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Extraction and partial purification of antimicrobial gent produced by Streptomyces spp. in Babylon province

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ABSTRACT

The Strptomyces was isolated from soil samples and identified as Streptomyces spp.
The cultural characteristics of Streptomyces spp isolates showed grey aerial mycelium and was yellowish brown to green substrate mycelium with no pigmentation on YMD agar medium, and no melanin production.. The active metabolite was extracted using ethyl acetate (1:1, v:v).The crude extract showed high antibacterial activity against S.aureus with 17 mm inhibition zone. The UV spectrum of antibacterial agent showed a single peak with maximum absorption(λ max) at 285 nm .
The IR (KBr) spectrum exhibited a peak at position 3400 cm-1 which indicate presence of hydroxyl (OH) group , and anther peak at 2800-3000 cm-1 which refer to presence of C-H alphatic and aromatic group and in addition to third peak at 1760 cm-1 that indicates to presence of carbonyl group (C=O).
Keywords: Stretptomyces, antimicrobial, Extraction and purification, Iraq

Introduction:

Streptomycetes are Gram-positive aerobic members of the order Actinomycetales within the class Actinobacteria (Stackebrandt et al., 1997). Streptomycetes produce an extensive branching substrate and aerial mycelium.The members of Streptomyces are distinguished by their ability to produce an array of secondary metabolites (Berdy, 2005).

The biosynthesis of these substances is influenced by physiological and environmental signals. The production of secondary metabolites commonly precedes the development of aerial hyphae, when the growth rate of bacterial filaments has decreased and sporulation starts. Much of the published data indicate that the most important environmental signal triggering secondary metabolism is nutrient starvation, particularly that of phosphate). The signaling networks behind the regulation of secondary metabolism in Streptomycetes have recently been reviewed by (Sola-Landa et al., 2003,Bibb, 2005).Various antimicrobial substances from Streptomyces sp. and actinomycetes bacteria have been isolated and characterized including aminoglycosides, anthracyclins,glycopeptides, β-lactams, macrolides, nucleosides, peptides,polyenes, polyester, polyketides, actinomycins and tetracyclines. Most of the antibiotics are extracellular-secondary metabolites which are normally secreted in culture media and serve as intermediates from primary metabolisms as precursors for their biosynthetic process(Mellouh,2003).

There are many antibiotic that effect on fungi such as polyenes derived from Streptomyces sp. have a broad in vitro spectrum of activity against a wide range of fungi including the Aspergillus sp. and Candida sp. (Hay, 2003).

Materials and Methods

Samples collection

In this study, 102 different soil samples(from agricultural, sandy and mixed soil) were collected from Babylon province, during the period from May to August 2010.

Isolation of actinomycetes colonies from the soil

Soil samples collected from the local soils were pretreated with calcium carbonate and dried in hot air oven at 45^{o}C for 1 h in order to reduce the incidence of bacteria and molds (El-Nakeeb and Lechevalier, 1963). Soil dilution plate technique was employed to isolate the actinomycete strains on different media such as asparagine-glycerol-salts, asparagine-glucose and starch casein salts agar media with pH adjusted to 7.2 and the plates were incubated at 30^{o}C for 10 days. The

actinomycete strains predominant on different media were picked out, purified and preserved on yeast extract-malt extract-dextrose (YMD) agar medium at 4°C (Williams and Cross, 1971, Thakur, et al .,2007)

Isolation of antibacterial metabolites:

Culture medium was inoculated by Streptomyces and incubated at 30 °C for 7 days in a incubator. After the incubation period, the culture filtrate was separated from the mycelial cake using filter paper, After that traces of fermentation broth was separated from broth by centrifuged at 5000 rpm for 15 min . The solvent was added to the supernatant in 1 : 1 proportion (Augustine et al., 2005).

Extraction and purification of the antibacterial agent :

Antibacterial compounds were recovered from the filtrate by solvent extraction with ethyl acetate in the ratio 1:1 (v/v) and shaken well for 1 h. The ethyl acetate phase was separated and evaporated to dryness in water bath at 80 - 90°C. Residue was weighed and redissolved with little ethyl acetate. The absorption spectrum of each extract was determined in UV region by using UV/VIS spectrophotometer(Dharmaraj et al., 2010).

