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ANTIMICROBIAL ACTIVITY OF EXTRACELLULARLY SYNTHESIZED SILVER NANOPARTICLES FROM MARINE DERIVED *STREPTOMYCES ROCHEI*

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ABSTRACT

The process of development of reliable and eco-friendly metallic nanoparticles is an important step in the field of nanotechnology. To achieve this use of natural sources like biological systems becomes essential. In the present work, we have investigated extracellular biosynthesis of silver nanoparticles at two molar concentrations (10^{-3} and 10^{-4}) of $AgNO_3$ using *Streptomyces rochei* isolated from the marine sediment samples of Kanyakumari Coast , Tamil Nadu , India. The silver nanoparticles were analyzed by UV-Visible spectroscopy. The antimicrobial activities of silver nanoparticles were screened against common human pathogen *Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae, Enterobactor faecalis* and *Staphylococcus aureus.* The results showed silver nanoparticles synthesized at 10^{-4} molar concentration of $AgNO_3$ displayed a significant antibacterial activity against *S. aureus* and *P. aeruginosa.* This study gives an innovative approach to develop new formulations based on metallic nanoparticles with antimicrobial properties to reach the pharmaceutical companies searching for new unconventional antibacterial agents.

KEYWORDS: **Antibacterial activity, Marine sediment, silver nanoparticles and *Streptomyces rochei*.**

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INTRODUCTION

Marine microbial biotechnology has opened up unexpected new horizons for finding novel organism for trapping their potential resources. Oceans account for more than 70% of the earth's surface and the microorganisms growing in marine environments are metabolically and physiologically diverse from the terrestrial organisms[1].*Streptomyces* is the largest genus of Actinobacteria and the type genus of the family *Streptomycetaceae*[2]. Over 500 species of *Streptomyces* bacteria have been described[3]. The metabolic diversity of the actinomycetes family is due to their extremely large genome, which has hundreds of transcription factors that control gene expression, allowing them to respond to specific needs[4]. The temperate zones are, however, generally most favorable for their development[5]. Nanotechnology is a field that is burgeoning day by day making an impact in all spheres of human life[6]. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level[7]. Nanomaterials have a long list of applicability in improving human life and its environment. An important area of research in nanotechnology is the biosynthesis of nanoparticles such as nanosilver. Biologically synthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries[8]. Silver nanoparticles are used as antimicrobial agents in most of the public places such as elevators and railway stations in China. Besides, they are used as antimicrobial agents in surgically implanted catheters in order to reduce the infections caused during surgery and are proposed to possess anti-fungal, anti-inflammatory, anti-angiogenic and anti-permeability activities [9]. In the last few decades there has been increased interest in reducing the availability of commercial textile containing antibacterial agents due to environmental pollution. Since silver is a good antibacterial agent and non-toxic and natural inorganic metal, it appears as an interesting material to be used in different kind of textile fibers.In this direction, polypropylene/silver nanocomposite fibers were prepared and the antibacterial tests showed that the fibers containing silver nanoparticles in core-part (inside the fiber) had no nearly significant antibacterial activity. However, the fibers having silver nanoparticles (30 nm size) in sheath-part showed excellent antibacterial effects [10]. Similar results were achieved with nanosized colloidal silver particles on polyester nonwovens.The growth of bacteria colonies was absolutely inhibited with only 10 ppm colloidal silver when the mean diameter of the silver particles was 2–5 nm. Consequently, a smaller particle size yielded better bacteriostasis on silver-padded nonwoven fabrics [11]. Although various chemicals and biochemical methods are being explored for production of AgNPs. Microbes are exceedingly effective in this process. Biosynthesis of silver nanoparticles from bacteria, fungi, yeast, plants, fruits and so on have been reported [12,13]. Consequently, researchers have turned to biological synthesis because through this, biological synthesis obtaining particles with good control on the size distribution than the physical and chemical methods. The nanoparticles could also be stabilized directly in the process by proteins [14]. Ag-NPs (silver nanoparticles) are also used in hygienic products including water purificationsystems, linings of washing machine, dishwashers, refrigerators, and toilet seats [15]. Recently, the development of resistant or even multiresistant pathogens has become a major problem, for instance *Staphylococcus aureus* resistance to methicillin and *Candida albicans* resistance to fluconazole have to be mentioned [16]. It is well known that silver ions and silver-based compounds are highly toxic to microorganisms. Thus, silver ions have been used in many kinds of formulations [17]. And recently it was shown that hybrids of silver nanoparticles with amphiphilic hyper branched macromolecules exhibit effective antimicrobial

