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Biosynthesis, Characterization, and Antimicrobial Activity of Silver Nanoparticles from Actinomycetes.

Zeinat Kamel*, Mahmoud Saleh, and Noha El Namoury.

Botany and Microbiology Department, Faculty of Science, Cairo University, Giza, Egypt.

ABSTRACT

Biosynthesis of silver nanoparticles using some Streptomyces species was reported, out of 46 strains tested, 15 strains showed ability to synthesized AgNPs. Among which the two potential Streptomyces M-13 and Streptomyces M-24 showed high potency of production of silver nanoparticles. Biosynthesized AgNPs from potent strain was characterized using UV-visible spectroscopy, FTIR analysis, Transmission Electron Microscope (TEM). TEM study indicated the formation of spherical shape distributed silver nanoparticles without aggregation varying from 10-20 nm in size. The silver nanoparticles synthesized by potential Streptomyces M-13 and Streptomyces M-24 showed good antibacterial activity against Gram-positive and Gram-negative bacteria by well diffusion method. The two potential Streptomyces species M-13 and M-24 was characterized and identified as Streptomyces graminofaciens and Streptomyces catenulae, respectively. The two Streptomyces strains reported in the present study are a newly added source for the biosynthesis of silver nanoparticles and these nanoparticles can be used potentially for biomedical application.

Keywords: Silver nanoparticles, *Streptomyces graminofaciens, Streptomyces catenulae*, TEM, antimicrobial activity.

Corresponding author

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INTRODUCTION

Nanotechnology is emerging as a rapidly growing filed with its application in science and technology [1]. The synthesis of nanoparticles of different chemical compositions, sizes, and controlled monodispersity is an important area of research in nanotechnology. In addition, there is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocols. In modern nanoscience, the interaction between inorganic molecules and biological synthesis is one of the most exciting areas of research.

In this respect, many unicellular and multicellular microorganisms were viewed as ecofriendly nanofactories to produce inorganic materials either on intra- or extracellular level [2-6]. Biological approaches using microorganisms, such as bacteria, actinobacteria, moulds, yeast, algae [8], and plant or plant extracts have been suggested as valuable alternatives to chemical and physical methods. The biological methods are eco-friendly, low cost and provide good yields. Recently, actinobacteria isolated from different ecosystems were recognized as potential synthesisers of gold and silver nanoparticles [31, 35]. The spread of multidrug resistant bacteria is one of the most serious threads for successful treatments of infectious diseases [15].

It was reported that silver had been used as antimicrobial agents since ancient times [9]. With the advancements in nanotechnology, AgNPs have found its significant application as antimicrobial agents, in fileds of microelectronics, catalysis, and biomolecular detection [10-12]. Although the antibacterial activity of AgNPs has been proved in the recent years, the actual mechanism of action is not yet clear. They may inactive microorganisms by interacting with their enzymes, protein or DNA to inhibit cell proliferation [13]. it is also evident that the increased antimicrobial activity of AgNPs may be attributed to its special characteristics of small size and high surface area to volume ratio [14].

The current study involves the biosynthesis of AgNPs for biomedical applications by using actinomycetes isolate. The synthesized AgNPs were characterized and tested for their antimicrobial activity.

MATERIAL AND METHODS

Samples were collected for the isolation of actinomycetes from soil and rhizosphere samples collected from different locations in Egypt from a depth of 10-15 cm. The collected samples were carefully stored in polythene bags and transported to the laboratory for further uses. The isolation was carried out by serial dilution technique on starch casein agar medium supplemented with nalidixic acid (20 ug/ml) and and nystatin (100 ug/ml) to reduce bacterial and fungal population incubated for 6-7 days at 37oC. The actinomycetes isolated were identified based on their morphological, physiological, and biochemical characteristics. The obtained isolates were maintained and stored on Starch Casein Agar (SCA) medium for further use.