Antibacterial activity of crude extract:

The crude extract were screened for antibacterial activity against Stapylococous aereus, by well diffusion method. 100 µl of the crude was placed in wells made on Muller Hinton agar plates seeded with the test bacterial pathogen cultures. The plates were incubated at 37°C and observed for inhibition zone after 24 h. (NCCLS) (2003).

Physicochemical characteristic of antimicrobial agent:

Ultraviolet (UV) and Fourier transform infrared spectra(FT-IR):

Ultraviolet (UV) spectrum were recorded on Shimadzu UV-170 spectrophotometer. One milligram of sample was dissolved in 10 ml water and the spectra were recorded at 200–400 nm range. The infrared spectra were recorded on Shimadzu IR-470 model. The spectra were scanned in the 400 to 4000 cm–1 range. The spectra were obtained using potassium bromide pellet technique. Potassium bromide (AR grade) was dried under vacuum at 100°C for 48 h and 100 mg of KBr with 1 mg of sample was taken to prepare KBr pellet. The spectra were plotted as intensity versus wave number(Augustine et al., 2005).

Result and discussion

According to morphological and biochemical tests of the recovered isolates, twenty isolates of actinomycetes were recovered and finally identified as Streptomyces species (19.6%) of which 4 isolates were belonged to Streptomyces spp which represents (20%) of all Actinomyctes organisms recovered.

Morphological and physiological characteristics of Streptomyces spp

Cultural characteristics such as color of aerial mycelium, color of substrate mycelium and pigmentation of Streptomyces spp solates were recorded on YMD agar medium (Figure-1). In addition to that physiological characteristics of these isolates were also recorded according to Shirling and Gottlieb (1966) (Table 1).

Figure -1: Colonies of Streptomyces spp. on yeast malt extract agar (ISP2).

Table (1) Morphological and physiological characteristics of Streptomyces spp isolated from soil samples:

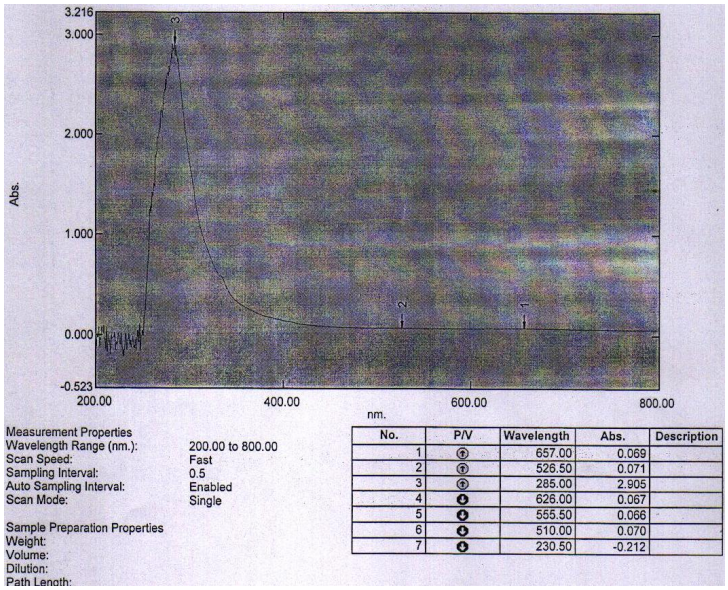
Characteristic	Results
Gram staining	positive
Aerobic growth	aerobic
Spore chain morphology-Sporophore)	Simple (straight) none segmented
Color of Aerial mycelium	grey
Melanin production	absent
soluble pigment	absent
Spore number	4
Substrate mycelium color (reverse color)	Yellow-brown +green pH not sensitive
Color of colony	grey
Texture of colony	Powdery (rigid)
Type of branch	straight
Earthy odor	+
Growth on : -yeast extract medium, -starch casein medium, -potato dextrose agar	 good + +
Sugar fermentation: glucose sucrose manitol D-xylose ribose fructose	 – – + - – –

Antibacterial activity of crude extract :

The antimicrobial activity of crude extract was most effect against S. auras with inhibition zone (17 mm) and this result agreed with result obtained by El-Naggar et al., (2001) showing the highest antimicrobial activity produced from Streptomyces violatus . Our results coincided with the findings of in which growth of S. aureus was inhibited by a substance produced from S. lydicus. Several species of Streptomyces from different soils and water samples are a virtually unlimited source of natural secondary metabolites and many kinds of which are used as pharmaceutical and agrochemical products [Ben-Fguira et al 2005, Pamboukain et al , 2004]