surface coating [18]. One of the most studied aspects of nanotechnology nowadays is their ability to offer the opportunity to fight microbial infections via synthesis of nanoparticles. The mechanism of prevention of bacterial growth by antibiotics is quite different from the mechanisms by which nanoparticles inhibit microbial growth. Therefore, nanoparticles have the potential to serve as an alternative to antibiotics and to control microbial infections such as those caused by MRSA [19]. The antimicrobial activity of silver nanoparticles (Ag-NPs) appears significantly high. Silver is more toxic element to microorganisms than many other metals in the following sequence: Ag > Hg > Cu > Cd > Cr >Pb> Co >Au > Zn > Fe >Mn> Mo >Sn[20]. Antimicrobial activities of silver nanoparticles have been studied by various researchers especially on *E. coli, S. aureus* [21]. The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed to eliminate microorganisms on textile fabrics [22] or they can be used for water treatment [23]. Thus, in the present study an attempt was carried out to see the antibacterial activity of silver nanoparticles synthesized from the isolated marine derived *Streptomyces rochei.*

MATERIALS AND METHODS

(i) Sample collection

The marine sediment sample was collected from Kanyakumari Coast, Tamil Nadu , India at 10 m length and 5 m depth in sterilized glass bottle. The collected marine sediment sample was stored in ice box and then transported to the laboratory within 3 hours.

(ii) Isolation of Streptomyces rochei from marine sediment

The medium used for the isolation and cultivation of marine actinomycetes was Bennett medium contains Beef extract 0.1g; Yeast extract 0.1g; Casein digest 0.2g; Agar 1.7g; Glucose solution(25%w/v) 2ml; Maltose solution (25% w/v) 2ml; sea water 50ml and distilled water 50ml. After autoclaving the medium was supplemented with 50 and 20 µg ml^{-1} of tetracycline and nystatin respectively as antibacterial and antifungal agents to inhibit the bacterial and fungal contamination. 20 grams of marine sediment was transferred into 250 ml conical flask containing 100 ml of sterilized physiological saline and this sample was serially diluted up to 10^{-4} dilutions [23]. Each test tube of diluted samples were vortexed vigorously for 15 minutes. From each suitable dilution, 0.1 ml was taken and spread evenly with sterile L-shaped glass rod over the surface of Bennett media and kept for incubation at 30ºC. Streak Plate method was used to purify the marine actinomycetes colonies [24]. The developed colonies that grow on petri plates can be individually purified by repeated streaking on Bennett medium by using separate petri plates and then subcultured to ensure for their axencity. Pure culture was transferred on slants and preserved at 4ºC for further analysis [25].

(iii) Biochemical characterization

Various biochemical tests were performed for the identification of the potent isolate *Streptomyces rochei*. These tests include growth on MacConkey agar, indole test, methyl red test, vogesproskauer test, citrate utilization, gas production from glucose, casein hydrolysis, urea hydrolysis, nitrate reduction, H_2O production, cytochrome oxidase test, catalase test, gelatin hydrolysis, arginine dihydrolase, tween-40, tween-60, tween-80 and esculin tests. To determine the production of acids by utilizing the different source of carbohydrates like adonitol, sorbitol, dextrose, fructose, inositon, lactose, maltose, raffinose, rhamnose, sucrose and xylose were tested[26] by inoculating the isolate in ISP1 broth supplemented respective sugars and incubated for 7 days at 30 ºC.

(iv) Preparation of cell free microbial extract

The culture medium was prepared, sterilized and inoculated with fresh culture of the strain.

