Antibacterial activity of Metal nanoparticles produced by *Streptomyces* spp. Isolated from soil samples

Samer M. Al-Hulu*
Department of Environment, Al-Qasim Green University Al-Qasim, Babylon, Iraq

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**Abstract**
Twenty-five soil samples were collected from Hilla city. (10) Actinomycetes isolates were isolated. Five *Streptomyces* spp. Late was diagnosed. Isolates positive for a gram and having grey Aerial Mycelium and yellow-green Substrate mycelium on yeast-malt extract medium. All *Streptomyces* spp. olates for nanoparticles production. *Streptomyces* spp.2 was able for producing of oymes spp.2 was, grey aerial, yellow-green substrate mycelium, unable for in producing, having the ability for using glucose, sucrose, mannitol, negative for fructose and mannose, negative for indole, and voga Proskauer, positive for methyl red test and citrate utilization, negative for catalase and urea test. UV spectrum for ZnO particles showed maximum absorption at 418 nm. FT-IR spectrum for ZnO nanoparticles represented absorption peak at 3425.58 cm⁻¹ is O-H group, 2360.87 cm⁻¹ is C=O group, 1678.07 cm⁻¹ is C-H group, 1089.78 cm⁻¹ is C-O bending, 1006.84 cm⁻¹ is C-O stretching, 615.36 cm⁻¹ is C-H, and 545.86 cm⁻¹ is C-Cl stretching. SEM shows the ability of *Streptomyces* spp.2 for spherical ZnO nanoparticles synthesizing with size (78.96) nm. *Streptomyces* spp.2 ZnO nanoparticles were having a great effect on E.coli, with inhibition zone (20 mm) and (15,18) mm against S.aureus, Klebsiella pneumonia.

*Corresponding Author
Name: Samer M. Al-Hulu
Phone:
Email: alhulusamer@ymail.com

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**INTRODUCTION**

Nanotechnology is a progressively step for the production of new materials science. It’s capable of providing new implementations in sciences and other technologies at the nanoscale level (Albrecht et al., 2006). It is taken into as the synthesis, characterization, materials in the nano region (1–100 nm). Generally, metal nanoparticles are less than 100 nm and having (20–15,000) atoms (Rai et al., 2009). Bacteria and fungi, examined for producing many metal nanoparticles include silver, gold, zinc, alginate, copper, and magnesium (Ghorbani et al., 2011). Metal nanoparticles from a biogenic source, having an antimicrobial activity (Kalimuthu et al., 2008). Decreasing in ionic concentration by biological systems due to size-controlled, stable, and scattered nanoparticle, having charming physicochemical features (He et al., 2007; Kim et al., 2007). Metal nanoparticles effect on a bacterial cell by bacterial cell penetration which causes membrane damage and the organism become dead. The production of half of the discovered bioactive secondary metabolites recorded by *Streptomyces* spp. (Vaurnakis and Blander, 1983) Antitumor agents (Radhakrishnan et al., 2011). *Streptomyces* are actinomycetes member, which occur in the natural environment (Actinomycetales 2000). It’s saprophytic organisms which stay most life cycles as semi-dormant spores. In the life cycle, *Streptomyces*...
spores germinate for producing substrate mycelium, which during maturation fragments into chains of spores (Berdy, 2005).

Streptomyces spp produces manganese, zinc nanoparticles (Waghmare et al., 2011). Morphologically characterizing at the nano-micrometre scale estimating by Scanning Electron Microscopy and TEM (Schaffer et al., 2009; Jayaseelan et al., 2012).

The study goal for finding Streptomyces spp, having the ability for producing a metal nanoparticle and study some character for these particles.

Figure 1: UV–Vis spectrum for Zno NPS Streptomyces spp.

Figure 2: FT-IR analysis of Zno nanoparticles synthesized by Streptomyces spp.

**MATERIALS AND METHODS**

**Streptomyces spp. Isolation**

Soil samples were collected from Hilla City and treated with Caco3 after draining by oven at 45°C (one hr.) due to reducing bacteria and mould occurring. Soil dilution plate was used for Streptomyces spp. By using YMD agar media. pH was done 7.2. and Incubate at 30°C (10 days) (Shirling and Gottlieb, 1966).