For screening of strains for synthesis of AgNPs, 46 isolates were freshly inoculated on 50 ml sterile starch casein medium in flasks and the flasks were incubated at 28◦C on rotary incubator shaker at 150 rpm for 5 days (pH 7.0). After incubation period, the culture was centrifuged at 5000 rpm for 30 min and the supernatant was used for the biosynthesis of AgNPs. Deionized water was used as a solvent in the synthesis of AgNPs. The collected supernatant (pH 7.0) was added separately to the reaction vessel containing silver nitrate at a concentration of 1% (v/v) and incubated on an orbital shaker at dark condition at 30◦C for 24 h . The reaction was carried out in the dark after the addition of the AgNO3, the flasks were observed for the synthesis of AgNPs by color change [16]. The cell free supernatant without the addition of AgNO3 was maintained as control. After desired reaction period, the reaction mixture containing silver nanoparticles was centrifuged at 10,000 rpm for 15 min, for disposing any impurities. The process of centrifugation and re-dispersion in sterile double distilled water was repeated thrice to ensure better separation of free entitles from the metal nanoparticles. The purified pellets were freeze dried using a lyophilizer [17]. According to the fast reduction of AgNO3 into AgNPs, a proficient Streptomyces strain was selected and used for further characterization.

The reduction of silver nitrate to silver using Streptomycetes extract was monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with deionized water,. The measurements are recorded on Shimadzu Dual Beam Spectrometer (Model UV-1650 PC) operated at a resolution of 1nm. FT-IR measurement was carried out for silver nanoparticles to identify the possible

bioactive molecules responsible for the reduction of the Ag+ ions, in the diffuse reflectance mode at a resolution of 4cm-1 using KBr pellets and the spectrum was recorded in the wavelength interval 4000 to 500cm-1. TEM technique was employed to visualize the size and shape of silver nanoparticles. The 200KV high resolution transmission electron microscope (FEITECNAI F- 20) was used. TEM grid was prepared by placing a drop of the particle solution and drying under a IR lamp [18].

Identification of potential two actinomyces was performed using phenotypic method and molecular identification. Morphological (macroscopic and microscopic), biochemical, and physiological characterization of potential Streptomyces species were tested following the standard protocol of the International Streptomyces Project (ISP) [19] and was identified with the help of keys of Bergeys manual of systematic bacteriology (1989, 1994).

Antimicrobial activity of the silver nanoparticles was checked by agar well diffusion method on mueller hinton agar plates. The concentrations of bacterial suspensions were adjusted to 0.5 McF using a spectrophotometer and were lawn cultured on mueller hinton agar (MHA) plates by using sterilized cotton swabs. In each of these plates, wells were cut out using a standard cork borer (6 mm diameter). Using a micropipette, 100µl of silver nanoparticle (100µg/ml) and 100µl of distilled water as control was added to separate wells. Plates were incubated for 24 hours at 37°C. Anti-bacterial activity was evaluated by measuring the zone of inhibition. Experiment was performed in triplicates [21].

RESULTS AND DISCUSSION

A total of 46 Streptomyces species samples were isolated from collected soil, rhizosphere and marine samples. The isolated Streptomyces spp are inoculated into the production starch nitrate medium and glycerol yeast extract agar for screening of biosynthesis of silver nanoparticles. Among 46 strains, tested 15 strains showed color change from yellow to brawn indicated the ability to synthesis AgNPs. The Ag+ ion reduction was evidently noticeable when $AgNO_3$ was added to the supernatant of Streptomyces species and the color changed from yellow to dark brown after few days. In control there was no color development (fig 1). Similar reports of color change during extracellular biosynthesis of AgNPs were also reported [22-25]. The color change of the Streptomyces filtrate from colorless to dark brown (test) on addition of $AgNO_3$ suggested the formation of silver of silver nanoparticles.

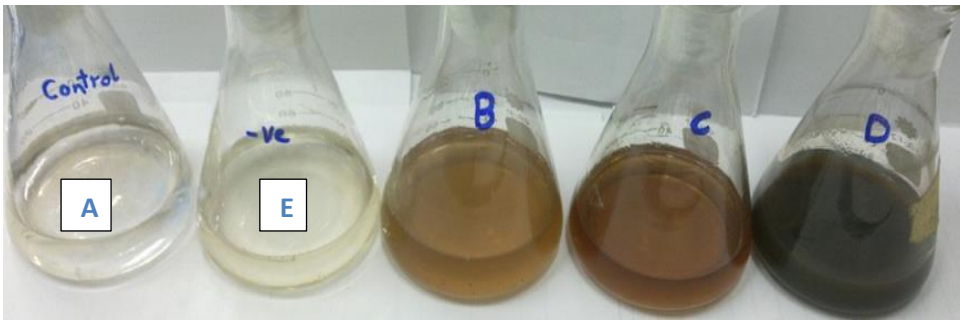

Figure 1: (A) control before addition of $AgNO_3$. (E) –Ve sample have no reaction after addition of $AgNO_3$ and incubation for few days. (B,C,D) After addition of $AgNO_3$ and incubation for few days showing different degrees in color changing from dark yellow to dark brown.