The cultured flasks were incubated at 37 ºC for 24 hours. After incubation time the cultures were centrifuged at 12000 rpm and their supernatant was used for further experiments.

(v) Biosynthesis of Ag-NPs

In a typical synthesis of silver nanoparticle extracellularly, 50 mL aqueous solution of 1 mM and 0.1 mM silver nitrate ($AgNO_3$) were separately treated with 50 mL of *Streptomyces rochei* supernatant solution in a 250 mL Erlenmeyer flask (pH adjusted to 8.5). The whole mixture was put into a shaker at 37°C (200 rpm) for 5 days and maintained in the dark and bright conditions. Control experiments were conducted with uninoculated media, to check for the role of bacteria in the synthesis of nanoparticles. The reduction of Ag^+ ions was monitored by sampling an aliquot (2 mL) of the solution at intervals of 24 hours and measuring the UV-Vis spectra of the solution. In each reaction vessels color change was observed to yellowish brown in the silver nitrate solution incubated with supernatant of *Streptomyces* species. The synthesized silver nanoparticles optical density was measured by UV-Vis spectrophotometer (Lazany) from wave lengths 300-600nm. The spectra were recorded at room temperature using quartz cuvette. Finally, the nanoparticles were separated from each half of the reaction mixture for further analysis by centrifugation at 10,000 rpm for 10 minutes at 4°C and remaining both silver nanoparticles solution were used for check the antibacterial activity.

(vi) Antibacterial activity

Muller Hinton Agar was prepared according to the manufacturer's instructions. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and was used to determine the antibacterial activity of Ag-NPs from *Streptomyces rochei*. Sterile molten cool (45°C) agar was poured aseptically into sterile petri plates (15 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with appropriate micro organisms by streaking evenly on to the surface of the medium with a sterile cotton swab and wells (8 mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 0.1ml of the each synthesized silver nanoparticles solution in respective wells. Tetracycline and double distilled water were used as positive and negative control respectively. Then the plates were incubated at 37°C for 24 hrs in the next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their confirmation. The results were read by the presence or absence of zone of inhibition.

RESULTS AND DISCUSSION

According to Bergey's manual of Determinative bacteriology, Ninth edition (2000)[27] and the Laboratory Manual for Identification of Actinomycetes, the organism was identified as *Streptomyces rochei* based on morphological, physiological and biochemical characteristics.

(i) Morphological, physiological and Biochemical characterization

Streptomyces rochei isolated on Bennett medium is Gram-positive, very long rod shaped, produce yellow pigments and possessing earthy odour characteristics of actinomycetes. The colony was light brown to gray in color and mycelium was aerial and white in color. Most of the actinomycetes were isolated from marine environment which required sea water for growth and these strains designated marine actinomycetes [28] and *Streptomyces* have been reported to grow well on starch casein agar by earlier workers [29] and the medium contains maximum amount of carbon sources, so, the maximum containing Bennett media supplemented with 50% natural sea water and 50% distilled water were used for the isolation of present strain *Streptomyces rochei*. *Streptomyces rochei* could utilize dextrose, fructose, lactose, maltose, mannitol, rhamnose and sucrose as the carbon source along with acid production. The biochemical tests like methyl red test, nitrate reduction test, citrate utilization, urea hydrolysis, cytochrome oxidase,

catalase test, gelatin hydrolysis, arginine dihydrolase, tween-60, tween-80 and esculin were positive, but indole test, vogesproskauer test, gas production from glucose, casein hydrolysis, starch hydrolysis, H_2S production and tween-40 were negative. These characteristics were useful to identify *Streptomyces rochei* isolate to the species level by based on its pigment production, morphological and physiological characteristics, additional feature such as studying the microscopic micrographs of the spore surface and biochemical properties can provide more details, that can be used for identification purposes, was also reported [30].

(ii) Extracellular biosynthesis of silver nanoparticles using culture supernatant of Streptomyces rochei

Production of silver nanoparticles by the culture supernatants of *Streptomyces rochei* with aqueous silver nitrate solution, 1mM and 0.1 mM were investigated. The picture of test tubes of silver nitrate solution after exposure to culture supernatant of *Streptomyces rochei* is shown in the of Figure 1.

