Characterization of Silver nanoparticles produced by *Streptomyces* spp. isolated from Soil Samples

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Abstract:

(24) soil samples were collected from Hilla city. Ten *Actinomyctes* were isolated. Four *Streptomyces* spp. isolate were diagnosed. Theses isolates were examined for production of sliver nanoparticle. *Streptomyces* spp.2 isolate were able on sliver nanoparticle production. All *Streptomyces* spp. isolates having Aerial Mycelium grey in color and Yellow-green Substrate Mycelium. *Streptomyces* spp.2 was selected for study characterization of sliver nanoparticle production. These isolate was grey aerial mycelium and yellow-brown substrate mycelium, glucose and sucrose using ,negative for mannitol and mannose. The characteristics of sliver nanoparticle was studied. The UV spectrum showed maximum absorption(λ max) at 422 nm. The FT-IR spectrum analysis for AgNPs represented absorption peak at 3402 cm^{-1} which refer to OH group,3080 cm^{-1} refer for C-H aromatic group,2924 indicate for presence of C-H aliphatic group,1749,1724 cm^{-1} indicate to C=O group (carbonyl group), 1651 indicate to presence C=C group,1375 indicate to presence of C-O-C, 794 indicate to presence C-Cl or C-Br. Biosynthesized AgNPs are spherical in shape with size (78.96 nm) and having purity (75.09%)as examined by EDXA combined with FE-SEM.

Key words: *Streptomyces*, Sliver nanoparticles, Production, Characterization.

Introduction:

Nanoparticles are sub nano sized colloidal structures created from natural and synthetic polymers with size range from 10-1000 nm [1]. Silver nanoparticles synthesized by using bacteria [17].Silver nanoparticles have many applications such as antimicrobials and therapeutics, bimolecular detection, biolabeling, catalysis and microelectronics, nonlinear optics and intercalation materials for electrical batteries [32]. *Streptomycetes* are *Actinomycetales* member and its *Actinobacteria* class [29]. *Streptomyces* having Aerial and Substrate mycelium, and have ability for producing a lot of secondary metabolites [2]. A big number of *Streptomyces* spp. that different physiologically, morphologically, and biochemical activity. Synthesis of nanoparticle by actinomycetes explain good stability and polydispersity. Actinomycetes having an important biocidal activity against many pathogens[8].Biologically synthesized AgNPs by *Streptomyces* sp. VITBT7 isolated from soil samples was recorded by [30]. These AgNPs showed SPR peak at 420 nm and having spherical shape with the size (20–70) nm. Antimicrobial activity of synthesized AgNPs against fungal and bacterial pathogens were recorded[30].Many techniques for characterizing of nanoparticles such as: UV–visible (UV–vis) Spectrophotometry, Dynamic Light Scattering (DLS), Field Emission Scanning Electron Microscopy (FE-SEM), Energy Dispersive X-ray (EDX) Analysis, Transmission Electron Microscopy (TEM) Fourier Transform Infrared Spectroscopy (FTIR), and powder X-ray diffraction (XRD) [24,26].UV–vis Spectroscopy is a generally used technique for detection of AgNPs[15] .Scanning Electron Microscopy and TEM used for morphologically characterizing at nano- micrometer scale [20]. Metal nanoparticles elemental composition is determined by Energy Dispersive Spectroscopy (EDS)[28]. FTIR spectroscopy is very important for surface chemistry characterizing [4].

This study aimed to isolation of *Streptomyces* spp. having ability for producing of sliver Nano particle and study characterization of these particle.

Materials and Methods:

<u>Isolation of *Streptomyces* spp.from soil samples:</u>

Soil samples gathered from the Hilla City, treating with Caco3 and dry by oven at 45^{o}C (1 hour) to reducing bacteria and mold presence. Soil dilution plate technique was used for isolating of *Steptomyces*spp. on (YMD) agar media. The pH was regulated to 7.2. The plates incubate at 30^{o}C (10 days) [27].

