouk, M. I., and Saleh, N. M. (2014). Molecular identification and characterization of antimicrobial active actinomycetes strains from some Egyptian soils. DNA, 11, 12.

[4] Chevrette, M. G., Carlson, C. M., Ortega, H. E., Thomas, C., Ananiev, G. E., Barns, K. J., ... and Grubbs, K. J. (2019). The antimicrobial potential of Streptomyces from insect microbiomes. Nature communications, 10(1), 1-11.

[5] Inderiati, S., and Franco, C. M. (2008). Isolation and identification of endophytic actinomycetes and their antifungal activity. J Biotechnol Res Trop Reg, 1, 1-6.

[6] Kumar, N., Singh, R. K., Mishra, S. K., Singh, A. K., & Pachouri, U. C. (2010). Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. International Journal of Microbiology Research, 2(2), 12.

[7] Mitsuiki, S., Sakai, M., Moriyama, Y., GOTO, M., and Furukawa, K. (2002). Purification and some properties of a keratinolytic enzyme from an alkaliphilic Nocardiopsis sp. TOA-1. Bioscience, biotechnology, and biochemistry, 66(1), 164-167.

[8] Narayana, K. J., and Vijayalakshmi, M. (2009). Chitinase production by Streptomyces sp. ANU 6277. Brazilian Journal of Microbiology, 40(4), 725-733.

[9] Todar, K. (2002). Antimicrobial agents used in treatment of infectious disease. Textbook of Bacteriology, University of Wisconsin-Madison, 1-11.

[10] Kuti, J. L., Capitano, B., and Nicolau, D. P. (2002). Cost-effective approaches to the treatment of community-acquired pneumonia in the era of resistance. Pharmacoeconomics, 20(8), 513-528.

[11] Verma, N., Kumar, K., Kaur, G., and Anand, S. (2007). L-asparaginase: a promising chemotherapeutic agent. Critical reviews in biotechnology, 27(1), 45-62.

[12] Hendriksen, H. V., Kornbrust, B. A., Østergaard, P. R., and Stringer, M. A. (2009). Evaluating the potential for enzymatic acrylamide mitigation in a range of food products using an asparaginase from Aspergillus oryzae. Journal of agricultural and food chemistry, 57(10), 4168-4176.

[13] Basha, N. S., Rekha, R., Komala, M., and Ruby, S. (2009). Production of extracellular anti-leukaemic enzyme lasparaginase from marine actinomycetes by solidstate and submerged fermentation: Purification and characterisation. Tropical Journal of Pharmaceutical Research, 8(4).

[14] Gupta, R., Saxena, R. K., Chaturvedi, P., and Virdi, J. S. (1995). Chitinase production by Streptomyces viridificans: its potential in fungal cell wall lysis. Journal of Applied Bacteriology, 78(4), 378-383.

[15] Faramarzi, M. A., Fazeli, M., Yazdi, M. T., Adrangi, S., Al-Ahmadi, K. J., Tasharrofi, N., and Mohseni, F. A. (2009). Optimization of cultural conditions for production of chitinase by a soil isolate of Massilia timonae. Biotechnology, 8(1), 93-99.

[16] Ordentlich, A., Elad, Y., and Chet, I. (1988). The role of chitinase of Serratia marcescens in biocontrol of Sclerotium rolfsii. Phytopathology, 78(1), 84-88.

[17] Gohel, V., Singh, A., Vimal, M., Ashwini, P., and Chhatpar, H. S. (2006). Bioprospecting and antifungal potential of chitinolytic microorganisms. African Journal of Biotechnology, 5(2), 54-72.

[18] Hamza, A. A., Ali, H. A., Clark, B. R., Murphy, C. D., and Elobaid, E. A. (2013). Isolation and characterisation of actinomycin D producing Streptomyces spp. from Sudanese soil. African journal of biotechnology, 12(19).

[19] Waksman, S. A. (1961). The Actinomycetes. Vol. II. Classification, identification and descriptions of genera and species. The Actinomycetes. Vol. II. Classification, identification and descriptions of genera and species.

[20] Shirling, E. T., and Gottlieb, D. (1966). Methods for characterization of Streptomyces species. International journal of systematic bacteriology, 16(3), 313-340.

[21] Locci, R. (1989). Streptomyces and related genera. Bergey's manual of systematic bacteriology, 4, 2451-2508.

[22] Buchanan, R. E., and Gibbons, N. E. (1974). Bergey's Manual of Determinative Bacteriology (8th edn.) The Williams and Wilkins Co. Baltimore, MD, 1246.

[23] Prescott, L. M., Harley, J. P., and Klein, D. A. (1993). Microbiology, Wm. C. Brown Communication. Inc., 2nd. Edition, USA, 912.

