Antibacterial activity of *Streptomyces Gelaticus* Isolated from Iraqi Soils

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Abstract

A total of 102 soil samples were collected from different sites in Babylon province, Iraq during the period from May to August 2010. According to morphological and biochemical tests, twenty isolates of actinomycetes were recovered and finally identified as *Streptomyces* species (19.6%) of which 4 isolates were belong to *Streptomyces gelaticus* which represent (20%) of all Actinomyctes organisms.

The cultural characteristics of *Streptomyces gelaticus* isolates showed that the color of aerial mycelium was grey, substrate mycelium was yellowish brown to green with no pigmentation on YMD agar medium, and no melanin production. The antibacterial activity of *S. gelaticus* SAM 10 isolate against three pathogenic bacterial species; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, was assessed using well diffusion method. The inhibition zone diameter for *S. aureus* was 19.9 mm while it was 10 mm for both of *E. coli* and *P. aeruginosa* respectively. The present study represents the first record of isolation of *Streptomyces gelaticus* in Iraqi soil.

Introduction

Actinomycetes are an important group of filamentous, gram positive bacteria which comprise a group of branching unicellular microorganisms. It produce branching mycelium which may be of two kinds: substrate mycelium and aerial mycelium. Among actinomycetes, the streptomycetes are the dominant, members of the actinomycetes, which live in natural environment [23].

Streptomyces spp. are ubiquitous in soil microbial communities, and plant materials, waters and marine sediments [27]. The streptomycetes are generally saprophytic organisms which spend the majority of their life cycles as semidormant spores [10]. During the life cycle, streptomycete spores germinate to produce substrate mycelium, which during maturation fragments into chains of spores. The substrate mycelium uses extracellular hydrolytic enzymes to gain nutrition from organic compounds that resist degradation by many other microbial groups, e.g. plant and fungal cell wall polysaccharides and insect exoskeletons. The members of *Streptomyces* are distinguished by their ability to produce an array of secondary metabolites [12,3,14].

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 [9,20,2] and they have a wide variety of chemical structures, including tetracyclines, macrolides, quinocyclines and meroparamycin. These antibiotics show antibacterial activity against Gram-positive bacteria

and Gram-negative bacteria [25,11,9]. In addition to that, *E. coli* ATCC 8739 could be inhibited by the active substance (P3-1) produced from *Streptomyces* spp. [4,17]. In addition, the antibiotics from *S. lydicus* were seen to inhibit the growth of *P. aeruginosa* [24].

This study was aimed at isolation of a new *Streptomyces* spp in local soil samples of Iraq because there is a need to search for new and efficient antibiotic in local soil samples of Iraq especially from the area of the study.

Materials and Methods

Sample collection:

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

Isolation of actinomycetes

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 [8]. 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 redominant on different media were picked out, purified and preserved on yeast extract-malt extract-dextrose (YMD) agar medium at 4^{o}C [26].

Morphological examination

Microscopic examination

A small peripheral portion of well-grown mature parts of the colony was picked using a sterile inoculation loop [22,26]. Micromorphology of the actinomycetes isolates was examined by slide culture method [15]. The morphology of the spores was determined by light microscopy.

Cultural, physiological and biochemical characteristics:

The morphological and cultural characteristics and biochemical tests of isolates were made according to the guidelines of the International Streptomyces Project, ISP [22,26,6]. The cultural aspect of the pure isolates was studied on different ISP medium after 14 days of incubation at 28°C. Carbohydrates utilization was determined by growth on carbon utilization medium (ISP9) supplemented with 1% carbon sources and incubated at 30°C. Melanin production was observed on peptone yeast iron agar and tyrosine agar [21].

Determination of color of the actinomycetes isolates:

Colour of aerial mycelium, substrate mycelium, soluble medium, were determined from mature, sporulating aerial mycelia of the actinomycetes colonies on different media such as starch casein agar, glycerol aspargine agar, yeast extract, malt extract agar, and oat meal agar. Colour of the soluble pigments was determined visually by observing the colour changes in the medium due to the diffusing pigments produced by actinomycete isolates [22,18].