Figure 1
Silver nanoparticles synthesized from Streptomyces rochei

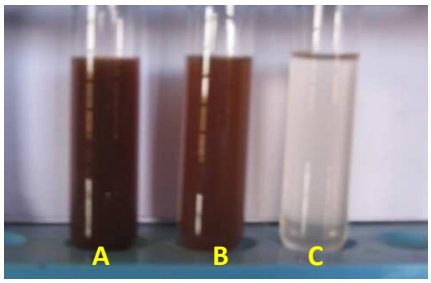
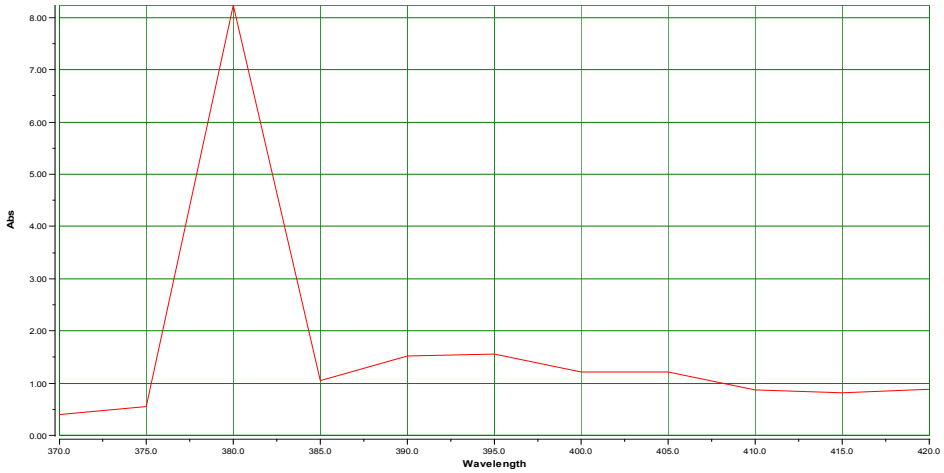


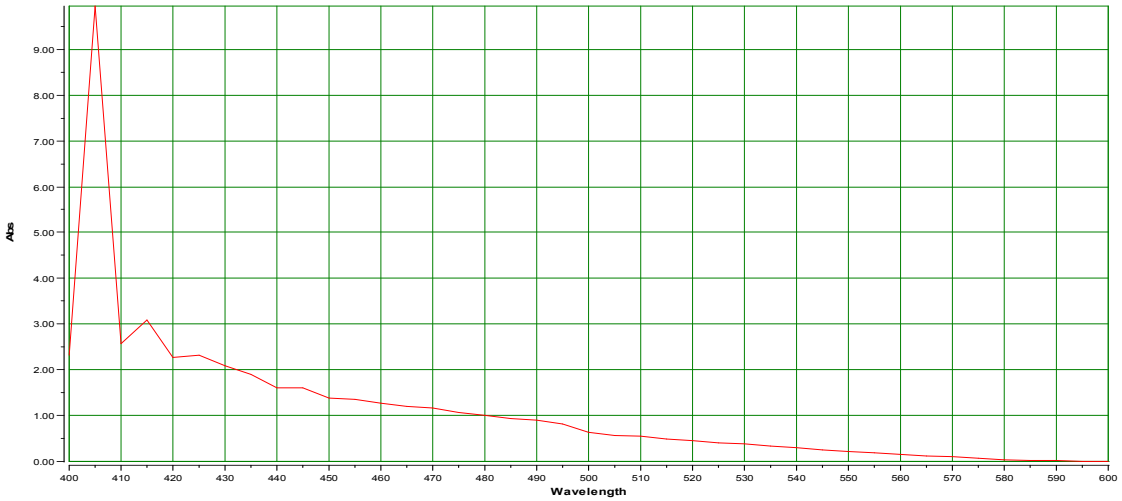
Figure 1
A- silver nanoparticles synthesized from Streptomyces rochei with the concentration of 0.1mM $AgNO_3$. B- Silver nanoparticles synthesized from Streptomyces rochei with the concentration of 1mM $AgNO_3$. C-Supernatant of Streptomyces rochei.