[24] Embley, T. M., and Stackebrandt, E. (1994). The molecular phylogeny and systematics of the actinomycetes. Annual review of microbiology, 48(1), 257-289.

[25] Collins, T., Read, M. A., Neish, A. S., Whitley, M. Z., Thanos, D., and Maniatis, T. (1995). Transcriptional regulation of endothelial cell adhesion molecules: NF-κB and cytokine-inducible enhancers. The FASEB Journal, 9(10), 899-909.

[26] Korn-Wendisch, F., and Kutzner, H. J. (1991). The family Streptomycetaceae. The Prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, Chap. 41.

[27] Pridham, T. G., Anderson, P., Foley, C., Lindenfelser, L. A., Hesseltine, C. W., and Benedict, R. G. (1957). A selection of media for maintenance and taxonomic study of streptomycetes. A selection of media for maintenance and taxonomic study of streptomycetes.

[28] Priya, C. S., Jagannathan, N., and Kalaichelvan, P. T. (2011). Production of chitinase by Streptomyces hygroscopicus VMCH2 by optimisation of cultural conditions. International Journal of Pharma and Bio Sciences, 2(2).

[29] R. Gulati, R. K. Saxena, and R. Gupta, "A rapid plate assay for screening L-asparaginase producing micro-organisms," Letters inAppliedMicrobiology,vol.24, no.1, pp.23-26,1997.

[30] Kamat, N., and Velho-Pereira, S. (2012). Screening of actinobacteria for antimicrobial activities by a modified" Cross-Streak" method. Nature Precedings, 1-1.

[31] Lertcanawanichakul, M., Pondet, K., and Kwantep, J. (2015). In vitro antimicrobial and antioxidant activities of bioactive compounds (secondary metabolites) extracted from Streptomyces lydicus A2. Journal of Applied Pharmaceutical Science, 5(2), 17-21.

[32] Ismail, A., Tiong, N. W., Tan, S. T., and Azlan, A. (2009). Antioxidant properties of selected non-leafy vegetables. Nutrition & Food Science.,39: 176-180.

[33] Alimuddin, A., Widada, J., Asmara, W., and Mustofa, M. (2011). Antifungal Production of a Strain of Actinomycete spp Isolated from the Rhizosphere of Cajuput Plant: Selection and detection of exhibiting activity against tested fungi. Indonesian Journal of Biotechnology, 16(1).

[34] Williams, S. (1984). A taxonomic approach to selective isolation of streptomycetes from soil. Biological, biochemical, and biomedical aspects of actinomycetes., 553-561.

[35] Arasu, M. V., Duraipandiyan, V., Agastian, P., and Ignacimuthu, S. (2008). Antimicrobial activity of Streptomyces spp. ERI-26 recovered from Western Ghats of Tamil Nadu. Journal de Mycologie Médicale, 18(3), 147-153.

[36] Bakheit, S. E., and Saadabi, A. M. (2014). Antagonistic affects of Actinomycetes isolated from Tuti island farms (Central Sudan) against Fusarium oxysporum f. sp. vasinfectum a phytopathogenic fungus. International Journal of Advanced Research, 2(2), 114-120.

[37] Taddei, A., Rodriguez, M. J., Márquez-Vilchez, E., and Castelli, C. (2006). Isolation and identification of Streptomyces spp. from Venezuelan soils: Morphological and biochemical studies. I. Microbiological Research, 161(3), 222-231.

[38] Claessen, D., De Jong, W., Dijkhuizen, L., and Wösten, H. A. (2006). Regulation of Streptomyces development: reach for the sky!. Trends in microbiology, 14(7), 313-319.

[39] Benedict, R. G., Pridham, T. G., Lindenfelser, L. A., Hall, H. H., and Jackson, R. W. (1955). Further studies in the evaluation of carbohydrate utilization tests as aids in the differentiation of species of Streptomyces. Applied microbiology, 3(1), 1.

[40] Antonova-Nikolova, S., Tzekova, N.,and Yocheva, L. (2005). Taxonomy of Streptomyces sp. strain 3B. Journal of Culture Collections, 4(1), 36-42.

[41] Urzì, C., and Realini, M. (1998). Colour changes of Notos calcareous sandstone as related to its colonisation by microorganisms. International biodeterioration & biodegradation, 42(1), 45-54.

[42] Walsh, D. A., Papke, R. T., and Doolittle, W. F. (2005). Archaeal diversity along a soil salinity gradient prone to disturbance. Environmental Microbiology, 7(10), 1655-1666..