The generation of dark brown color is due to the surface Plasmon resonance (SPR) exhibited by the nanoparticles [25]. The UV-Vis spectrum in fig (a,b) showed SPR peak of silver nanoparticles at 425 and 417 nm . It is well known that the size and shape of the silver nanoparticles reflects the absorbance peak [26]. The absorption spectrum obtained showed a strong surface Plasmon resonance band maximumal 425&417 nm fig 2 (a,b) a characteristic peaks of silver nanoparticles [23]. Although actionmycetes are known for production of 10000 microbial bioactive metabolites, only three genera viz. Thermomonospora, Rodococcus and Streptomyces are involved in nanoparticles biosynthesis [44]

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UV-visible spectrophotometry

The absorption spectra of AgNPs synthesized by two Streptomyces species showed a surface Plasmon absorption band with maximum of 417 nm for Streptomyces -13 and 425 for Streptomyces -24, indicating the resonance of AgNPs (Fig 2 a,b). Similarly Bhainsa reported that the biosynthesized AgNPs are primarily conformed by UV-Vis spectroscopy and peak was noted at 420 nm [27]. Priyaragini reported that AgNPs biosynthesized by actinobacteria with sharp narrow absorption peak located between 420-450 nm [24]. Similar results was found that silver nanoparticles produced by an actinomyces isolated from mangrove soil showed a strong surface Plasmon resonance band maximum at 432 nm [25]. The absorption peak depends on particle size and stabilizing molecules [28].

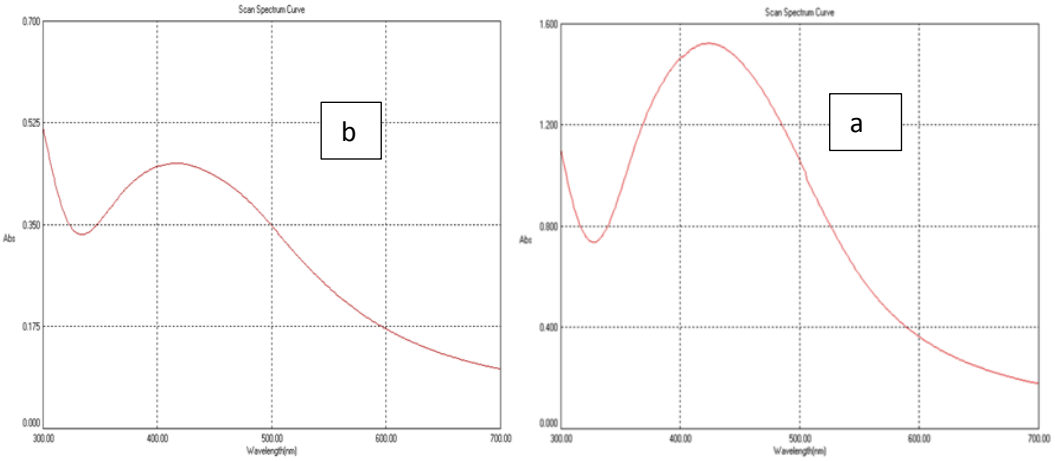

Figure 2: (a) show UV-Vis spectrum of silver nanoparticles produced by *Streptomyces-24* peak at 425 nm. (b) show UV-Vis spectrum of silver nanoparticles produced *Streptomyces-13* at 417 nm.

FTIR Analysis of AgNPs

FTIR spectrum analysis of AgNPs showed intense absorption bands at 3452 and 1635 cm−1 (fig3 a,b). The intense broad absorbance at 3452 cm−1 (O–H stretch) is the characteristic of the H-bonded functional group in alcohols and phenolic compounds. The intense medium absorbance at 1635 cm−1 (–C=C– stretch) is the characteristic of the alkenes group. A previous report reveals that the alcohols, phenolic, and alkanes groups have a strong ability to interact with nanoparticles.