Ultraviolet (UV) spectrum of antibacterial agent

The uv spectrum of antibacterial agent produced by Streptomyces gelaticus SAM 10 showed that it have a single peak with maximum absorption(λ max) 285 nm (figure 2). This result agreed with result obtained by Swaadoun et al. (1999) who found that The UV spectral data for the ethyl acetate extract of the selected strains from fermented broth have a maximum absorbance peaks range between 215 to 320 nm and the characteristics of absorption peaks indicate production of highly polyene natural compounds. The spectral data were consistent with those obtained by Atta et al., (2009) who found that the The UV absorbtion for antibacterial agent produce from Streptomyces spp A2-NIOFD1have a maximum absorbance peaks range between 270 to 302 nm.



Figure(2) Ultraviolet absorbance of antibacterial agent produced by Streptomyce spp

Infrared (IR) spectrum

The IR (KBr) (v-, cm-1) spectrum figure (1) had a peak about 3400 cm-1 which indicate presence of OH group absorption , and a peak appearing at 2800-3000 cm-1 that indicated the presence of C-H alphatic and aromatic group. The peak at 1760 cm-1 that indicates to presence of carbonyl (C=O) fuction of aster or an amide group . this result was read according to Robert et al., (2005). The microbial metabolites from soil sample in Bangladesh, Streptomyces species, isolated an active metabolite 2-hydroxy-3(hydroxymethyl)- 4Hpyran-4-one and the structure of the metabolite was confirmed by chemical and spectroscopic techniques including IR, (Bytul, et al. 2002). Similar functional group and spectra profiles were exhibited by these extracts

suggesting similarity with these extracts and this could be attributed to antifungal activity demonstrated by some of the extracts during preliminary screening .The distribution of the antibiotic inhibition phenotype of Streptomycetes with great antibacterial and antifungal activity which gave a similar spectra profile has also been reported (Illic et al.,2007).

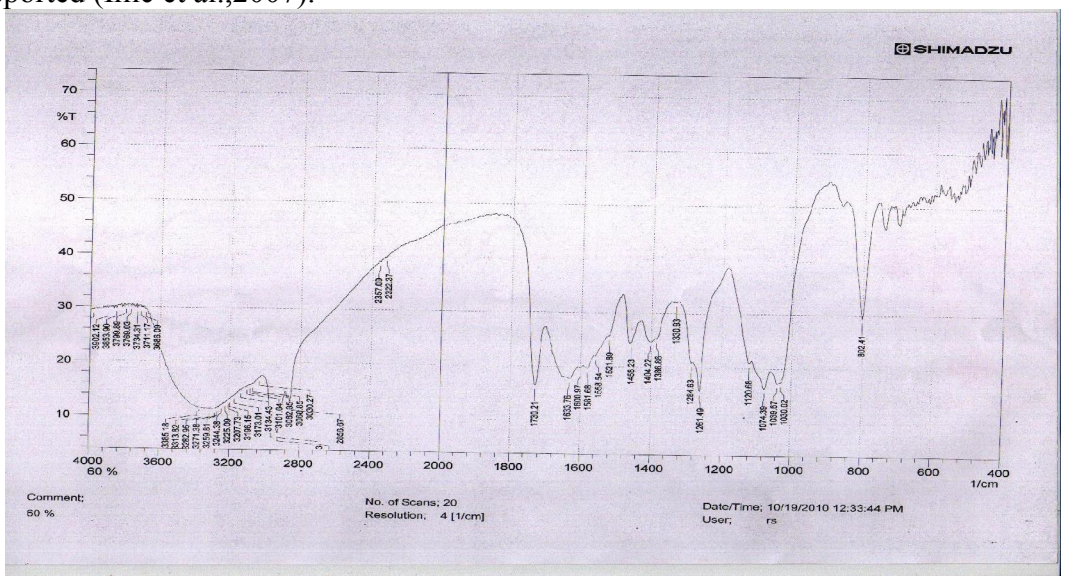


Figure (1): IR spectrum of antibacterial agent produced by Streptomyces spp.

References

Arwa,A.(2007).Production of Antimicrobial Agent by Streptomyces violachromogenes. Saudi .j. Biol., **14** (1): 7-16.

