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Biosynthesis of silver nanoparticles using culture supernatant of pseudomonas aeruginosa and antibacterial activity

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Abstract: Biosynthesis of nanoparticles employing microbes has emerged as an alternative method to complex chemical synthesis. In present study, biosynthesis of silver nanoparticles (AgNPs) by using culture supernatant of pseudomonas aeruginosa was investigated, the formation of silver nanoparticles was confirmed by the change in color of the culture filtrate from yellow to brown after addition of silver nitrate. Furthermore, the silver nanoparticles were characterized by means of UV-Visible spectroscopy, it showed absorption peak at (414-416) nm which corresponds to the Plasmon resonance of silver nanoparticles. The silver nanoparticles were effectively synthesized (produced) by pathogenic pseudomonas aeruginosa, which it is showed highest antimicrobial activity against both of them Staphylococcus aureus, Staphylococcus epidermis(22mm),followed by pathogenic Escherichia coli(20mm),also it have similar antimicrobial activity toward environmental Salmonella typhi and E. coli (18) mm. While, it showed the lowest antimicrobial activity against Enterococcus spp. (14) mm.

Keyword: Antimicrobial activity, Nanoparticles, Pseudomonas aeruginosa, UV_visible spectroscopy.

Introduction

Outbreak of the infectious diseases caused by different pathogenic bacteria and resistant to commercially available antimicrobial agent has become serious problem .Therefore, there is a pressing need to search for new antimicrobial agent from natural and inorganic substance, nanoscale materials have emerged as novel antimicrobial agents due to their high surface area to volume ratio and its unique chemical and physical properties[8,15]. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new material [2], this term was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974to describe precision manufacturing of materials at the nanoscale level [22]. Biosynthetic methods employing microorganism such as bacteria and fungi or plant extracts, have emerged as a simple and viable alternative to more complex chemical methods [17]. Recently a few microorganisms have been explored as potential bio- factories for synthesis of metallic nanoparticles such as cadmium sulfide, copper, zinc, titanium, magnesium, gold, and silver [20].

Silver nanoparticles (Ag NPs) are among the most commercialized inorganic nanoparticles, it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganism. Of these, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine. The metal microbe interactions have several roles in biotechnological applications including the fields of bioremediation, bio mineralization, bioleaching, and microbial corrosion [4,9] The biomedical applications of silver nanoparticles can be effective by the use of biologically synthesized nanoparticles which minimize the factors such as toxicity and cost and are found to be exceptionally stable [17]. Silver ion or metallic silver as well as, silver nanoparticles in medicine had been used for burn treatment, as dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. and it has low toxicity to human cells, high thermal stability and low volatility [5].

Aim of the work

The aim of this study was to synthesize a new material at nanoscale level, that include inorganic nanoparticles (Ag NPS), with antimicrobial activity using p. aeruginosa.

Material and Methods

Source of Microorganisms:

The bacteria P. aeruginosa was obtained from biology department, college of science, cultured on nutrient agar slant incubated at 37C°for 24 h.

Synthesis of silver Nanoparticles:

Sterilized nutrient broth was inoculated with a fresh culture of P. aeruginosa. Then, incubated at 37C° for 24 h in orbital shaker at 150 rpm. The culture was centrifuged at 5000 rpm for 15 min and the supernatant was used for synthesis of silver nanoparticles[8]. The supernatant of P. aeruginosa culture was separately added to a reaction vessels containing silver nitrate solution $AgNO_3$(1mM) and incubated in brown bottle for 3 days at 37C°in an orbital shaker at 150 rpm.The metal processed bacterial filtrate was centrifuged at 8000 rpm for 20 minutes [3,10].

UV-Visible spectro photometric analysis:

The supernatant was tested qualitatively by UV-Visible spectrophotometer to confirm the reduction of silver ions. Silver nitrate solution was used as blank , absorption spectrum of the solution was measured using (Visible Spectrophotometer-UV-1650PC, UV) at wavelength between 100-1000 nm[10].

Antibacterial activity of silver nanoparticles:

The silver nanoparticles synthesized from P. aeruginosa were tested for their antimicrobial activity using the agar well-diffusion method against pathogenic and environmental isolates of E. coli , salmonella. typhi , Staphylococcus aureus, staphylococcus epidermis and enterococcus spp. (obtained from biology department ,college of science) .These organisms were grown in nutrient broth for 24 h, bacterial concentration was fixed at $5*10^8$ by comparison with McFarland NO.0.5 Approximately 20 ml of Muller Hinton agar was poured in sterile Petri dishes and allowed to solidify. Wells of 6mm diameter were made on Muller Hinton agar plates using sterile cork borer, each strain was swabbed uniformly onto the surface of plates. Ten µL of silver nitrate solution, supernatant of silver nanoparticles, pellet of silver nanoparticles (precipitation) and liquid culture filtrate separately was loaded into the well using micropipette. After incubation for 24h at 37C°, zones of inhibition were measured.