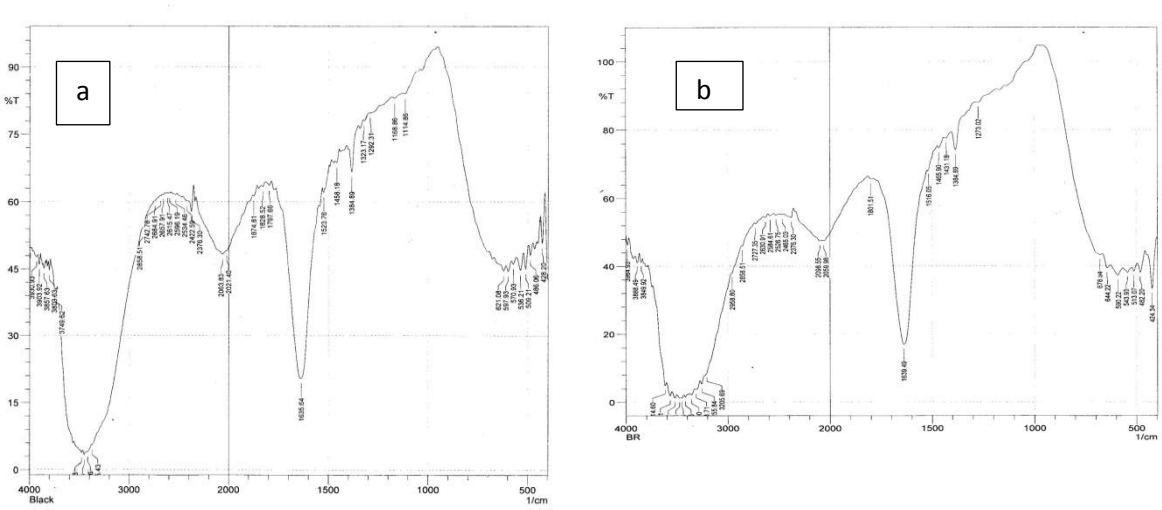

Figure 3: (a) FT- IR analysis of AgNps produced by *Streptomyces-24.* (b) FT- IR analysis of AgNps produced by *Streptomyces-13*

Similarly Panchanathan reported that the biosynthesized AgNPs have (O–H stretch) is the characteristic of the H-bonded functional group in alcohols and phenolic compounds and (–C=C– stretch) is the characteristic of the alkenes group [29]. Jasmine reported that FTIR data confirm the presence of C=C ring stretching [30]. Similar results was found that FTIR analysis data confirms the presence of O–H stretching which may be responsible for reducing metal ions into their respective nanoparticles [31].

Transmission Electron Microscopy (TEM) Analysis

Silver nanoparticles were observed under transmission electron microscope (TEM), observation at higher magnification (100000x). TEM studies image of silver nanoparticles derived from Streptomyces 13 and Streptomyces 24 was shown in Fig 4 and 5. The morphology of biosynthesized silver nanoparticles was spherical in shape, well distributed without aggregation in solution (fig 3 a,b). The approximate size of the prepared AgNPs was estimated in range from 10-20 nm. Microbial synthesis of nanoparticles with different size and shapes depends on the organisms involved concentration of metal ions and duration of metal incubation period. An actinomycetes Rhodococcus sp synthesized gold nanoparticles intracellularly 5-15 nm size. Where other actinomycetes Thermomonospora sp synthesize gold nanoparticles extracellular with 8 nm size. *Fusarium oxysporum* synthesize 5-15 nm silver and 20-40 nm gold nanoparticles extracellularly [32,33].The obtained nanoparticles from an actinomycetes are in the range of sizes approximately 5-50 nm [25]. Streptomyces viridogens strain HM10 synthesizes gold nanoparticles with 18-20nm size [35]. Very few studies have been reported on actinomycetes capable of synthesizing nanoparticles [24,33, 34]. The average gold nanoparticle size produced by Streptomyces viridis ranged from 18-20 nm [35]. Nelly reported that the production rate of silver nanoparticles by Streptomyces glaucus depends not only on the initial concentration of AgNO3 but also varies with time of silver action [36]. They added that the mean size of nanoparticles observed is about 27 nm. Some bacteria like pseudomonas stutzeri synthesize silver nanoparticles up to 200nm size [37]. Larger nanoparticles are formed when P.stutzeri AG259 is placed in concentrated aqueous solution of $AgNO_3$ [38].

