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EXTRACELLULAR SYNTHESIS OF POLYGONAL SILVER NANOPARTICLES USING EXTRACT OF *ESCHERICHIA COLI* ATCC 25922 AND ITS ANTIBACTERIAL ACTIVITIES

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In the present investigation, we report the extracellular biosynthesis of polygonal silver nanoparticles (Ag-NP) employing the extract of *Escherichia coli* and its antibacterial activities against some bacteria. The extracellular solution of *Escherichia coli* was used for the reduction of $AgNO_3$ solution to AgNP. The present study includes formation of AgNP employing UV–Vis spectrophotometer, size and morphology by employing TEM (Transmission Electron Microscopy), compositional analysis by EDX (Energy Dispersive X-Ray microanalysis). The AgNPs were 10–50nm in dimensions as measured using TEM images. Antibacterial activity against some bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae*. AgNPs were exhibiting good micbicidal activity.

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1. Introduction

Nanotechnology is an emerging branch that is growing rapidly in bringing out new materials with novel properties, which could be used in every walks of life [1-2]. The process of development of reliable, energy efficient, cost effective and eco-friendly method to synthesize metallic nanoparticles is an important avenue in the field of nanotechnology. The process is only possible if we use plants and microorganisms to synthesize the nanomaterials, so biological systems are becoming essential in this regard [3]. Microorganisms play vital role in the geochemical cycles of metals such as Mg, Na, Fe, Co, Cu, Mo, Ni, W, V and Zn. These metal cycles are driven by microbial metabolism, because metals are essential for microbial nutrition [4].

Exploiting this mechanism to material science research now a days people are involving themselves in microbial syntheses of nanoparticles. Since, unlike other processes in physical and chemical methods, which involve hazardous chemicals, microbial biosynthesis of nanoparticles is non-hazardous and easy to carry out. Therefore, microbial synthesis of nanoparticles has emerged as an inevitable branch of Nanotechnology. Diversified microbes have the innate potential for the synthesis of nanoparticles and they could be regarded as potential biofactories for nanoparticles synthesis [5].

Among the range of microorganisms, prokaryotic bacteria have received the most attention in the area of bio-synthesis of nanoparticles. Microbial resistance against heavy metal ions such as Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb or U have been explored for bioleaching processes

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of ores and biological recovery of metals [6-8]. The knowledge has come from biomineralization process possessed by most of the organisms, in combination with complex biomolecules such as proteins, carbohydrates and lipids the organisms form hard skeletal materials for its mechanical support. Hence today scientists have been concentrating in biological systems mediated synthesis the nanomaterials. The underlying mechanisms of synthesis of nanoparticles were also reported recently [9-12]. Bacteria have been recognised as a natural factory to synthesise nanoparticles largely viz. silver [13], gold nanocubes and truncated cubes [14], CdS [15-16], *Escherichia coli* is a well known bacterium and has been used to synthesise silver and other nanoparticles [17-18]. By controlling growth kinetics of bacteria, shapes of the silver nanoparticles were controlled, the shape anisotropy of silver nanoparticles was achieved using *Morganella psychrotolerans*, a silver-resistant psychrophilic bacterium. In the previous report on *Escherichia coli* ATCC 8739 was forming spherical shaped silver nanoparticles, in the present study the selected strain for the synthesis has formed polygonal shaped particles [19].

Inorganic nanoparticles have opened new formulation of bacteriocidal agents; it is well known that silver based compounds are highly toxic to microorganisms, having strong biocidal effects [20]. Silver nanoparticles are used since time immemorial, in silver coated medical devices and textiles fabrics & silver dressings. Since it has large surface area, silver nanoparticles exerts efficient antimicrobial property compared to other salts and hence it has been used severally in therapeutics, for instance in gel for topical use. Different strains exhibit distinct response on exposure with silver nanoparticles [21-22] four strains of *Escherichia coli* were used in the previous report, there was a difference found out in terms of strain variation as well.

