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Detection on sliver nanoparticles production by *Streptomyces* spp. isolated from soil samples in Hilla city

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Abstract : (22) soil samples were collected from Hilla city. Ten isolates of *Actinomycetes* were recovered. Five isolates were identified as *Streptomyces* spp. Depending on morphological and biochemical assay. *Streptomyces* spp. Isolates were tested for sliver nanoparticle production. The results showed that one *Streptomyces* spp. Isolate have ability for producing of sliver nanoparticle. Silver nanoparticles (Ag NPs) production by *Streptomyces* spp. was detectedby adding (1mM silver nitrate) to supernatant culture, yellowish- brown colour production after incubation indicate to formation of Silver nanoparticles. Cultural characteristics of *Streptomyces* spp.4 isolate was aerial mycelium with grey color, yellowish-brown substrate mycelium on yeast malt agar. Atomic force microscopy (AFM) results showed that AgNPs are spherical in shapes with the particle diameter (121.30nm). The surface thickness is 191 nm ,it represents thickness of the film surface roughness. Antimicrobial activity of particle was determined. The results showed that *Streptomyces*.4with high activity against*E.coli* with (14 mm) inhibition zone compared (11mm) against *S.aureus* ,(8 mm) against *C. albicans.*
Key words : *Streptomyces*spp., sliver nanoparticle production,Antimicrobial activity.

Introduction

Nanotechnology research is one of emerging areas of research with its using in science for manufacturing of new compounds at nanolevel [2]. Generally Nanoparticles are 0.1–1000 nm in size and made by two methods:top–down and bottom–up [19].Silver nanoparticles have exclusive optically, electric, and thermal featureswhich incorporate into products which range from photovoltaics to biological and chemical sensors [10]. Silver nanoparticles have a great importance among metal nanomaterials, because physicochemical properties[1].

Streptomycetes are actinomycetes member, which live in natural environment[3].It's a saprophytic organisms which spend the most life cycles as semi dormant spores. In life cycle, *Streptomycetes* sporesgerminate for producing substrate mycelium, which during maturation fragments into chains of spores[8].

Generally, synthesize many different metabolites, antibioticis represent the most known product which used in veterinary and pharmaceutical side [17].*Streptomyces* spp. positive for gram stain, filament bacteria and produce different compound with having biologically active compounds such ashydrolytic enzymes, antibiotics, and enzyme inhibitors [21].A biologically synthesized AgNPs using *Streptomyces* sp. VITBT7 isolated from soil was recorded[24]. These AgNPs showed SPR peak at 420 nm and spherical shape with 20–70 nm in size .AgNPs showed antimicrobial activity against fungal and bacterial pathogens [23].An extracellular synthesizes AgNPs by *Streptomyces sp*. JAR isolatedfrom the soil samples and with size AgNPs was 68.13 nm, and showed antimicrobial activity against bacterial and fungal pathogens[5].

This study aimed for isolation of *Streptomyces* spp. having ability for producing of sliver nanoparticle and study antibacterial activity for these particle.

Materials and Methods

Isolation of *Streptomyces* Spp.

(22) Soil samples gathered from Hilla city. Samples treated with calcium carbonateLdehydrate in hot air oven (45^{o}C) for 1 hr. to decreasing the bacteria and mold incidence. Dilution plate technique was used for isolating of *Streptomyces* spp .on (YMD) agar medium .The pH was made(7.2).The plate incubated at 30^{o}C for ten days [20-25].

Screening for *Streptomyces* spp with silver nanoparticles production:

Streptomyces was cultivated to Luria Broth medium and incubate at 37ºC on closing rotary shaker (100 rpm for 72 hrs). Broth was centrifuging (7500 rpm for 15 min). Pellet and supernatant were collect separately. Ten ml of 1mM (AgNO3) add into fifty ml of supernatant[1].

Characteristics of *Streptomyces* spp.4 isolate:

Streptomyces cultural characteristics examined on YMD agar,such as aerial mycelium color, substrate myceliumcolor and pigment production by *Streptomyces* spp.isolate. Morphological characteristics of *Streptomyces* spp. checked [20].Carbon utilization sources was made [4].