<u>Biosynthesis of silver nanoparticles (AgNPs):</u>

1 mM silver nitrate (50 ml of aqueous solution) mixing with *Streptomyces* spp. supernatant (50 ml) . The pH was regulated to 8.5. Mixture incubate in rotary shaker at 37 ^{o}C (200 rpm) for Five days in the dark. Color change was observed, yellowish brown was appeared when incubated with *Streptomyces* supernatant [23].

Characters of silver nanoparticle :

UV–Visible Spectroscopy (UV–Vis):

Ultraviolet (UV) spectrum was measured by using UV–Vis spectrophotometer (Double Beam Spectrophotometer T80 UV/Vis spectrometer).An aliquot of the tested solution was putted in a cuvette , and monitored for wave length (200 to 700) nm [25].

Fourier Transform Infrared Spectroscopy (FT-IR):

FT-IR for AgNPs was tested by using (Shimadzu IR-470 model) apparatus at chemical department Babylon University. Sample preparing by nanoparticles dispersing in matrix of dry KBr pressed to disc formation. The spectrum for measuring (400–4000) cm^{-1} [5].

Scanning Electron Microscopy (SEM):

The morphology, shape and size of nanoparticles produced was tested by SEM. SEM measurements was achieved in College of Pharmacy, Babylon university by using SEM (FEI QUANTA 450) apparatus,at (10,000 V) [12].

Energy dispersive X-ray analysis (EDX):

EDX was made by using of X-ray microanalysis system coupled with scanning electron microscope (SEM). It was achieved at the College of Pharmacy, Babylon university. (EDX) analysis was achieved by same instrument to confirming silver presence in particles and to detecting for other sample elementary compositions [21].

Results and Discussion:

Streptomyces spp. isolation:

Twenty four soil samples collected from Hilla city. Ten *Actinomyctes* isolates were isolated. Four *Streptomyces* spp. isolate were diagnosed (Table 1).All *Streptomyces* spp. isolates was gram positive and having grey Aerial Mycelium and Yellow- green Substrate Mycelium when cultured on yeast-malt extract medium. *Streptomyces*, Gram-positive bacteria, Actinobacteria phylum. It having similar lifestyle to filamentous fungi. *Streptomycetes* live as soilsaprophytes[13]. It produce branching substrate and aerial mycelium. *Streptomyces* having ability for producing different of secondary metabolites [2].

Table (1): Characteristics of *Streptomyces* spp. isolates.

Characteristics	*Streptomyces* spp.1	*Streptomyces* spp.2	*Streptomyces* spp.3	*Streptomyces* spp.4
gram stain	+	+	+	+
aerial mycelium	grey	grey	grey	grey
substrate mycelium	yellow-brown	yellow-brown	yellow-brown	yellow-brown
Oxidase test	+	+	_	+
Catalase test	+	_	_	+
Sugar fermentation				
glucose	+	+	_	+
sucrose	_	+	+	+
manitol	+	_	_	+
mannose	_	+	+	_

<u>Screening for biosynthesis of silver nanoparticles:</u>

Four *Streptomyces* spp. isolates were checked for extracellular synthesis of AgNPs. *Streptomyces* spp.2 isolate was able to producing of sliver nanoparticle. *Streptomyces* spp.2 supernatant was yellow pale before silver ions add and become yellowish-brown at the reaction end with silver ions(Table 2).Yellowish-brown color indicate to production of sliver nanoparticle when supernatant mixed with silver nitrate. Yellowish-brown color was a clear indication of silver nano particle formation [22]beacause reduction of Ag+ ions and formation of surface plasmon resonance in the reaction mixture [32].*Streptomyces albogriseolus* having ability for producing of AgNPs with size 16.25±1.6 nm) [19]. Anther researcher showed that the biologically synthesized AgNPs*Streptomyces* spp. VITBT7 isolated from soil samples. These AgNPs showed SPR peak at 420 nm and spherical shape with size ranged (20 –70 nm) [30]. Extracellular production for AgNPs using by *Streptomyces* spp. JAR isolated from the soil samples [3]. Biosynthesized AgNPs was 68.13 nm in size.