**Biosynthesis of metal nanoparticles**

**Biomass preparation**

Streptomyces spp.2 were put in Erlenmeyer flask (500 ml) containing (malt extract glucose, yeast extract peptone broth (100 ml)) and growth supplied with (griseofulvin 50 µg/ml) on a shaker (200 rpm) at 35°C (four days). Remove the flasks from shaker, stored at five to ten °C; mycelial biomass becomes settled. Streptomyces supernatant neglected, One hundred ml of Distilled Water add for washing the cells. Flasks were kept at five to ten °C to settle the biomass, for 30 min. The supernatant was ignored with slow, one hundred ml of DW second added in the flask, and its do again for (3) times. Mycelial mass removed from the DW by centrifuge (1500 rpm) for ten minutes, Mycelial pellets were weighed and utilize for zinc nanoparticles.

synthesis. (5) a gram of Streptomyces wet biomass exposure to 50 ml znso4 in (250) ml Erlenmeyer flask after that flakes keeps on the shaker at (200 rpm) for 35°C for four days. Colour changing observe after 12, 24, 48 and 72 h. The formation of yellow colour indicates the formation of zinc nanoparticles indicate a positive result (Waghmare et al., 2011).

**Characteristics of Streptomyces spp.2 isolates**

Cultural characteristics of Streptomyces spp.2 were recorded on YMD (yeast-malt dextrose agar which includes aerial, Substrate mycelium and pigment production (Shirling and Gottlieb, 1966). Morphological characterization of Streptomyces spp.2 isolates was tested (Williams and Cross, 1971; Actinomyctales, 2000) . Carbon sources using and melanin pigment producing for Streptomyces spp. The strain was recorded (Shirling and Gottlieb, 1966).

**Characters of ZnO nanoparticles**

**UV–Visible Spectroscopy (UV–Vis)**

Ultraviolet (UV) spectrum was examined by UV–Vis spectrophotometer (Double Beam Spectrophotometer T80 UV/Vis spectrometer) (200 to 700) nm (Waghmare et al., 2011).

**Fourier Transform Infrared Spectroscopy (FT-IR)**

FT-IR for ZnO nanoparticles was tested by using (Shimadzu IR-470 model) apparatus at college of Science, chemistry department, at Babylon University. Sample preparing by nanoparticles dispersing in a matrix of dry KBr pressed to disc formation. The spectrum for measuring (500- 4000 )cm⁻¹ (Isaa et al., 2015).

**Scanning Electron Microscopy (SEM)**

Morphology, shape and size nanoparticles were tested by SEM, at Babylon university Pharmacy College, SEM (FEI QUANTA 450) apparatus, at (10,000 V).

**Antibacterial activity for Streptomyces spp.2 nanoparticle**

Streptomyces spp.2 nanoparticle tested against E.coli, S.aureus, Klebsiella pneumoniae, by using
Figure 3: SEM analysis of Zno nanoparticles for Streptomyces spp.

Table 1: Screening of Streptomyces spp. Isolate for Zno nanoparticle production

| Streptomyces spp. isolates | Results |
|---------------------------|---------|
| Streptomyces spp.14       | -       |
| Streptomyces spp.2        | +       |
| Streptomyces spp.5        | -       |
| Streptomyces spp.18       | -       |
| Streptomyces spp.10       | -       |

Table 2: Tests for Streptomyces spp.2

| Streptomyces spp.2        | Result     |
|---------------------------|------------|
| Gram stain                | +          |
| aerial mycelium           | Grey color |
| Substrate mycelium        | Yellow-green color |
| melanin                   | -          |
| Catalase                  | -          |
| Urea test                 | -          |
| IMVIC test                | -          |
| Indole                    | -          |
| Metyl red                 | +          |
| Vogaes proskauer          | -          |
| Citrate                   | +          |
| Sugar utilization:        |            |
| Glucose                   | +          |
| Sucrose                   | +          |
| Fructose                  | -          |
| Mannitol                  | +          |
| Mannose                   | -          |
Table 3: Antibacterial activity for Streptomyces spp.2 Zno nanoparticles

| Bacterial test | Inhibiton zone (mm) |
|----------------|---------------------|
| S.aureus       | 15                  |
| K. pneumoniae  | 18                  |
| E.coli         | 20                  |

well diffusion plate methods. 100μ of the agent was put in well made on (Muller Hinton agar) seeded with bacterial tests. The diameter of the inhibition zone after 24 hr incubation at 37 °C (NCCLS, 2003).