2. Materials and methods

2.1 Source of microorganism

The bacterial strain *Escherichia coli* (ATCC 25922) was obtained from PG research centre, J.J College of Arts and Science, Pudukkottai, Tamilnadu, India. The obtained pure culture was maintained in nutrient agar medium slant at 32ºC as well as sub-cultured from time to time to regulate its viability in the microbiology laboratory during their study period. The confirmatory test for *Escherichia coli* was done using EMB agar (Eriochrome methylene blue). The EMB agar medium was prepared under standard microbiological procedure 50ml and poured on to the plates keeping one as test plate and one as control plate. The test plate was quadrant streaked and kept at 35ºC in an incubator for 24 hrs. The *Escherichia coli* was confirmed by the formation of green colour metallic sheen.

2.2 Production of Biomass

The bacterial strain *Escherichia coli* were cultured, to produce the biomass for biosynthesis in LB (Luria Bertani) broth medium. The culture flask were incubated on an orbital shaker at room temperature and agitated at 220 rpm. And then biomass was harvested after 24 hrs of growth. With this harvested cells synthesis of nanoparticles was carried out.

2.3 Synthesis of Silver Nanoparticles

The bacterial strain *Escherichia coli* were cultured, to produce the biomass and the extract of cultured biomass was added to the reaction vessel containing silver nitrate solution in the concentration of 0.5 mM, 1 mM, 3 mM, 5 mM and 7 mM respectively and the control (without the silver nitrate, only biomass) was also run along the experimental condition. The reaction between this supernatant and the Ag+ ions was carried out in bright condition for 24 hrs.

2.4 UV-Vis Spectral Analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 24hrs of the reaction period by diluting the sample into distilled water. The

spectral analysis was done using UV-Vis spectrophotometer (UV-1700 series, Shimadzu Corporation Ltd., Kyoto, Japan) with the samples in the quartz cuvette.

2.5 Transmission Electron Microscopy

TEM samples of the silver nanoparticles synthesized were prepared by placing drops of the product solution onto carbon-coated copper grids and allowing the solvent to evaporate. TEM measurements were performed on a JEOL model JEM-2000FX instrument operated at an accelerating voltage of 200 kV.

2.6 Growth kinetics of *Escherichia coli*

The bacterial strain of *Escherichia coli* was inoculated in the LB medium and the growth process was monitored every one hour once by keeping it in a shaker at 220rpm at room temperature for 24hrs and the growth was measured in the UV-Visible spectrophotometer (UV-1700 series, Shimadzu Corporation Ltd., Kyoto, Japan) by which the optical density of the bacterium was measured with the time intervals in the x axis. The bacterial growth was determined by measuring optical density after every 1 hour at 600 nm.

2.7 Antibacterial activity assay

Antibacterial activities of the synthesized Ag nanoparticles were determined, using the agar well diffusion assay method. Using sterile micropipette 20 µl (0.002mg) of the sample of nanoparticles solution was poured onto each of the wells in all three concentration (0.5, 1.0, 3.0, 5.0, 7.0 mM) onto the plates. After incubation at 35ºC for 24hrs the different levels of zone of inhibition were measured. The silver nanoparticles synthesized from *Escherichia coli* were tested against multiple strains like *Staphylococcus, Pseudomonas, Bacillus, Klebsiella* and *Escherichia coli*. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

3. Results and discussion

3.1 UV-Vis spectra analysis on AgNP formation and Bacterial growth kinetics

Surface Plasmon Resonance (SPR) is a collective excitation of the electrons in the conduction band around the nanoparticle surface. Electrons confirm to a specific vibration mode by particle size and shape. Therefore, metallic nanoparticles display characteristic optical absorption spectra in the UV-Visible region. Reduction of Ag ions into silver particles after exposure to biomass followed by the colour change. Ag nanoparticles exhibit dark brown colour in the aqueous solution due to the Surface Plasmon Resonance phenomenon. The result obtained in this investigation is by the identification of the visible colour change from yellowish to dark brown colour. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at (420-440 nm). The stability in the surface plasmon band in the silver nanoparticles indicates that the particles are dispersed into the aqueous solution with no sign of aggregation. The stability of the silver nanoparticles could be due to the capping agent which probably may be proteins released by the bacterium in the medium which results in peak near the y axis.