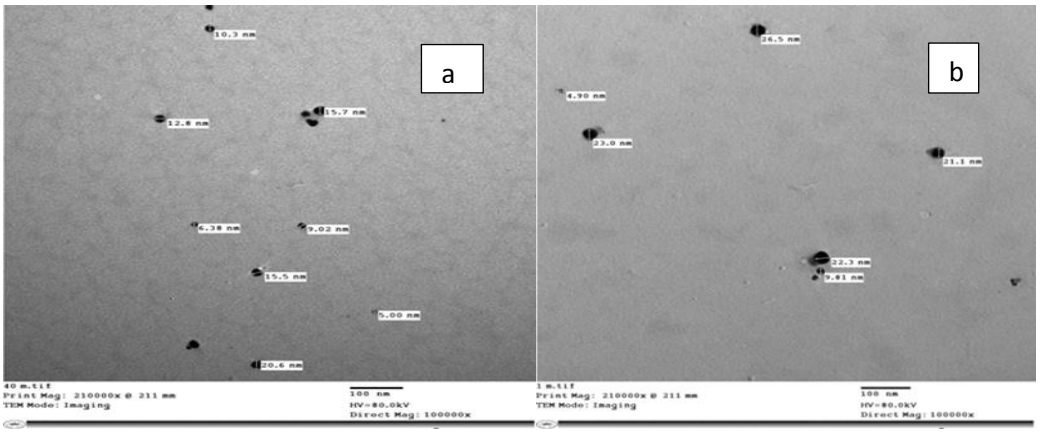

Figure 4: (a) TEM electron micrograph of silver nanoparticles produced by *Streptomyces-13*. (b) TEM electron micrograph of silver nanoparticles produced by *Streptomyces-24*.

Characterization and Identification of potent *Streptomyces* species

Morphological characteristics of the two *Streptomyces* isolates were studied using inorganic salt agar medium (ISP-4) according to the ISP methods [19]. The results of the present investigation show that *Streptomyces M-13* and *Streptomyces M-24* are active in biosynthesis of silver nanoparticles and selected for identification and for the biosynthesis of AgNPs .The identification was carried out according to [39-42].The identification was confirmed using rRNA sequence analysis based on 16s rRNA sequence.

Identification of *Streptomyces* M-13

Spore chain are spiral (fig 5) spore surface is smooth (fig 6(a)) and spore mass is gray. Physical and

biochemical characteristics of *Streptomyces M-13* strain are given in table 1:

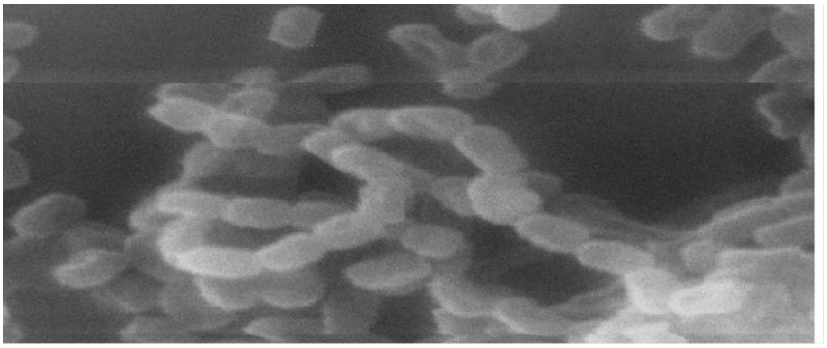

Figure 5: light microscope micrograph showing spiral shape sporophore.

Table 1: Some morphological, biochemical and physiological characteristics of *Streptomyces isolate M-13*

Characters	Results
Morphological characteristics	
Spore chain	Spiral
Spore surface	Smooth
Colour of aerial mycelium	Gray
Colour of substrate mycelium	Brownish
Diffusible pigment	Not detected
Cell wall hydro lysate	
Diaminopimelic acid (DAP)	L-L DAP
Sugar patterns	Not detected
Melanin pigment	-ve
Nitrate reduction	+ve

On the basis of previously collected data it could be showed that *Streptomyces M-13* is suggestive of being belonging to *Streptomyces graminofaciens* (table 1) and thus given the tentative name *Streptomyces graminofaciens.*

