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Effects of Some Conditions on Antibacterial Activity of Actinomycetes Spp. Isolated from Soil Samples

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ABSTRACT

Thirty soil samples were collected from Hilla town. Five isolates of actinomycetes were found. The antimicrobial activity of these isolates were determined against *S.aureus*. The results showed that *Actinomycetes* spp.2 was active against *S.aureus* with 20mm inhibition zone. Cultural characteristics of *Actinomycetes* spp.2 isolate were recorded. According to morphological and biochemical test, these isolate was belong to *Streptomyces* spp. *Streptomyces* spp.2 was selected for study of effects of various carbon and nitrogen source on antibacterial activity and extraction of antibacterial agent. The results showed that glycerol was given higher inhibition zone when used as carbon source and peptone was best nitrogen source with higher inhibition zone against *S.aureus* and *E.coli.* The antibacterial agent was extracted from *Streptomyces* spp.2. and it active against *S.aureus* with 21 mm inhibition zone and 18 mm against *E.coli*, and lowest inhibition zone against *P.aeruginosa* with 12mm.

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INTRODUCTION

Actinomycetes are positive for gram stain. These filamentous bacteria having highly DNA ratio with G+C more than 55 % .Properly, thy are considered as a mid cluster between bacteria and fungi , at present they are known as prokaryotic, and they are having a wide spreading in soil, water and colonizined with plants (Pragya *et al*., 2012).

Higher proportion of actinomycetes are Free in life style and producing for spores. It saprophytic bacteria and habitat in soil , water and colonized in plants. The Actinomycetes distribution and occurrence depending on soil sort and its play a major role in decomposition for organic material in soil and converted to humus (Jeffery *et al*., 2007).

Streptomycetes are member that affiliate to *Actinomycetales* member and located at class of *Actinobacteria* (Stackebrandt *et al*., 1997).It generate a branching aerial and substrate mycelium, and it recognized by capability for generating for many of secondary metabolites (Berdy, 2005).

More than of 500 *Streptomyces* species was recognized by forming of aerial and spores on solid medium. It is uncomplicated for diagnosis of *Streptomycetes* depending on difference in color and the colony texture which as dry and wrinkle.

Streptomycetes are Actinomycetes which having type 1 cell wall and it belong to *Streptomycestaceae* family which considered as member in order Actinomycetales that having a complex live cycle (Vimal *et al*.,2009).

There are many active metabolite which creating by actinomycetes such as antibiotic ,antitumor , immune changing and enzyme inactivating(Radhakrishnan *et al*., 2011).Other secondary metabolites haven been creating by *Streptomyces which includes* antifungal and anti cancer agent (Atta, *et al*., 2012, Lucas *et al*., 2013).

This study aimed to isolation of *Streptomyces* spp with antibacterial activity and effects of different carbon and nitrogen source against test pathogens and extraction of antibacterial agent.

MATERIALS AND METHODS

Isolation of Actinomycetes:

Soil samples were collected from Hilla town. The samples were treated with calcium carbonate and drying in oven at one hour for 45^{o}C to decreasing the occurrence of bacteria and molds. *Actinomycetes* was isolated on

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yeast malt dextrose agar medium after dilution by using Soil dilution plate method. The pH was controlled to 7.2 and the plates were incubated at 30^{o}C for 10 days (Shirling, and Gottlieb**,** 1966).

Properties of Actinomycetes isolates:

Cultural properties of *Actinomycetes* spp. were observed on YMD (yeast-malt dextrose) agar which encompass color of aerial, substrate mycelium furthermore, pigmentation actinomycete isolates was noted (Shirling and Gottlieb,1966).Actinomycete morphology were examined by slide culture method (Williams and Cross, 1971, Bergey's ,2000). Carbon sources assimilations and melanin production was tested (Shrilling and Gottlieb,1966).

Effect of carbon and nitrogen sources on antibacterial activity:

Impact of many carbon and nitrogen sources on antibacterial activity was examined these includes, (glucose, glycerol, starch, maltose and sucrose) as carbon source and (meat extract, yeast extract, peptone, tryptone and ammonium sulfate) as nitrogen sources. These sources was add to medium in 1% (w/v) for nitrogen and carbon source before the sterilization of medium. The active carbon and nitrogen source which supporting the maximum production of metabolite were chosen . Temperature and pH were controlled to 30^{o}C and at 7.5 (Oskey *et al*., 2011).

Extraction of the antibacterial agent:

Antibacterial agent was extracted from culture filtrate was made by using ethyl acetate as solvent at ratio 1:1 (v/v) after that it shaken well for 1 hour. The solvent was separated and evaporated till drying in water bath. The weigh for residue was recorded and it re dissolved with ethyl acetate in little amount(Dharmaraj *et al*., 2010).

Antibacterial activity of antibacterial agent:

Screening of antibacterial agent were made Against *Escherichia coli, Stapyllococous aureus,* and *Pseudomonas aeruginosa* as test pathogen by using well diffusion method.100 µl of the actinomycetes agent was putted in wells made on Muller Hinton agar plates inoculate with the test pathogen. The inhibition zone was noted after incubation at 37°C for 24 hr. (NCCLS) (2003).

RESULTS AND DISSCUSION

Isolation of actinomycetes:

Thirty soil samples were collected from Hilla City. Five isolates of actinomycetes were detected according morphological test that showed these isolates are gram positive, earthy odor, rigid colony, finding of aerial and substrate mycelium, don't have ability for melanin production. *Actinomycetes* are free in your living, saprophytic. Highly number from actinomycetes were distributed in soil. *Actinomycetes* community represent as one of the most group of soil population which different with type of soil (Flärdh, 2003).