The UV-Vis Spectra recorded from *Streptomyces rochei* reaction vessels shown in graph 1(A and B). Graph 1(A) indicates the maximum absorbance (380nm) value of synthesized silver nanoparticles from the precursor concentration of 1mM $AgNO_3$and graph 1(B) indicates the maximum absorbance (405nm) value of synthesized silver nanoparticles from the precursor concentration of 0.1mM $AgNO_3$. The appearance of brown color shown in test tubes (A and B) clearly indicates the formation of silver nanoparticles in the reaction mixture [31].

Graph 1(A)
UV-Vis Spectra absorbance value of synthesized silver nanoparticles



Graph 1(B)
UV-Vis Spectra absorbance value of synthesized silver nanoparticles



The formation and stability of the reduced Ag-NPs in the colloidal solutions were monitored by UV-Vis spectral analysis. UV-Vis spectrum is one of the important technique ascertain of the metal nanoparticles, provided surface Plasmon resonance exits for the metal. It can be observed that the silver Surface Plasmon Band occurs at around 410 nm and steadily increase in intensity as a function of time of reaction. The surface plasmon band in the silver nanoparticles solution remains close to 410 nm throughout the reaction period, suggesting that the particles are dispersed in the aqueous solution with no evidence for aggregation [32].

(iii) Antibacterial activity of silver nanoparticles (Ag-NPs) against pathogenic bacteria:

The antibacterial activity of silver nanoparticles were investigated against some selected Gram negative (*Pseudomonas aeruginosa, E.coli ,Klebsiella pneumonia , Enterobactorfaecalis*) and Gram positive (*Staphylococcus aureus)* human pathogenic bacteria by agar well diffusion method (figure2(a-e)).

Antibacterial activity of silver nanoparticles (Ag-NPs) against pathogenic bacteria