Extraction and purification of the antibacterial agent:

Antibacterial compounds were recovered from bacterial filtrate by solvent extraction with ethyl acetate in a ratio of 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 [5].

Antibacterial activity:

Determination of antibacterial activities of pure extract of *Streptomyces* cultures performed using streak-plate method. Mueller Hinton agar plates were prepared and inoculated with *Streptomyces* cultures by a single streak of inoculum in the center of the petri dish and incubated at 27°C for 4 days. Later, the plates were seeded with test organisms by a single streak at a 90 angle to actinomycetes isolates. Antagonism was measured by the determination of the size of the inhibition zone in millimeters [16].

Minimum Inhibitory Concentration (MIC):

MIC of the antibacterial agent produced by *S. gelaticus* isolate was determined by the broth two-fold dilution method. The crude extract was serially diluted in Mueller Hinton broth (Difco, USA). Different concentrations of the pure extracts (µg\ml) were prepared from the stock solution. The tubes were incubated aerobically at 37°C for 24 hrs for growing of bacteria. After incubation, the tube with lower concentration of extract shows no growth was taken as the MIC value for the respective organism [19].

Results 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 gelaticus* which represents (20%) of all Actinomyctes organisms recovered. According to our knowledge, this species has not been isolated or recovered in Iraq and in many countries.

Morphological and physiological characteristics of *S. gelaticus*:

Cultural characteristics such as color of aerial mycelium, color of substrate mycelium and pigmentation of *Streptomyces gelaticus* isolates were recorded on YMD agar medium (Figure-1). In addition to that physiological characteristics of these isolates were also recorded according to [22] (Table 1).

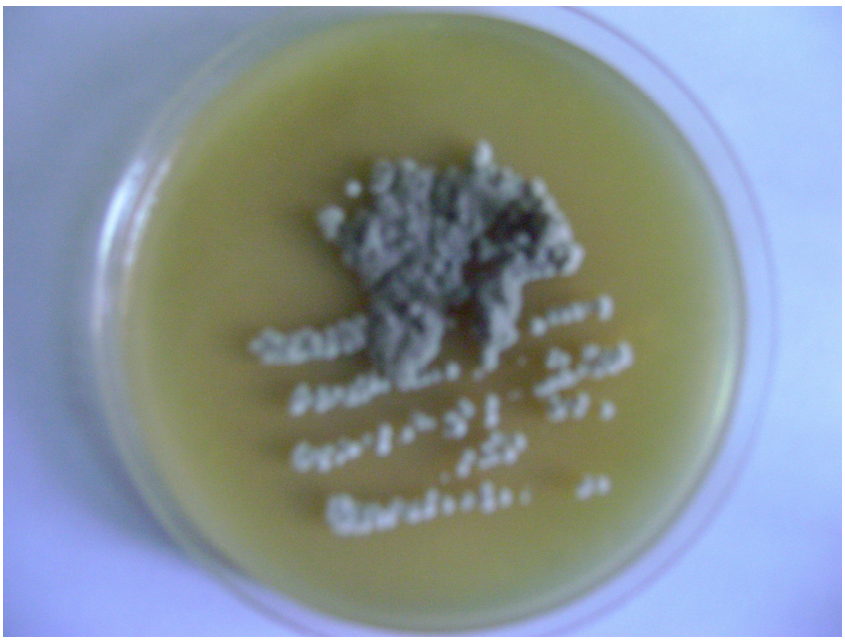

Figure 1 Colonies of *Streptomyces gelaticus* SAM 10 on yeast malt extract agar (ISP2).