[43] Antony-Babu, S., Stach, J. E., and Goodfellow, M. (2008). Genetic and phenotypic evidence for Streptomyces griseus ecovars isolated from a beach and dune sand system. Antonie Van Leeuwenhoek, 94(1),

[44] Narayana, K. J., Peddikotla, P., Krishna, P. S. J., Yenamandra, V., and Muvva, V. (2009). Indole-3-acetic acid production by Streptomyces albidoflavus. J Biol Res, 11, 49-55.

[45] Ding, L., Münch, J., Goerls, H., Maier, A., Fiebig, H. H., Lin, W. H., and Hertweck, C. (2010). Xiamycin, a pentacyclic indolosesquiterpene with selective anti-HIV activity from a bacterial mangrove endophyte. Bioorganic and medicinal chemistry letters, 20(22), 6685-6687.

[46] Mostafa SA. Zentralbl Bakteriol Naturwiss. Production of L-asparaginase by Streptomyces karnatakensis and Streptomyces venezuelae.1979; 134(5): 429-36.

[47] Kolla J.P. Narayana; Muvva Vijayalakshmi Chitinase Production by Streptomyces sp. ANU 6277Braz. J. Microbiol. vol.40 no.4 São Paulo Oct./Dec. 2009.

[48] Meriem, G., and Mahmoud, K. (2017). Optimization of chitinase production by a new Streptomyces griseorubens C9 isolate using response surface methodology. Annals of Microbiology, 67(2), 175-183.

[49] Narayana, K. J. P., Kumar, K. G., and Vijayalakshmi, M. (2008). L-asparaginase production by Streptomyces albidoflavus. Indian Journal of Microbiology, 48(3), 331-336.

[50] Amena, S., Vishalakshi, N., Prabhakar, M., Dayanand, A., and Lingappa, K. (2010). Production, purification and characterization of L-asparaginase from Streptomyces gulbargensis. Brazilian journal of Microbiology, 41(1), 173-178.

[51] Quecine, M. C., Araujo, W. L., Marcon, J., Gai, C. S., Azevedo, J. L., and Pizzirani-Kleiner, A. A. (2008). Chitinolytic activity of endophytic Streptomyces and potential for biocontrol. Letters in Applied Microbiology, 47(6), 486-491.

[52] Ghadin, N., Zin, N. M., Sabaratnam, V., Badya, N., Basri, D. F., Lian, H. H., and Sidik, N. M. (2008). Isolation and characterization of a novel endophytic Streptomyces SUK 06 with antimicrobial activity from Malaysian plant. Asian Journal of Plant Sciences.

[53] Robinson, T., Singh, D., and Nigam, P. (2001). Solid-state fermentation: a promising microbial technology for secondary metabolite production. Applied microbiology and biotechnology, 55(3), 284-289.

[54] Baniya, A.; Singh, S.; Singh, M.; Nepal, P.; Adhikari, M. Aryal, S. and Adhikari, A. (2019). Isolation and Screening of Antibiotics Producing Streptomyces spp from the Soil Collected around the Root of Alnus nepalensis from Godawari. Nepal Journal of Biotechnology. 6. 46-56.

[55] Hassan, M. A., El-Naggar, M. Y., and Said, W. Y. (2001). Physiological factors affecting the production of an antimicrobial substance by Streptomyces violatus in batch cultures. Egyptian Journal of Biology, 3(1), 1-10.

[56] Anansiriwattana, W., Tanasupawat, S., Amnuoypol, S., and Suwanborirux, K. (2006). Identification and antimicrobial activities of actinomycetes from soils in Samed Island, and geldanamycin from strain PC4-3. Thai J Pharm Sci, 30, 49-56.

[57] Al-Hulu, S. M., Al-Charrakh, A. H., and Jarallah, E. M. (2011). Antibacterial activity of Streptomyces gelaticus isolated from Iraqi soils. Medical Journal of Babylon, 8(3), 404-411.

[58] Kekuda, P. Shobha K. Onkarappa, R.(2012) Studies on antioxidant and anthelmintic activity of two Streptomyces species isolated from Western Ghat soil of Agumbe, Karnataka. Journal of Pharmacy Research. 3(1): 26-29.

[59] Rabah, F. L., Elshafei, A., Saker, M., Cheikh, B., & Hocine, H. (2007). Screening, isolation and characterization of a novel antimicrobial producing actinomycete, strain RAF10. Biotechnology, 6(4), 489-496

[60] Oskay, M. (2009). Antifungal and antibacterial compounds from Streptomyces strains. African Journal of Biotechnology, 8(13).

[61] Sunanda, K. K., Uma, K. D., and Apparao, A. (2010). Characterization of marine Streptomyces from Visakhapatnam coast. Drug Invention Today, 2(1).

[62] Inbar, J. and Chet, I. (1991) Evidence that chitinase produced by Aeromonas caviae is involved in the biological control of soil-borne plant pathogens by this bacterium. Soil Biol Biochem 23, 973-978.