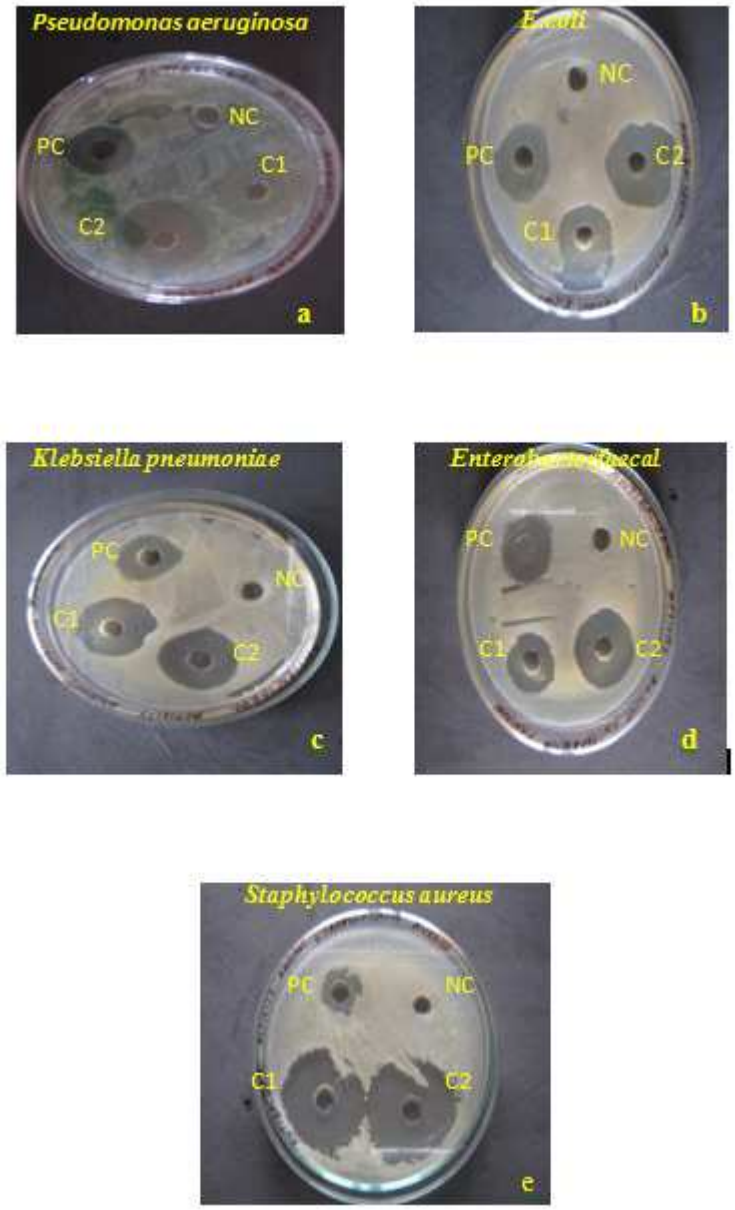


Figure2
(PC-Positive Control; NC-Negative Control; C1- Ag-NPs Sample synthesized from 1mM Concentration of $AgNO_3$; C2-Ag-NPs Sample synthesized from 0.1mM Concentration of $AgNO_3$)

In the positive control wells zone of inhibition were observed and in the negative control wells no zone of inhibition were observed. The diameter of inhibition zones around each well with Ag-NPs is represented in table-1.

Zone of inhibition of Ag-NPs against bacterial strains

Table1

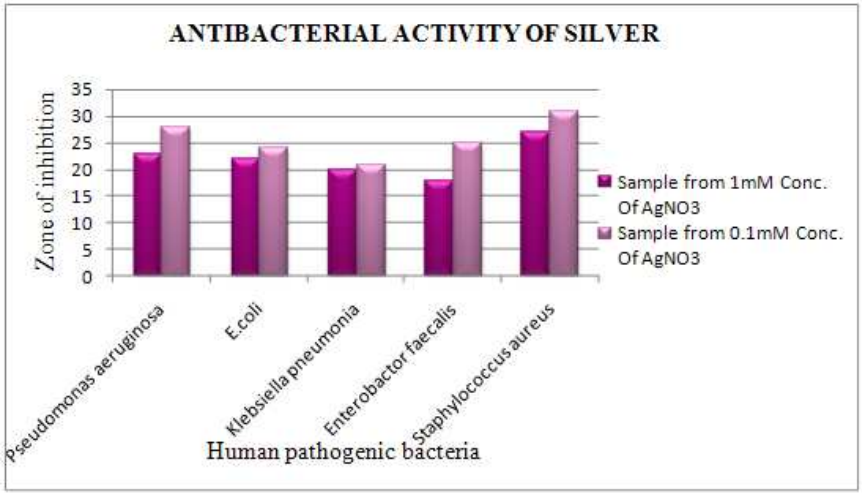
Zone of inhibition range (18mm-31mm) of the Ag-NPs synthesized from Streptomyces rochei with 1mM and 0.1mM concentrations of $AgNO_3$.

Strain name	zone of inhibition(mm) (Ag-NPs synthesized with conc. of 10^{-3}M $AgNO_3$)	zone of inhibition(mm) (Ag-NPs synthesized with conc. of 10^{-4}M $AgNO_3$)
Pseudomonas aeruginosa	23	28
E.coli	24	22
Klebsiella pneumonia	20	21
Enterobactorfaecalis	18	25
Staphylococcus aureus	27	31

The maximim antimicrobial activity was observed against *Staphylococcus aureus* followed *by Pseudomonas aeroginosa, Enterobactor faecalis, E. coli* and the least was noticed against *Klebsiella pneumonia*. Silver has been used for its well known antimicrobial properties since roman time however the advances in generating Ag-NPs have made possible a revival of the use of silver as a powerful bactericide. Many researchers [33] used *Escherichia coli* as a model for gram negative bacteria and proved that Ag-NPs may be used as an antimicrobial agent. Other workers [34] also opined that the Ag-NPs have an antimicrobial effect on *S. aureus and E. coli*. In the present study the silver nanoparticles was synthesized in various concentration (1mM and 0.1 mM) of silver nitrate ($AgNO_3$) and each synthesized nanoparticles were used for antibacterial assay. The Ag-NPs sample from 0.1mM concentration of $AgNO_3$ showed the greater antibacterial activity than Ag-NPs sample from 1mM concentration of $AgNO_3$ and the inhibition zone range from 21mm to 31mm in diameter represented in graph 2.