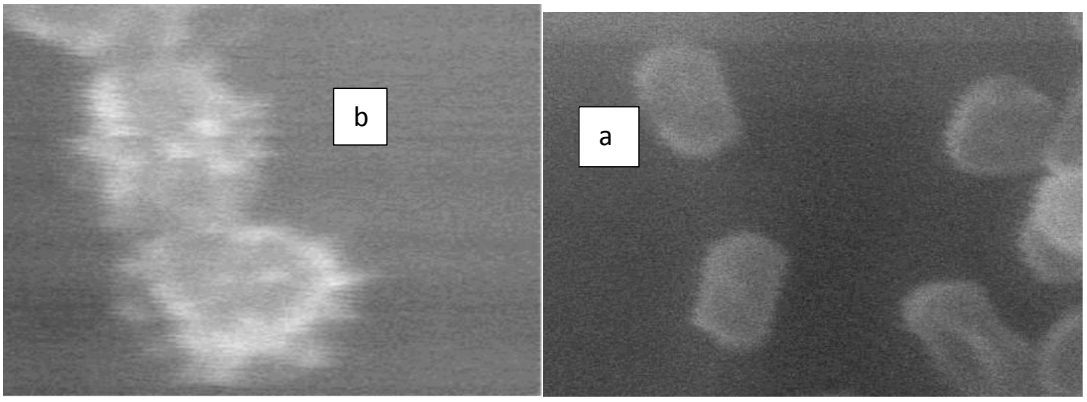

Figure 6: (a) Scanning electron micrograph of *Streptomyces isolate -13* showing smooth spore surface. (b) Scanning electron micrograph of *Streptomyces isolate -24* showing spiny spore surface.

Identification of *Streptomyces* M-24

Spore chain are looped spiral (fig 7) spore surface is spiny (fig 6(b)) and aerial mycelium showed greenish color on all media. Some morphological physical and biochemical characteristics of *Streptomyces M-24* strain are given in table 2.

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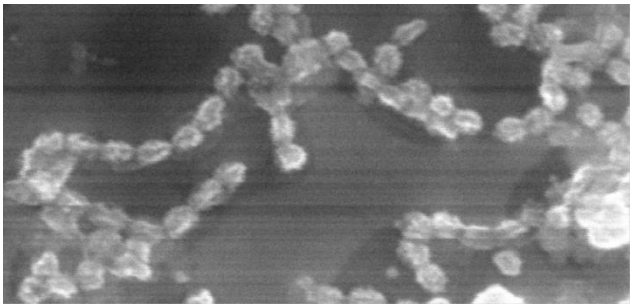

Figure 7: light microscope micrograph showing looped spiral shape sporophore.

Table 2: Some morphological, biochemical and physiological characteristics of *Streptomyces isolate M-24*

Characters	Results
Morphological characteristics	
Spore chain	Looped-flexuous
Spore surface	Spiny
Colour of aerial mycelium	Greenish gray
Colour of substrate	Brownish
Diffusible pigment	Not detected
Cell wall hydro lysate	
Diaminopimelic acid (DAP)	L-L-DAP
Sugar patterns	Not detected
Melanin pigment	+ve
Nitrate reduction	+ve

According to the above mentioned results of morphology, physiological, biochemical properties ,and following the diagnostic working keys for classification and identification of actinomycets [43] and following the description of the Streptomyces species included in the international Streptomyces project(ISP)[19],this isolate was characterized to be *Streptomyces catenulae* and given the names *Streptomyces catenulae-24.*

Antibacterial activity of biosynthesized AgNPs

The antibacterial activity of AgNPs synthesis by two potent *Streptomyces* strain was performed. The results in table (3) and fig(8 (a,b)) revealed that both gram positive and gram negative bacteria were inhibited by AgNPs. The biosynthesized AgNPs by both *Streptomyces* species proved effective against the tested bacteria but the inhibitory effect varied from one another. *Klebsiella*, *E.coli*, *Bacillus.cereus*, and *S.aureus* were more affected by AgNPs compared to *Salmonella*, *Pseudomonas*, *Moraxella, Acientobacter, Enterococcus,and St.pneumonia.*

Table 3: Antibacterial activity of biosynthesized AgNPs by two *Streptomyces* spp against resistant bacteria.

Diameter of inhibition zone (mm)			
Code number	microorganisms	Ag NPs. *Streptomyces graminofaciens-13*	Ag NPs. *Streptomyces catenulae-24*
Gram negative isolates			
E-8	*Salmonella sp.*	14	15
E-1	*Pseudomonas sp.*	14	12
E-3	*Klebsiella sp.*	18	19
E-2	*Moraxella sp.*	12	13
E-5	*E.coli*	19	18
E-10	*Acientobacter sp.*	11	12
Gram positive isolates			
E-7	*S.aureus*	17	18
E-9	*Enterococcus sp.*	12	13
E-6	*Bacillus.cereus*	18	19
E-4	*St.pneumonia*	11	12