Antibacterial activity for silver nanoparticles:

The biosynthesized activity of AgNPs was tested by well diffusion method. Wells were made in Muller Hinton agar. Plates inoculated with (*S.aureus,E.coli, C.albicans*) as test pathogens . Fifty microliters of AgNPs were pipetted into each well. After incubation at 37 ^{o}C overnight. Inhibition zone diameter was measured in mm [16].

Atomic force microscopy for silver nanoparticles:

Silver nanoparticles topography examined by AFM (Model AA2000 , made in USA) University of Babylon, College of Science, physical department Thin film for sample prepareon a glass slide (100 µL of the sample) was taken and dropped onto the slide, and drying for 5 min. [9].

Results and Discussion

Isolation of *Streptomyces* spp. isolates:

(22) soil samples gathered from different places in Hilla town. Ten of these were identified as *Actinomycetes* spp.Out of these five *Streptomyces* spp. isolates were identified as *Streptomyces* spp..All isolates positive forgram stain with aerial mycelium grey in color and yellowish- green substrate mycelium when cultivated on yeast malt extract agar.*Actinomycetes* are positive for gramexhibit a filament growth like fungi. Its aerobic and wide spread in nature[13]. *Streptomyces* are, gram positive, aerobic actinomycetes which form branch, substrate mycelium, aerial hyphae that differentiate tostrings of spores, with LL-diaminopimelic acid andno major characteristic sugars in whole-organism hydrolysates[12] and having DNA rich in guanine plus cytosine[14].

Screening for biosynthesis of silver nanoparticles:

Five isolates of *Streptomyces* spp. were checked for production of nanoparticle. One *Streptomyces* spp. isolate have ability for sliver nanoparticle production .*Streptomyces* spp.4 supernatant was pale-yellow in color before the addition of silver ions, after that changed to yellowish-brown at the end of the reaction with silver ions (Figure 1).

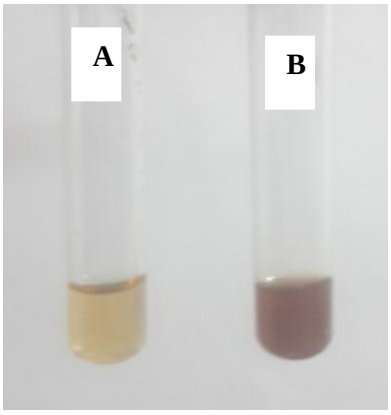


Figure (1): *Streptomyces* spp.4Supernatant mixed with 1Mm $AgNO_3$ .A: Atfirst of incubation (a pale yellow color),B: after 72 hour of incubation (a yellowish- brown color).

Yellowish-brown color which appearin the flasks mixed with silver nitrate was a indication forformation of silver nano particle [22]by reducing of Ag+ ions and formation of surface plasmon resonance in the reaction mixture, no colour change appeared in culture filtrates without silver nitrate[15- 26]. The Nanoparticlessynthesis by actinomycetes has many importance e.g. they are safe to handle, easily available, and possess variable metabolites that may help in reduction. Moreover, these particles have unnumbered applications[6].

The cultural features of*Streptomyces* spp.4

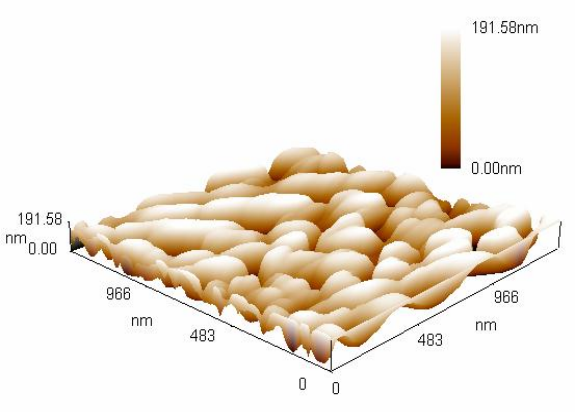
The features of *Streptomyces* spp.4culturewas examined.It was grampositive and aerial myceliumgrey in color when grown on yeast malt extract agar, it was unable melanin producing on tyrosine broth medium. It diagnosed as *Streptomyces*depending on morphology and sugar fermentation (Table1).