Graph 2

Antibacterial activity of silver nanoparticles synthesized from Streptomyces rochei



CONCLUSION

Synthesis of silver nanoparticles using marine source has been unexplored, which aroused our interest in the present investigation. The results indicated that the natural marine derived *Streptomyces rochei* is a good source for the extracellular synthesis of silver nanoparticles. The silver nanoparticles exhibited a tremendous potential antibacterial activity against common human pathogenic bacteria. Further research is required to fully understand the processes involved and to safely exploit the tremendous antibacterial properties of silver without jeopardizing human health, critical infrastructure and the environment.

REFERENCE

1. Takizawa, M., Colwell, R.R., Lill, I., Isolation and diversity of actinomycetes in the Chesapeake Bay. Applied Environ. Microbiol, (59): 997-1002, (1993).
2. Kämpfer, P., Dworkin, M., "The Family Streptomycetaceae, Part I: Taxonomy". The prokaryotes: a handbook on the biology of bacteria Berlin: Springer, 2006, pp. 538–604.
3. Euzéby, J.P., “genus streptomyces”. List of Prokaryotic names with Standing in Nomenclature, academic press, US 2008.
4. Goshi, K., Uchida, T., Lezhava, A., Yamasaki, M., Hirastu. K., Shinkawa, H., Kinashi H., Cloning and analysis of telomere and terminal inverted repeat of the linear chromosome of Streptomyces griseus. Journal of biotechnology, (184):3411-3415, (2002).
5. Mustafa, S. A., Tamer, U. A., Azer, C., Antimicrobial activity of some actinomycetes isolated from farming soils of Turkey. African Journal of biotechnology, 3(9): 441-446, (2004).
6. Vaidyanathanh, R., Kalishwarlal, K., Gopalram, S., Gurunathan, S., Biotechnol. Advances. African Journal of biotechnology, (27):924-937, (2009).
7. Albrecht, M. A., Evan, C. W.,Raston, C. R., Green Chemistry, (8):417-432, (2006).
8. Klaus-Joerger, T., Joerger, R., Olsson, E., Granqvist, C.G., Trends in biotechnology, (19): 15, (2001).
9. Kalishwaralal, K., Banumathi, E., Pandian, S.B.R.K., Deepak, V., Muniyandi, J., Eom, S.H., Silver nanoparticles inhibit VEGF induced cell proliferation and mgration in bovine retinal endothelial cells. *Colloids Surf B,* (73): 51–7, (2009).
10. Yeo, S. Y., Lee, H. J., Jeong, S. H., Prepaation of nanocompositefibers for permanent antibacterial effect. Journal of material science,*(*38): 2143,(2003).
11. H.J.Lee and S.H.Jeon, Bacteriostasis of nanosized colloidal silveron polyester nonwovens. *Text. Res. J.* 74, 442 (2004).
12. Nanda, A., Saravanan, M., Nanomedicine.Journal of nanotechnology, 5 (4): 369-480, (2009).
13. Mokhtari, N. S., Daneshpajkeh, S., Seyedbagheri, S., Atashdehghan, R., Abdi, K., Sarkar, S. Minaian, S., Shaverdi, H. R., Materials Research Bulletin. 44, 1415, (2009).
14. Durán, N., Marcato, P.D., Alves, O.L., De Souza, I.H., Esposito, E., Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusariumoxysporum*strains. Journal of nanotechnol,*(*3): 8, (2005).
15. Rai, M., Yadav, A., Gade, A., Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, (27): 76–83, (2009).
16. Schaller, M., Laude, J., Bode waldt, H., Hamm, G., orting, H.C.K., Toxicity and antimicrobial activity of a hydrocolloid dressing containing silver particles in an *ex vivo* model of cutaneous infection. Skin Pharmacology Physiology, 17, 31 (2004).
17. Sondi, I., Salopek-Sondi, B., Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-