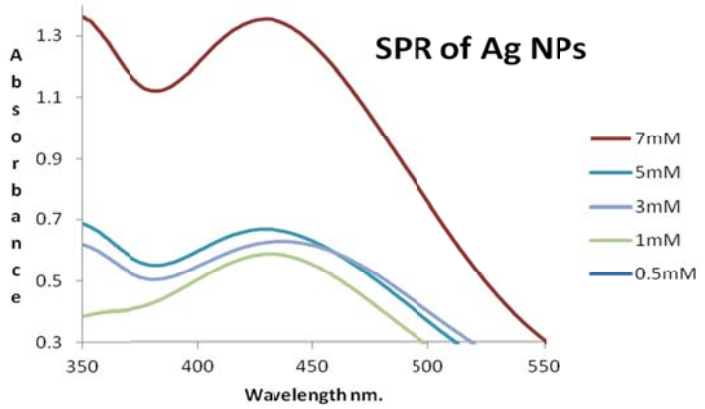


Fig.1. UV-Vis absorption spectra of Ag nanoparticles synthesized from Escherichia coli for all five concentration.

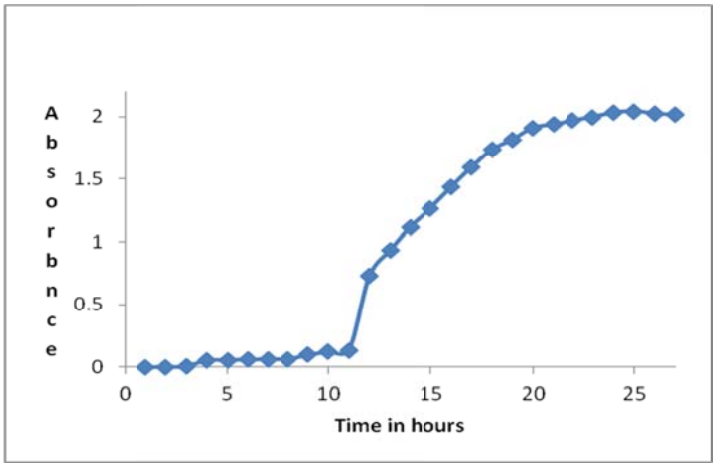


Fig. 2. UV-Vis absorption spectra of growth kinetics of Escherichia coli

3.2 TEM analysis of Ag nanoparticles and energy dispersive X-Ray microanalysis (EDX):

The silver nanoparticles synthesized with the help of bacterial biomass were scanned using Transmission Electron Microscopy from which we can conclude that the silver nanoparticles synthesized were seems to be spherical in morphology as shown in figure. TEM pictures as captured for the silver nanoparticles film spread over a carbon –coated copper grid is presented. The TEM image clearly depicts a dense assembly of silver nanoparticles mostly of spherical shapes. With regards to the silver nanoparticles, their distribution appeared to conform to a polydispersed pattern. The shapes of the nanoparticles are found to be most of spherical and surprisingly one with the pentagonal shaped nanoparticles. The average size of the nanoparticle was found to be 10-50nm ranges.