Table (2): Screening of *Streptomyces* spp. for sliver nanoparticle production

Streptomyces spp. isolates	Results
Streptomyces spp.1	–
Streptomyces spp.2	+(yellowish-brown)
Streptomyces spp.3	–
Streptomyces spp.4	–

Characterization of silver nanoparticles synthesized by *Streptomyces* spp.2

UV–Visible spectroscopy:

UV spectra for *Streptomyces* spp.2 particles showed that maximum absorption at 422 nm Figure (1).Spectro photometric absorption for AgNPs measurements at wavelength ranged of 400–450 nm which used for characterizing of silver nanoparticles [10]. Absorption spectra at 420 nm, indicating for AgNPs presence. AgNPs were spherical shape (20–30) nm and 70% purity as detected by FE-SEM, [18] .Biological method for the AgNPs synthesis by *Streptomyces* sp. ERI-3 cell-filtrate recorded by [7] . UV–vis spectroscopy for theses nanoparticles showed a maximum absorbance at 430 nanometer.

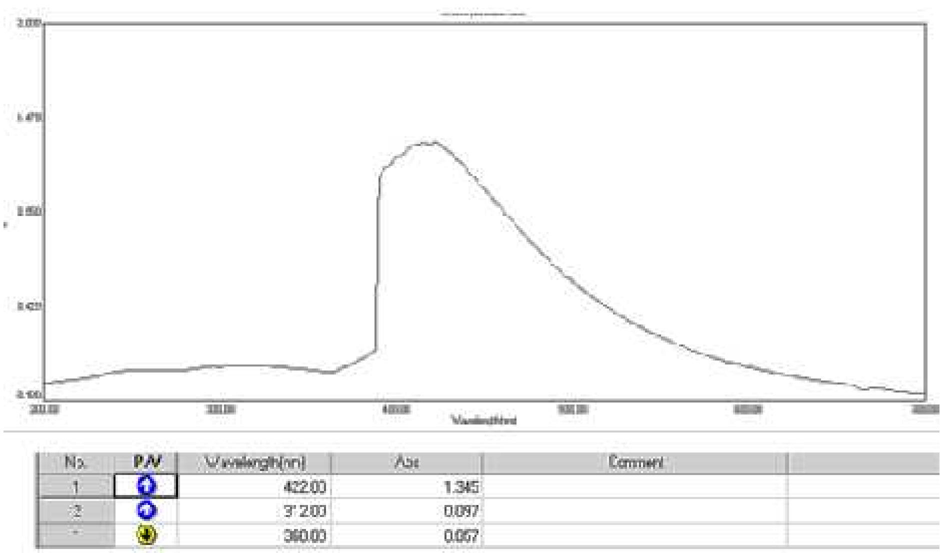


Figure (1) :UV–Vis absorption spectrum of AgNPs synthesized by *Streptomyces* spp.2

Fourier Transform Infrared Spectroscopy (FT-IR):

FT-IR spectrum for AgNPs represented absorption bands at 3402 cm^{-1} which refer to OH group,3080 cm^{-1} refer to C-H aromatic group, 2924 refer to presence of C-H aliphatic group,1749,1724 cm^{-1} refer to presence C=O group (carbonyl group), 1651 refer to C=C group, 1375 refer to C-O-

C,794 refer to C-Cl or C-Br (Figure 2). Similarly, [16] found that the biosynthesized AgNPs have (O–H stretch) is the features of H-bond functional group in compounds (alcohols and phenolic) and (–C=C– stretch) is the features of alkenes group [16]. Results was found by [11] showed that the FTIR analysis refer to findinds of O–H stretching, its responsible for metal reducing ions into their respective nanoparticles.