Antibacterial activity of Actinomycetes isolates:

The antimicrobial activity of these isolates were detected against *S.aureus* (Table 1). The results found that *Actinomycetes* spp.2 was higher activity 22 mm against *S.aureus*. According this results *Actinomycetes* spp.2 was chosen for study influence of various carbon and nitrogen source on antibacterial activity and extraction of antibacterial agent.

Table 1: Antibacterial activity of Actinomycetes isolates.

Actinomycetes isolates	Inhibition zone (mm)
Actinomycetes spp.1	18
Actinomycetes spp.2	20
Actinomycetes spp.3	16
Actinomycetes spp.4	12
Actinomycetes spp.5	10

Characterization of Actinomycetes spp.2:

Cultural descriptions which include, aerial and substrate mycelium color for *actinomycetes* spp.2 isolates were recorded on yeast malt dextrose agar medium. According to morphological and biochemical test, Actinomycetes spp.2 were coming back to *Streptomyces* spp. (Table 2).

Antibacterial activity of Streptomyces spp. 2 by using different carbon sources:

The antibacterial activity of culture filtrate of *Streptomyces* spp. 2 showed that the using of glycerol as a carbon source was giving top antibacterial activity with inhibition zone 25 mm against *S.aureus* and 20 mm

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against *E.coli* contrast with other carbon source. Our results similar to results noted by (Wu *et al*.,2008; Oskay, 2009; Oskay, *et al*., 2010) which found that the greatest growth and antibiotic production were seen by using glycerol and glucose.

Table 2: Morphological and biochemical test of *Streptomyces* spp. 2.

characteristics	results
Gram stain	+
aerobic	+
Rigid colony on agar	+
Earthy odor	+
Color of aerial mycelium	grey
Color of substrate mycelium	Yellow brown- green
Melanin producing	-
Carbon utilization: glucose	+
maltose	+
sucrose	+

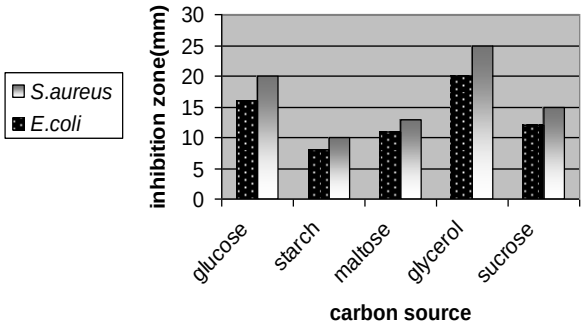

Fig. 1: Influence of different carbon sources on antibacterial activity of *Streptomyces* .2 against test pathogens.

The creating of secondary metabolites are effected by different carbohydrate sources (Ruiz *et al*., 2010).

Antibacterial activity of Streptomyces .2 by using different nitrogen sources:

The results showed peptone was given higher activity against test pathogens with 24 mm inhibition zone against *S.aureus* and 18 mm against *E.coli* when used as nitrogen source. These results agreed with results recorded by (Oskay *et al.,* 2011) who found using of peptone as a nitrogen source was significantly effect on production for antibiotic (Figure 2).

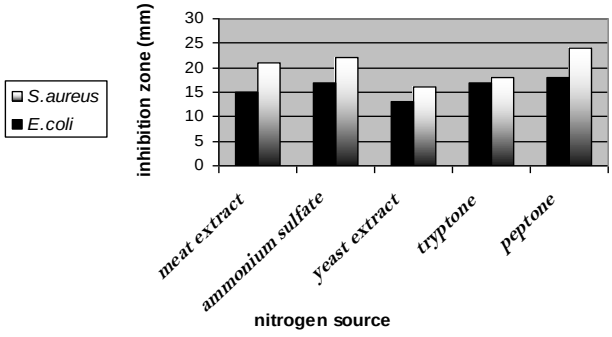

Fig. 2: Influence of various nitrogen sources on antibacterial activity of *Streptomyces* .2 against test pathogens.

Secondary metabolites production during fermentation are effected by various environmental parameters such as nutrients (phosphorous, nitrogen and carbon source), growth proportion, feedback control, enzyme inactivation (Lin *et al*., 2010; Ruiz *et al*., 2010).

Extraction of the antibacterial agent:

The agent which having antibacterial activity was isolated from *Streptomyces* spp.2.The finding of results proved that this agent was active against check pathogens, with inhibition zone 21mm against *S.aureus* and 18 mm against *E.coli*, and 12 mm against *P.aeruginosa*(Figure 3).

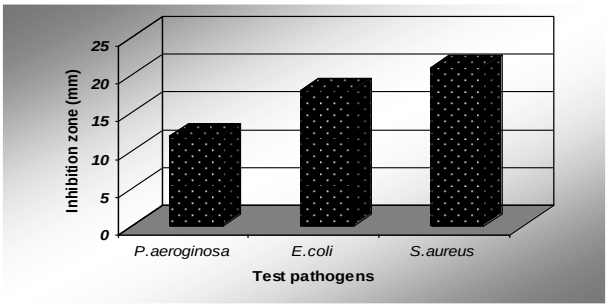

Fig. 3: Antibacterial activity of agent produced by *Streptomyces* spp.2.

Different types of antimicrobial substances from *Streptomyces* sp. and actinomycetes bacteria have been isolated and described including aminoglycosides, macrolides, nucleosides, anthracyclins, glycopeptides, β-lactams, peptides, polyketides, actinomycins, polyenes, polyester, and tetracyclines (Mellouhi, 2003).

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