negative bacteria. J. Colloid Interf. Sci, 275, 177, (2004).
18. Ymonier, C.A., Scholotterbeck, U., Antonietti, L., Zacharias, P.,Thomann, R., Iller, J.C.T., Mecking, S., Hybrids of silvernanoparticles with amphiphilichyperbranched macromolecules exhibiting antimicrobial properties. Chem. Commun, 24, 3018(2002).
19. Luo, P.G., Tzeng, T.R., Shah, R.R., Stutzenberger, F.J., Nanomaterials for Antimicrobial Applications and Pathogen Detection. Current Trends in Microbiology, In Press. 2007.
20. Zhao. G.J., Stevens, S.E., Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion. Bimetals, (11): 27–32, (1998).
21. Baker, C., Pradhan, A., Paktis, L., Pochan, D.J., Shah, S.I., Antimicrobial activity of silver nanoparticles J. Nanosci. Technol,(5): 244-249, (2005).
22. Zhu, J.J., Lhao, X.N., Hen, H.Y., Silver containing materials used in textile fabrics. Materials Letters, (49): 91-95, (2001).
22. Chou,W.L., Yu, D.G., Yang, M.C.,Silver nanoparticles in waret treatment. Polym. AdV.Technol, (16): 600-608, (2005).
23. Sahu, M.K., Kumar, K.S., Kanan, L., Isolation of actinomycetes from various samples of the vellar estuary, South east coast of India. Proc. Ecotech, (24): 45-48, (2004).
24. Williams, S.T., Cross, T., Actinomycetes Isolation from soil. In: Methods in Microbio. Booth, C. (Ed.)., Vol. 4, Academic Press, London, New York, pp:295-334 , 2003.
25. Kokare, C.R., Maadik, K.R., Kadam,S.S., Isolation of bioactive marine actinomycetes from sediments isolated from goa and Maharashtra coast lines (West Coast of India). Ind. J. Marine Sci, (33): 248-256, (2004).
26. Nonomura, H., Key for classification and identification of 458 species of the *streptomyces* Included in ISP. J. Ferment. Technol, (52): 78-92, (1974).
27. Bergey, D. H. (David Hendricks), Breed, Robert S. (Robert Stanley), Laboratory Manual for Identification of Actinomycetes. pp 1860-1937; 1877-1956, 2000.
28. Mincer, T.J., Jensen, P.R., Kauffman, C.A., Fenical, W., Widespread and persistent populations of a major new marine actinomycetes taxon in ocean sediments. Applied Environ. Microbiol.,(68): 5005-5011, (2002).
29. Laidi, R.F., Sifour, M., Sanker,M., Hocine, H., A new actinomycetes strain SK4-6 producing secondary metabolite effective against methicillin –resistant *Staphylococcus aureus.*World J. Microbiol. Biotechnol., (24): 2235-2241, (2008).
30. Oskay, M., Tamer, U.A., Azer,C., Antibacterial activity of some actinomycetes isolated from farming soils of Turke. Afr. J. Biotechnol., (3): 441-446, (2004).
31. Durán, N., Marcato. P. D., Alves, O. L., Gabriel, I. H., Souza, D. E., Esposito, E. J., Extracelluar biosynthesis of silver nanoparticles using culture supernatant of *Streptomyces* species. J. Nanobiotechnol., (3): 1-7, (2005).
32. SAIFUDDIN, N., WONG, C.W., NUR YASUMIRA, A.A., Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation. E-Journal of Chemistry,6(1): 61-70, (2009).
33. Feng, Q.L., Wa, J., Chen, G.Q., Cui, K. Z., Kim, T.M., Kim, J.O., Antimicrobial activity of silver nanoparticles against bacterial species. J. Biomed. Mater. Res. (52): 662-668, (2003)
34. Yamanaka, M., Hura, K., Kudo, J., Study of Antibacterial activity. Appln. Env. Microbiol. (**71):** 7589-7593, (2005)
35. Alexander, M., Introduction to soil Microbiology. John Wiley and sons, Inc., New York 1961