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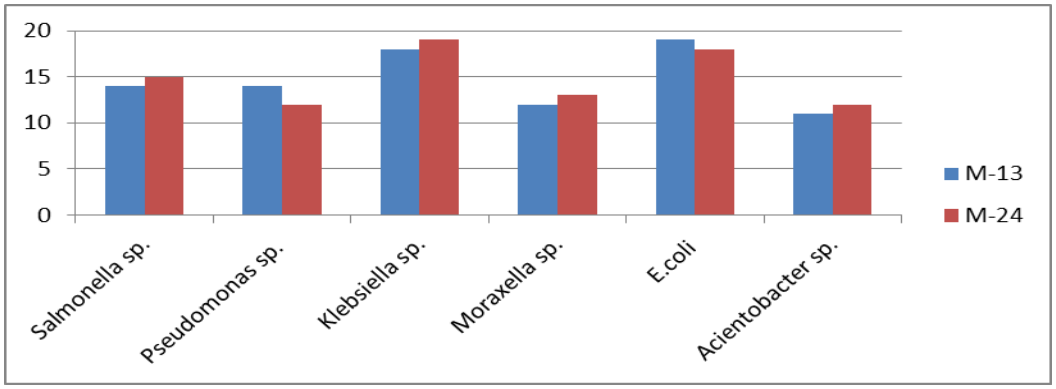

Fig 8: (a) Antimicrobial activity of AgNPs produced by two *Streptomyces sp* against six gram negative clinical bacterial isolates.

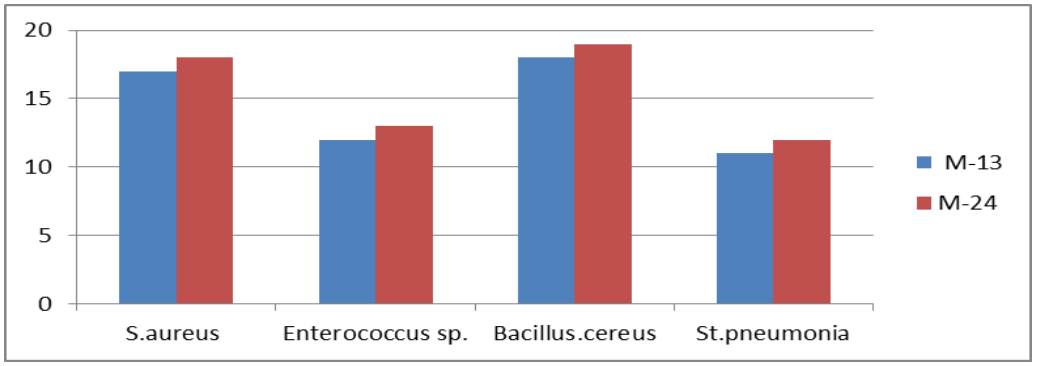

Fig 8: (b) Antimicrobial activity of AgNPs produced by two *Streptomyces sp* against four gram positive clinical bacterial isolates.

REFERENCES

[1] Albrecht, MA, Evans, CW, and Raston, CL. Gr Chem 2006; 8(5): 417–432.
[2] Beveridge, TJ, Hughes, MN, Lee H. et al., Adv Micr Phys 1997; 38, 177–243.
[3] Simkiss, K. and Wilbur, KM. Academic. Press, New York, NY, USA 1989.
[4] Mann, S. and Ozin, GA. Nature 1996; 382: 313–318.
[5] Bhainsa, KC. and D'Souza, SF. Coll Surf B 2006; 47(2): 160–164.
[6] Shahverdi, AR. Minaeian, S. Shahverdi, HR. Jamalifar, H. and Nohi, AA. Proc Biochem 2007; 42(5): 919–923.
[7] Jha, AK and Prasad, K. Biotechnol J 2010; 5(3): 285–290.
[8] Khouloud BM, Mattar MZ, Sabae SZ, Darwesh OM and Sahar HH Res J Pharm Biol Chem Sci 2015; 6(5): 933- 943.
[9] Sadhasivam, S. Shanmugam, P. and Yun, K. Coll Surf B 2010; 81. 1. 358–362.
[10] Liong, M. France, B. Bradley, KA and Zink, JI. Advanc Mat 2009; 21. 17. 1684–1689.
[11] Cho, KH. Park, JE. Osaka, T. and Park, SG. Electrochim Acta 2005; 51. 5. 956–960.
[12] Wei, H. Chen, C. Han, B. and Wang, E. Anal Chem 2008; 80. (18): 7051–7055.
[13] Singh, AK. Talat, M. Singh, DP and Srivastava, ON. J Nanop Res 2010; 12. 5. 1667–1675.
[14] Shahverdi, AR. Fakhimi, A. Shahverdi, HR and Minaian, S. Nanomed 2007; 32: 168–171.
[15] Tenover, F.C Amer J Med 2006; 119: 3-10.
[16] Panchanathan M., Jayachandran V., Kalimuthu S., Kannan S. and Se-Kwon K. BioMed Res Intern 2013; 1-9.
[17] Tripathy. A. Ashok M. Raichur N. Chandrasekaran .T.C. Prathna. J Nanopart Res 2010; 12:237–246.
[18] Thangavel S., Manikkam R., Venugopal G., Raasaiyah P., Ramasamy B. Coll Surf B: Biointerf 2013; 111: 680– 687.
[19] Shirling, EB. and Gottlieb, D., Int J Sys Evol Microbiol 1966; 16: 313–340.
[20] Noura E, Nayera AM and Darwesh OM J Microbiol Biotechnol 2014; 24(4): 453–464.
[21] Dinesh Kumar S, Karthik L, Gaurav Kumar, Bhaskara Rao K.V Pharm online 2011; 3: 1100-1111.