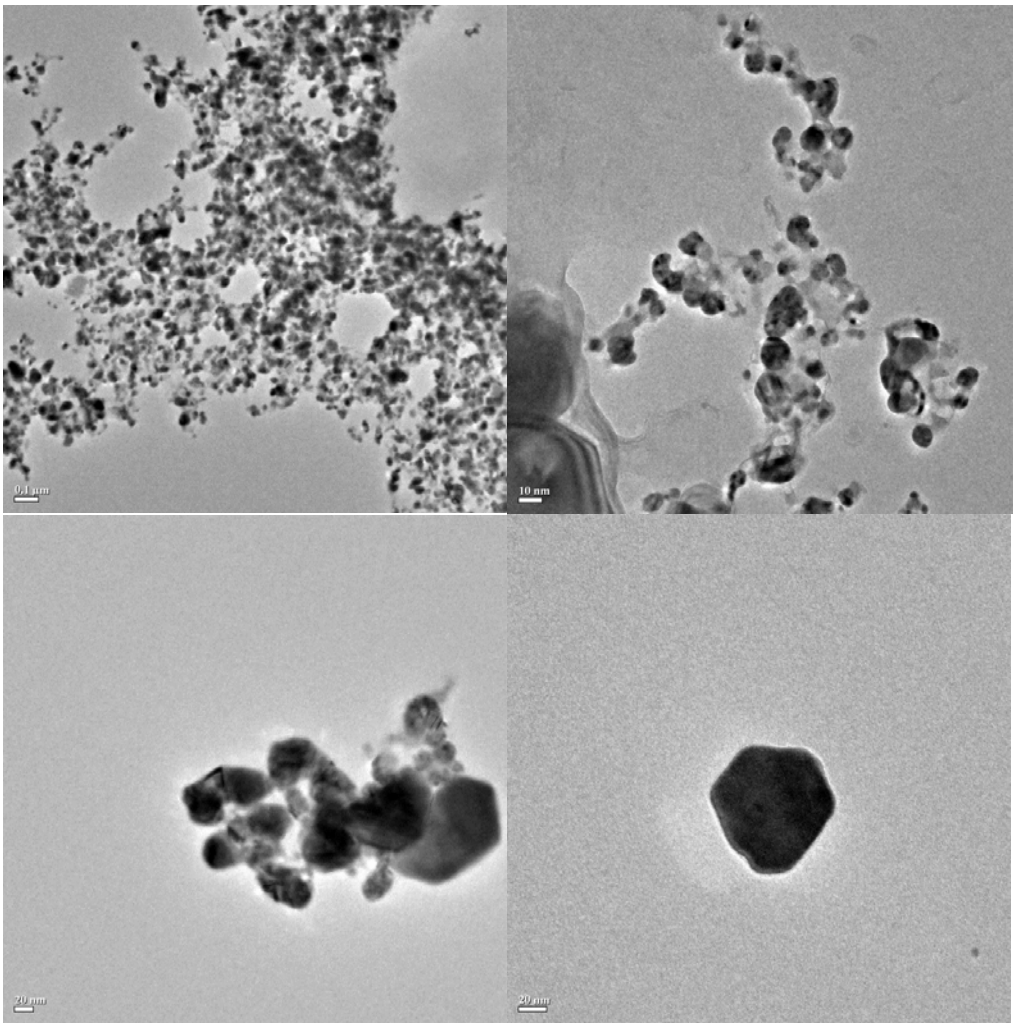


Fig. 3. TEM images of polygonal silver nanoparticles in different magnifications

3.3 Energy dispersive X-ray

The particles were verified using EDX analysis to ensure the presence of Ag as shown in fig. The EDX spectrum shows that 40.21% by weight was found to be silver nanoparticles. The peak between 2 to 4 keV can be assigned to Ag nanoparticles and the other peaks correspond to the Cu, Si and Cl. And these peaks may be from the capping agents originated from the bacterial biomass. The quantitative weight percentages of the other peaks are tabulated.