Atta, H.M., and **Ahmad, M.S**. (2009). Antimycin-A Antibiotic Biosynthesis Produced by Streptomyces Sp. AZ-AR-262: Taxonomy,Fermentation, Purification and Biological Activities. Aust. J. Basic

Augustine S K, Bhavsar S P, Baserisalehi M and **Kapadnis B P** (200°). A non-polyene antifungal antibiotic from Streptomyces albidoflavus PU 23 ; Indian J. Exp. Biol.,**42** :928–932

Ben-Fguira, L.F., Fosto, S, Mehdi, R.B, Mellouli L, and **Laatsch H** (2005).Purification and structure elucidation of antifungal and antibacterial activities of newly isolated Streptomyces sp. strain US80. Microbiol.Res., **156**: 341-347

Berdy, J .(2005) Bioactive microbial metabolites: a personal view.J Antibiot,**58**:1–26.

Bibb MJ (2005) Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol, **8**:208–215

Boudjelle, H, Bouti K, Zitouni A, Mathieu F, Lebrihi A, and **Sabaou N** (2006).Taxonomy and chemical characterization of antibiotics of Streptosporangium Sg 10 isolated from a Saharan soil.Microbiol.Res.,**161**:288-298.

Bytul, M.R., M.S.A. Bhuiyan, M.A.A. Al-Bari and A.S.M Anisuzzaman, (2002). Susceptibility of crude metabolites and two isolated compounds of soil Streptomyces species to pathogenic organisms.

Dharmaraj, S., Ashokkumar, B. and **Dhevendaran, K.** (2010).Isolation of marine Streptomyces and the evaluation of its bioactive potential African Journal of Microbiology Research Vol. **4** (4), pp. 240-248.

El-Nagger, MY, El-Assar SA, and **Abdul-Gawad M** (2006). Eroparamycin production by newly isolated Streptomyces sp.strain MAR01:Taxonomy, Fermentation, Purification and Structural elucidation. J.Microbiol.**44**(4):432-438.

El-Nakeeb, M.A, and **Lechevalier, H.A** .(1963). Selective isolation of aerobic actinomycetes.Appl. Microbiol. **11**:75-77

Hay,R.J.(2003).Antifungal drugs used for systemic mycoses.Dermatol.Clin.**21**:577-587.

Ilic SB, Konstantinovic SS, Todorovic ZB, Lazic ML, Veljkovic VB, Jokovic N, and **Radovanovic BC** (2007). Characterization and Antimicrobial Activity of the Bioactive Metabolites in Streptomycete Isolates. Microbiol. **76**: 421–428.

Ilic Slavica B, Sandra, .S, Konstantinovic Zoran B, and Todorovic (2005).UV/Vis analysis and antimicrobial activity of Streptomyces isolates.Med. Biol.,**12**:44-46

Mellouli, L., Ameur-Mehdi R.B., Sioud, S, Salem M, and **Bejar S** (2003).Isolation, purification and partial characterization of antibacterial activities produced by a newly isolated Streptomyces sp. US24 strain.Res. Microbiol. **154**: 345-352.

National Committe for Clinical Laboratory Standards (NCCLS) (2003). Performance standards for antimicrobial susceptibility testing: Approved standard. M2-A8. National Committee for Clinical Laboratory Standards. Wayne, Pa.

Pamboukain CRD, Facciotti MCR (2004). Production of the antitumeral retamycin during continuous fermentations of Streptomyces olindensis. Process Biochem. **39**: 2249-2255.

Shirling EB, and **Gottlieb D** (1966). Methods for characterization of Streptomyces species. Int. J. Syst. Baceriol., **16**: 313-340.

Stackebrandt, E., Rainey, F. A. and **Ward-Rainey, N. L.** (1997).Proposal for a new hierarchic classification system, Actinobacteria classis nov. Int J Syst Bacteriol, **47**:479-491.

Swaadoun, I., Hameed, K.M,and **Moussauui, A**. (1999). Characterization and analysis of antibiotic activity of some aquatic Actinomycetes.Microbios., **99**:173-179.

Thakur, D., Yadav, A., Gogoi, B.K.,and **Bora, T.C.** (2007). Isolation and screening of Streptomyces in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. J. Mycol.Med.. **17**:242-249.

Williams, S.T. and T. Cross, (1971). Isolation, purification, cultivation and preservation of actinomycetes. Methods Microbiol., 4: 295-334.