RESULTS AND DISCUSSION

Streptomyces spp. Isolation

A total of (25) soil samples collecting from Hilla city, 10 Actinomyces isolates were isolated. (5) Streptomyces spp. The isolate was diagnosed (Table 1) — all Streptomyces spp. Strains were 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 had a similar lifestyle to filamentous fungi. Streptomycetes live as soils saprophytes (Flärdh, 2003). It had a substrate, and an aerial mycelium branched (Berdy, 2005).

Screening for the biosynthesis of ZnO nanoparticles

All Streptomyces spp. Isolates were screened for ZnO nanoparticles production. The result found Streptomyces spp.2 was able for producing of the nanoparticle. The changing of colour after 72 hr is an indicator for nanoparticles production. (Table 1).

Streptomyces sp. HBUM171191 was converted progressively after 72 hours. It was dark yellow when treated by an aqueous solution of (10-3 mM) ZnSO4 (Waghmare et al., 2011).

Characteristic for Streptomyces spp.2

Streptomyces spp.2 was gram-positive, grey aerial mycelium, yellow-green substrate mycelium, when cultured on YMD agar, negative for melanin-producing. Streptomyces spp.2 having the ability for using glucose, sucrose, negative for fructose and mannose (Table 2).

UV–Visible spectroscopy

UV spectra for Streptomyces spp.2 particles showed that maximum absorption at 418 nm (Figure 1). The ZnSO4 treated Streptomyces sp. HBUM171191 having maximum 350 nm after 72 hr (Waghmare et al., 2011).

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectrum for Zno nanoparticles represented absorption peak at 3425.58 cm⁻¹ is O-H group, 2360.87 cm⁻¹ is C=O , 1678.07 cm⁻¹ is C=O group, 1388.75 cm⁻¹ is C·H group.1089.78 cm⁻¹ is C-O bending, 1006.84 cm⁻¹ is C-O stretching, 615.36 cm⁻¹ is C-H, and 545.86 cm⁻¹ indicate to C-cl stretching (Figure 2).

SEM analysis of ZnO nanoparticle synthesized by Streptomyces spp.2

Scanning electron microscope analysis (SEM) was made. The SEM shows that the bacterium has the ability for synthesizing of ZnO nanoparticles which were spherical, with size ranging (78.96) nm. Extracellular nanoparticles formation with size (10–75) nm and spherically shape (Isaa et al., 2015).

Antibacterial activity for ZnO nanoparticles

Streptomyces spp.2 ZnO nanoparticles were having a significant effect on E.coli, with inhibition zone (20 mm) compared with (15,18) mm inhibition zone against S.aureus, Klebsiella pneumonia. (Table 3). ZnO-NPs with (80-100) nm size having activity against S.aureus and E.coli was recorded by Yamamoto O.(2001). ZnO-NPs show good antibacterial activity and generation of reactive oxygen species (ROS) (Janaki et al., 2015) by bacterial cell membrane penetration by the formation of pores and the cell contains is damage by ROS (Yamamoto, 2001; Brayner et al., 2006).

CONCLUSIONS

Streptomyces spp. Isolates were screened for nanoparticles production. Streptomyces spp.2 was able for producing of the nanoparticle. SEM shows the ability of Streptomyces spp.2 for spherical ZnO nanoparticles synthesizing with size (78.96) nm. Streptomyces spp.2 ZnO nanoparticles are having a significant effect on E.coli, with inhibition zone (20 mm) and (15,18) mm against S.aureus, Klebsiella pneumonia.
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Conflict of interest

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