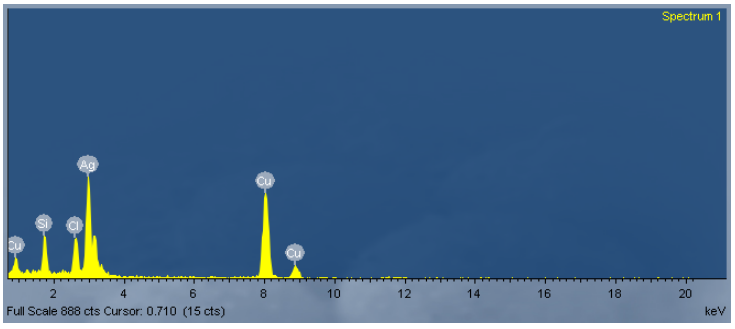


Fig. 4. EDX spectrum of polygonal silver nanoparticles with Cu as a standard

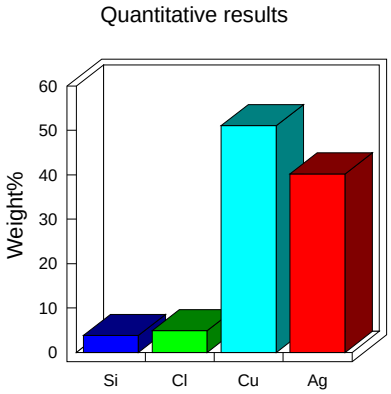


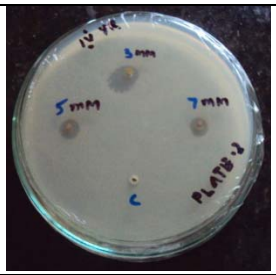
Fig. 5. Quantitative results of EDX spectrum of polygonal silver nanoparticles

3.4 Antibacterial activity study

Biosynthesized Ag nanoparticles were studied for its antibacterial activity against pathogenic microorganisms by using standard zone of inhibition microbiological assay , with a well size of 5mm diameter and 20 µl of samples of all three concentration (0.1 mM, 1 mM, 3 mM, 5 mM, 7 mM) with the Ag samples in three wells and the control with uninoculated broth. The Ag nanoparticles synthesized showed inhibition zone against all the test organisms. And the zone of inhibition for each concentration against the test organism is as shown in the tabulation.

Silver nanoparticles exhibited antibacterial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells. The antibacterial activity have been investigated against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae*. And the zone formation reported well in the pseudomonas sp compare to all other species. This clearly demonstrates that the antibacterial activity is only due to Ag-NP impregnated inside the bacterial and not due to individual bacterial. This findings shows that Ag-NP, require a lower concentration to inhibit development of the test bacterias used, and this probably due to the increasing surface area in Ag-NP. It shows good inhibition effect on *Pseudomonas, Staphylococcus* and *Bacillus* species and the inhibition effect based upon the concentration of silver used. And the activity was found to be quite strong and good.

Table1. Shows zone formation against various Bacteria

S.No.	Organism	Concentration	Zone of Inhibition (mm)	Photo of Agar well plate
1.	*Escherichia coli*	3mM	8mm	
		5mM	7mm	
		7mM	6.5mm	
		Control	-	
2.	*Pseudomonas*	3mM	14mm	
		5mM	13mm	
		7mM	13mm	
		Control	-	
3	*Klebsiella*	3mM	8mm	
		5mM	7mm	
		7mM	6mm	
		Control	-	
4	*Staphylococcus*	3mM	15mm	
		5mM	13mm	
		7mM	14mm	
		Control	-	
5	*Bacillus*	3mM	12.5mm	
		5mM	12mm	
		7mM	11mm	
		Control	-	

4. Conclusion

The study was carried out on the synthesis of silver nanoparticles extracellularly and confirmation of their presence using EDX and UV-Vis spectroscopy analysis. The TEM analysis was done in order to characterize the synthesized silver nanoparticle synthesized in their shapes and sizes. The use of bacteria as a system for nanoparticles bio-synthesis has come in vogue in recent past as an effective alternative to chemical synthesis. The ability to synthesis silver nanoparticles rapidly with the morphology control by eco-friendly biological methods is exciting and represents an important advance in making them viable alternatives to the more popular

chemical methods. Especially, extracellular synthesis offers the advantage of obtaining significant quantities that can be easily processed by filtering the cells and isolating the particles through cell-free filtrate. This study also exemplifies the application of silver nanoparticles synthesized in the anti-bacterial assay. And this findings shows that Ag-NP, require lower concentration to inhibit development of the test bacterias used and this probably due to the increasing surface area in Ag-NP. By this application we can utilize this Ag-NP in antibacterial creams in a concentration non-toxic to the human cell and impregnated to the inside of the bacteria and kills them effectively. Hence it has wide application in medical field .

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