Table (1):Morphology and biochemical test of *Streptomyces* spp.4

Results	*Streptomyces* spp.4
+	gram stain
grey	aerial mycelium
yellow-green	substrate mycelium
-	Melanin producing
+	Earthy odor
-	Indole
+	Methyl red production
-	Vogesproskauer
+	Citrate utilization
Sugar fermentation	
+	glucose
-	sucrose
+	mannitol
-	ribose

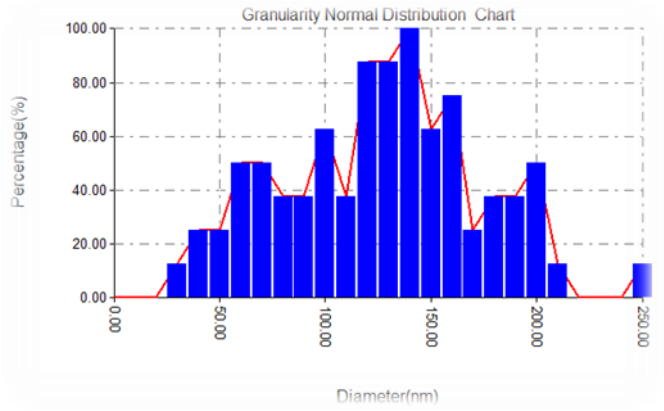
Atomic force Microscope (AFM) of sliver nanoparticles:

The 3D images results of AFM indicate to formation of homogeneous distribution of sliver nanoparticles and spherical in shape. The image topography of AgNps indicates the formation of nanoparticles with agglomeration.



Figure(2):3D picture of Atomic force Microscope (AFM) of sliver nanoparticles synthesized by *Streptomyces* spp.4.

The surface thickness is 191 nm ,itillustrate thickness of film surface roughness, which account for the highest crystalline granular tops on the surface. The regularity in the grown film. The granules with a vertical arrangement on the crystalaxis and equal heights.



Avg. Diameter:121.30 nm

Figure (3):Atomic force Microscope image with nanoparticles size distribution of biological synthesized AgNPs synthesized by *Streptomyces* spp.4.

Figure (3)shows on the granular aggregates distribution on film surface. Average diameter for sliver nanoparticle producing by *Streptomyces* spp.4 equal 121.30nm. Faghri Zonooz and Salouti [7] showed that *Streptomyces* spp.producing sliver nanoparticle with spherical in shape and size ranged (10-100 nm). Subashini[23], record that a biologically synthesized AgNPs using *Streptomyces* spp. VITBT7 isolated from soil samples. These AgNPs having spherical shape and the size is (20–70) nm in range.

Antimicrobial activity:

Antimicrobial activity for sliver nanoparticle produced by *Streptomyces* spp.4 was tested by well diffusion method. Results showed that *Streptomyces* spp.4 have higher activity against *E.coli* with inhibition

zone (14)mm compared with (11mm) against *S.aureus* and (8) mm against *candida albicans*. Silver nanoparticle display a broad bactericidal against gram positive and gram negative bacteria and multi resistant strains [18]. Antibacterial activity of silver nanoparticles was detected [15].Kamel *et al.*[11]record antimicrobial activity of silver nanoparticles.

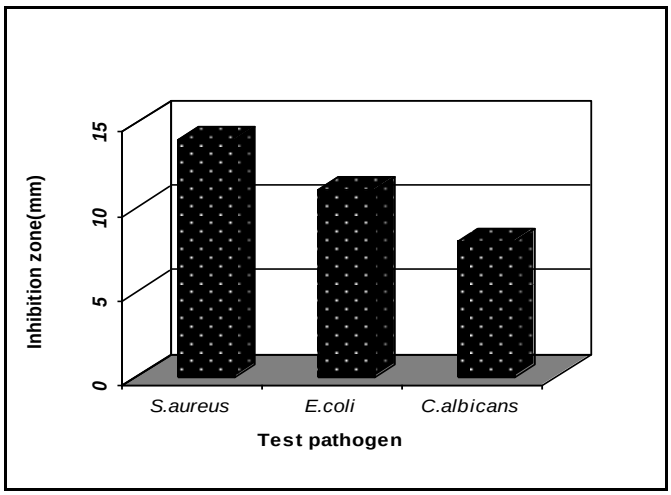


Figure (4):Antimicrobial Activity of Silver Nanoparticles against test pathogens

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