Results

Biosynthesis of Ag NPs:

Biological synthesis of silver nanoparticles by P. aeruginosa is confirmed by change of reaction mixture from pale yellow to brown color after completion of reaction mixture(72 hours incubation) ,Whereas no color change was observed in media with silver nitrate solution alone (figure 1) , the appearance of yellowish brown color clearly indicates the formation of silver nanoparticles in reaction mixture [20]. Brown color was noted in test tube containing supernatant of pathogenic P. aeruginosa treated with $AgNO_3$ solution more intensely than the test tube containing supernatant of environmental P. aeruginosa. Increasing intensity may due to the formation of more nanoparticles [6] .The characteristic brown color of colloidal silver solution could be due to the excitation of surface Plasmon vibrations of Ag-NPs and provides a convenient spectroscopic signature of their formation [1,18].

This indicates that certain reducing agent released in the cultures of the tested bacteria are involved in the reduction of silver ions Ag^+ to silver nanoparticles Ag-NPs [6]. Thus, it was evident that electron shuttle or other reducing agents released by P. aeruginosa which are capable of reducing silver ions to silver nanoparticles. On the other hands, the reduction of silver ions did not occur in the absence of bacterial cells. This clearly indicates that reducing agents released into the cultures of P. aeruginosa were involved in the reduction process[19].

The mechanisms of biosynthesis of Ag-NPs has been hypothesized that silver ions required NADPH-dependent nitrate reductase enzymes for their reduction, which was secreted by the bacteria in its extracellular environment [11] .Nitrate reductase is known to shuttle electron from nitrate to metal group .Thus, these results substantiate the role of nitrate reductase enzyme in the biosynthesis of silver nanoparticles [7]. A similar observation was made by[8,10] in the biosynthesis of Ag-NPs by P. aeruginosa strain by extracellular process.

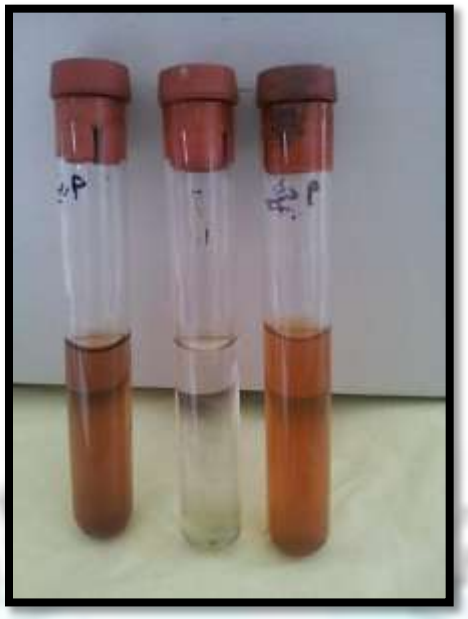
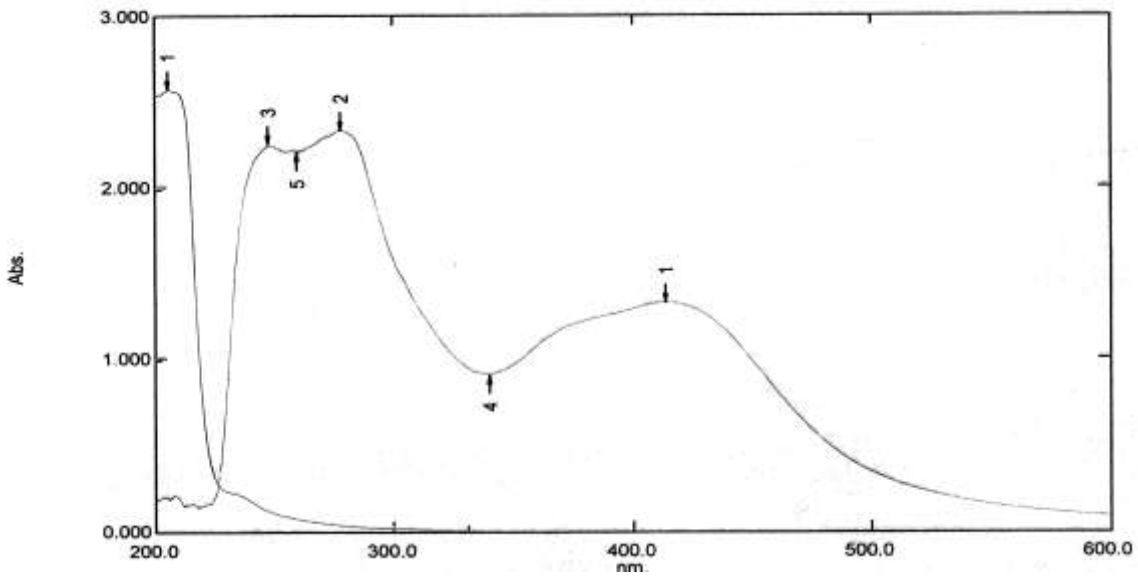