[22] Duran, N.; Marcato, DP.; De Souza, HI.; Alves, L.O.; Espsito, E. J Biomed Nanotechn 2007; 3: 203-208.
[23] Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH. Mater Lett 2007; 61: 1413-1418.
[24] Priyaragini S, Sathishkumar SR, Bhaskararao KV. Int J Pharm Sci 2013; 5(2):709-712.
[25] Narasimha G, Janardhan ,M. Alzohairy,H. Khadri, K. Mallikarjuna. Int J Nano Dimens 2013; 4(1): 77-83.
[26] Sosa, I.O., Noguez, C., Barrera, R.G. J Phys Chem B., 2003; 107, 6269–6275.
[27] Bhainsa, KC and D'Souza, SF. Coll Surf B., 2006; 47. (2): 160–164.
[28] Krishnaraj CG Jagan, S Rajasekar and P Selvakumar, PT Kalaichelvan, N Mohan, Coll Surf B: Biointerf 2010; 76, 50-56.
[29] Panchanathan M., Jayachandran V., Kalimuthu S., Kannan S., and Se-Kwon K. BioMed Res Intern 2013; 1-9.
[30] Jasmine Subashini V. Gopiesh Khanna K. Bioproc Biosyst Eng 2013; 1070-1078.
[31] Karthik L, Gaurav Kumar A, Vishnu Kirthi A, Rahuman K, Bhaskara Rao V, Bioproc Biosyst Eng 2013; 0994-1003.
[32] Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M. Chembio-chem 2002; 3: 461–463.
[33] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. Colloids Surf B Biointerfaces. 2003; 28:313–318.
[34] McLeod, R.S. McHenry, E.J. Beckman, C.B. J. Phys. Chem. B., 2003; 107, 2693–2700.
[35] Balagurunatghan R, Radhakrishnan M, Babu Rajendran R and Velmurugan D. J. Biochem. Biophy 2011; 48: 331-335.
[36] Nelly Yason Tsibakhashvilli, Elena Lvanovna Kirkesali et.al Adv sci lett 2011; 4(3), pp. 1-10.
[37] Joerger R, Klaus T, Granqvist CG: Adv Mater 2000; 12:407-409.
[38] Klaus T, Joerger R, Osson E, Granqvist CG. Proc Natl Acad Sci 1999; 96:13611–13614.
[39] Buchanan, R.E. & Gibbons, N.E. (eds.). 8th Edition, the Williams and Wilkins, Baltimore 1974.
[40] Williams, SM. Goodfellow, and G. Alderson. 1989; 4: 2452-2492.
[41] Hensyl, WR. 9th Edition. John. G. Holt and Stanley, T. Williams (Eds.)., Williams and Wilkins, Baltimore, Philadeiphia, Hong kong, London, Munich 1994.
[42] George, M., Anjumol, A., George, G., Hatha, A.A.M. Afr J Microbiol Res 2012; 6(10): 2265-2271.
[43] Szabo, S. Biro and M. Good fellow (Editors), Symp Biol Hung., 1985; 32. A, B).
[44] Sathya S, Sudhagar S, Vidhya PM, Bharathi RR, MuthusamyVS, Niranjali DS, Lakshmi BS Chem Biol Interact 2010; 188:412–420.