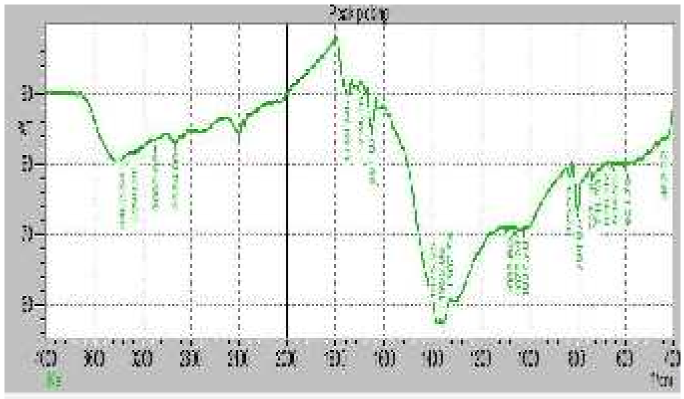
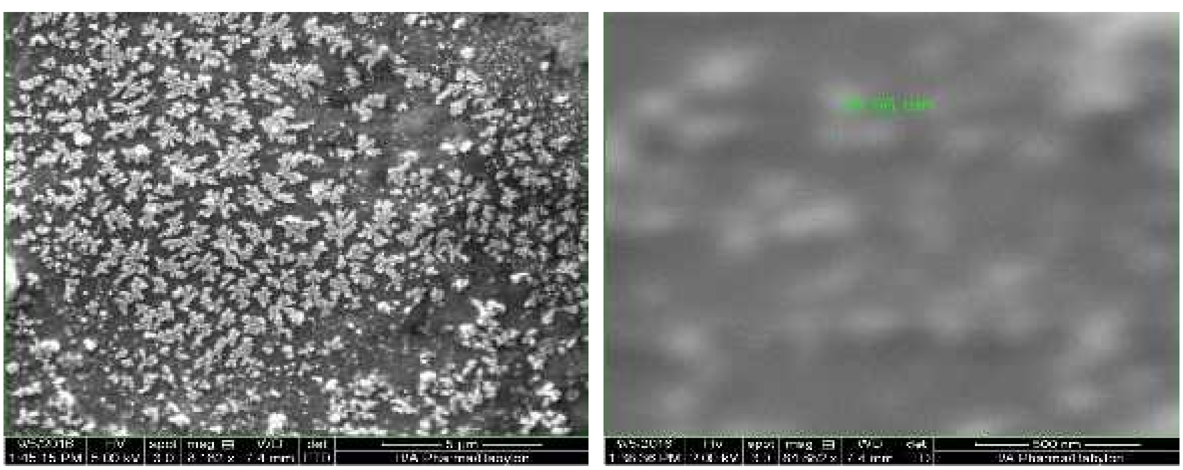


Figure (2): FT-IR analysis of AgNPs synthesized by *Streptomyces* spp.2

<u>SEM analysis of AgNPs synthesized by *Streptomyces* spp.2:</u>

Scanning electron microscope analysis (SEM) was executed to characterizing *Streptomyces* spp.2 (silver nanoparticles) shape and size .The SEM shows that the bacterium has ability for synthesizing of silver nanoparticles which were well break away disperse as much as possible, spherical in shape. Obtained nanoparticles having size ranging 78.96 nm (Figure 3).SEM pictures indicate to extracellullary nanoparticles formation with size (10–100) nm and spherically shape [7]. AgNps producing from *Streptomyces* were spherical in shape with size (20-70) nm recorded by [30].



Figure(3) : SEM analysis of AgNPs synthesized by *Streptomyces* spp.2

Energy dispersive X-ray analysis (EDX):

AgNPs purity was recorded by EDXA (Figure 4) cooperating with FE-SEM. EDXA spectrum detected a strong signal for silver with purity 75.09%.Other peaks observed includes Si, O, C, and Cl .Metal nanoparticles elemental content established by using EDS [28].X-ray microanalysis for silver peck in AgNPs synthesized by *S. rochei* having absorption peak at 3.5 keV, which is typically metallic silver nanoparticles absorption. Silver nanoparticles characterized by using X-ray microanalysis [14].