Table 1 Morphological and physiological characteristics of *Streptomyces gelaticus* SAM 10 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:

Antimicrobial activities of 20 *Streptomyces* isolates (including *S. gelaticus* isolates) were recorded (Table 2). *S. gelaticus* SAM 10 was showed highest activities against gram-positive bacteria, *Staphyllococus aureus* (19.9 mm), while it showed low activity against Gram-negative bacteria, *Escherichia coli* (10 mm) and *Pseudomonas aeuroginosa* (10 mm). The other *S. gelaticus* isolates showed low or no activity against pathogenic bacteria (Table 2).

Our results coincided with the findings of [24] 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 [9,2,20] and they have a wide variety of chemical structures, including tetracyclines, macrolides, quinocyclines and meroparamycin. These antibiotics show antibacterial activity against

Gram-positive bacteria and Gram-negative bacteria [9,11,25]. In addition to that, *E. coli* ATCC 8739 could be inhibited by the active substance (P3-1) produced from *Streptomyces* spp. [17,4]. In addition, the antibiotics from *S. lydicus* were seen to inhibit the growth of *P. aeruginosa* [24].

The differences in the susceptibilities of Gram positive and Gram negative bacteria to *Streptomyces* extracts have been observed by previous workers [13]. Gram negative bacteria are inherently more resistant to antimicrobials than Gram positive organisms and this has been ascribed to the combined exclusion of antimicrobial compounds by double membrane barrier and transmembrance efflux present in this group of organisms [28].

Minimum inhibitory concentration

Results of the (MIC) of *Streptomyces gelaticus* SAM 10 antibacterial agent showed that MIC values against *S. aureus, E. coli, P. auroginosa* were16, 32, and 32 µg/ ml respectively**.** These results coincided with the findings obtained by [1] who showed that biological activities (MIC) produced by *Streptomyces* sp. AZ-NIOFD1 was active against Gram-positive (MIC ranged from 11.7 to 31.25 ?g/ml) and Gram-negative bacteria (MIC ranged from 11.7 to15.6 ?g/ml).

The biological activities (MIC) of the crude extracts emphasizes that the extracts are active against Gram-positive and Gram-negative bacteria. The MICs values exhibited by all extracts in this study ranged between 0.039 mg/ml and 10 mg/ml. This was relatively higher than the MIC values obtained from marine *Streptomyces* strain Merv 1996 [7] against *B. subtilis* ATCC 6051, *S. aureus* ATCC 6538 and *M. luteus* with MICs of 0.0036 mg/ml, 0.0008 mg/ml and 0.002 mg/ml respectively; *Streptomyces* sp. AZ-NIOFD1 isolated from River Nile water, Egypt [1] with MIC range of 0.0117 mg/ml - 0.03125 mg/ml; and marine *Streptomyces* sp. Merv 8102 [7].

Table 2 The antimicrobial activity of crude extract of 20 *Streptomyces* isolates against pathogenic bacteria.

***Streptomyces gelaticus* isolates**	**Inhibition zone (mm)**		
	S. aureus	***E. coli***	***P. aeruginosa***
Streptomyces gelaticus 1	0	0	0
Streptomyces spp.2	6	6	0
Streptomyces spp. 3	7	6	0
Streptomyces spp. 4	8	7	0
Streptomyces spp. 5	6	7	0
Streptomyces spp. 6	6	0	0
Streptomyces spp. 7	7	10	0
Streptomyces spp. 8	7	9	0
Streptomyces spp.9	8	6	0
Streptomyces gelaticus 10	19.9	10	10
Streptomyces spp.11	10	6	0
Streptomyces spp.12	12	9	0
Streptomyces spp.13	14	8	0
Streptomyces spp.14	15	6	0
Streptomyces spp.15	16	7	0
Streptomyces spp.16	18	7	0
Streptomyces spp.17	17	8	0
Streptomyces spp.18	13	9	0
Streptomyces gelaticus 19	14	8	0
Streptomyces gelaticus 20	12	7	0

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