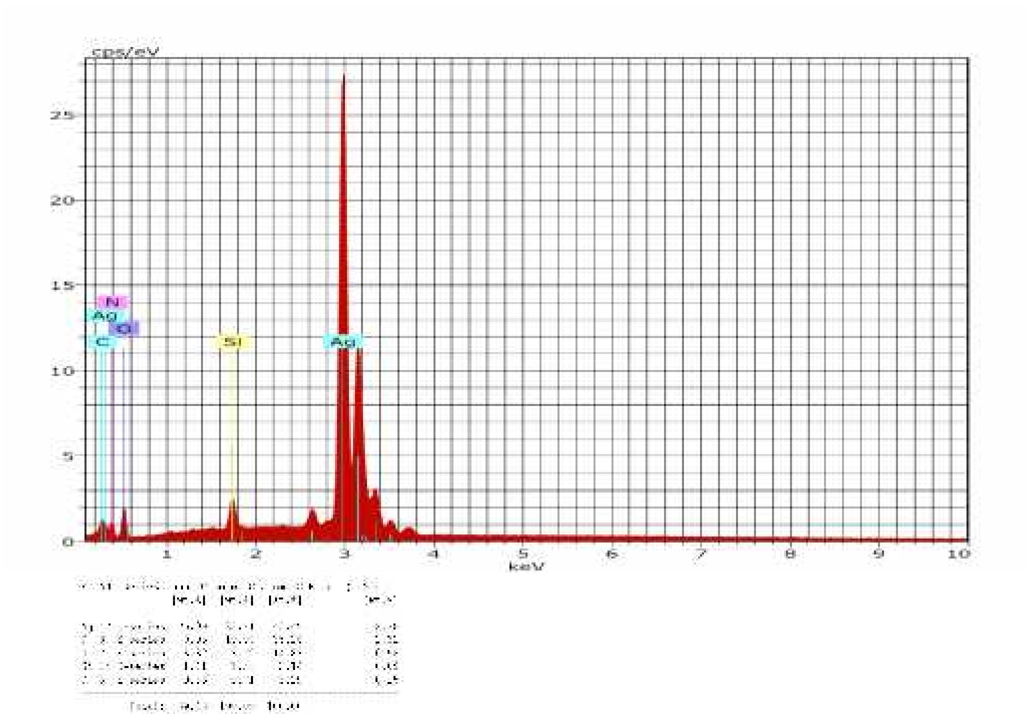


Figure (4) : EDX analysis of AgNPs synthesized by secondary metabolites of *Streptomyces* spp.2

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توصيف جزيئات الفضة النانوية المنتجة بواسطة الستربتومايسس المعزولة من عينات تربه

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الخلاصة :

جمعت أربع وعشرون نموذج تربة من مدينة الحلة . عزلت عشرة عزلات من الاكتينومايسيتات . شخصت أربعة عزلات ستربتومايسس .فحصت هذه العزلات لإنتاج جزيئات الفضه النانوية . وجد ان عزلة الستربتومايسس 2 لها القابلية على إنتاج جزيئات الفضة النانوية. امتلكت جميع عزلات الستربتومايسس المعزولة مايسيلم هوائي رصاصي اللون ومايسليم أساس اصفر – بني .اختيرت عزلة الستربتومايسس 2 لدراسة خصائص جزيئات الفضة النانوية المنتجة امتلكت هذه العزلة مايسيليوم هوائي رصاصي اللون ومايسليوم أساس اصفر وكانت قادرة على استغلال الكلوكوز والسكروز وغير قادرة على استغلال المانيتول والمانوز. اظهر طيف امتصاص الأشعة فوق البنفسجية أقصى امتصاص عند 422 نانوميتر . اظهر طيف امتصاص الأشعة تحت الحمراء قمة عند 3402سم$^{-1}$ والتي تشير إلى وجود مجموعة هيدروكسيل(OH) , 3080سم$^{-1}$ تشير إلى وجود مجموعة (C-H) اروماتيه , 2924 سم$^{-1}$ تشير إلى وجود مجموعة (C-H) اليفاتية , 1724 – 1749 سم$^{-1}$ تشير الى وجود مجموعة كاربونيل(C=O), 1651سم$^{-1}$ تشير الى وجود مجموعة (C=C) , 1375سم$^{-1}$ تشير الى وجود مجموعة (C-O-C) , 794سم$^{-1}$ تشير إلى وجود مجموعة (C-Br او C-Cl) . امتلكت جزيئات الفضة النانوية المنتجة شكلا كرويا وحجم مساوي إلى 78.96 نانوميتر وامتلكت نقاوة 75.09% كما فحصت بتقنية تحليل الأشعة السينية التفريقي والمجهر الالكتروني الماسح.

الكلمات ألمفتاحيه: ستربتومايسس,جزيئات الفضة النانوية , انتاج ,خصائص.