Figure 1: Biosynthesis of silver nanoparticles

(A) test tube containing environmental p. aeruginosa supernatant with $AgNO_3$ solution
(B) test tube containing aqueous$AgNO_3$ solution only
(C) test tube containing pathogenic p. aeruginosa supernatant in aqueous $AgNO_3$solution

The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV- visible spectrophotometric analysis .Thus, it is one of the most widely used techniques for structural characterization of silver nanoparticles. A strong peak (maximum absorbance) at 414,416 nm was observed for the silver nanoparticles prepared using environmental and pathogenic p. aeruginosa respectively, while no absorption band was observed for AgNO3 solution (Fig.2) .This event clearly indicates that the reduction of silver ion to metallic silver took place extracellulary through the reducing agent released into the solution [12]. Observation of this peak, assigned to surface Plasmon. The surface Plasmon resonance (SPR) band of silver nanoparticles remain in the range of (380_440) nm throughout the reaction period, suggesting that the particles were dispersed in the aqueous solution with no evidence for aggregation after completion of the reaction [14,17]



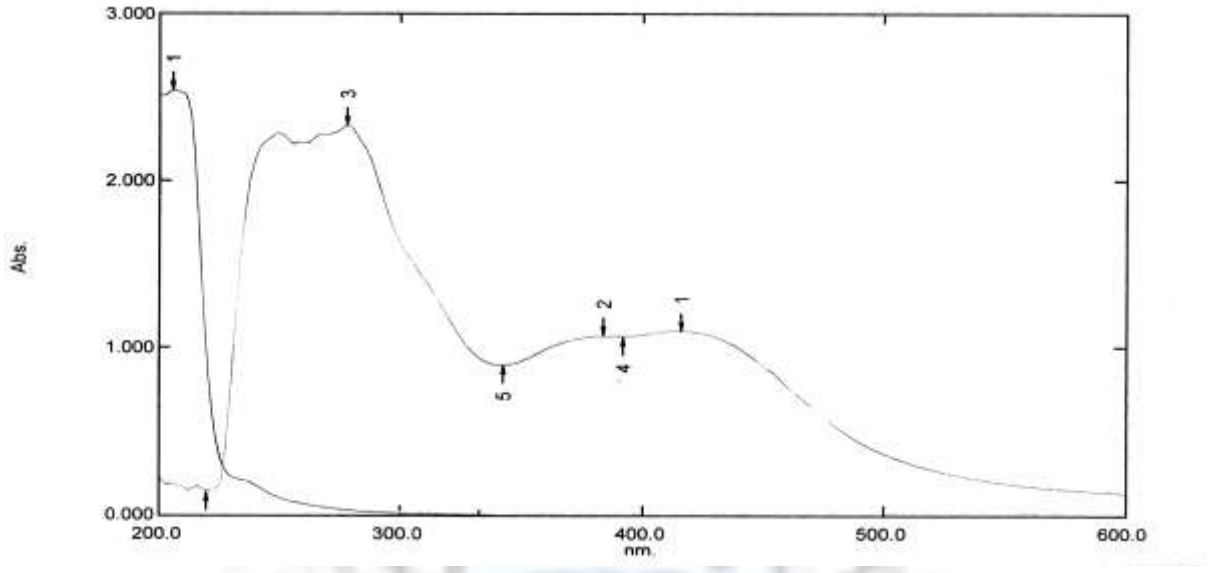


Fig (2): Absorption spectrum of silver nanoparticles synthesized by the culture supernatant of P. aeruginosa

The Antibacterial Activity of Silver Nanoparticles:

The antimicrobial activity of Ag NPs synthesized by p. aeruginosa against various pathogenic and environmental microorganisms such as Escherichia coli , Salmonella typhi ,Staphylococcus aureus , Staphylococcus epidermidis and Enterococcus spp. using well diffusion method is represented in (Figure 3,4,5,6).

Table (1) show the diameter of inhibition zone (mm) around each well with SNPs solution formed by pathogenic p. aeruginosa. The highest antimicrobial activity was observed against Staphylococcus aureus , Staphylococcus epidermidis (22) mm, followed by pathogenic Escherichia coli (20)mm ,also it have similar antimicrobial activity toward environmental Salmonella typhi and E. coli (18) mm .While, the lower antimicrobial activity was found against Enterococcus spp. (14) mm. This result corresponding to the work of [10], which investigated highest antimicrobial activity of Ag NPs against E. coli (18 mm), also corresponding to work of [8], which investigated antibacterial activity of Ag NPs synthesized by p. aeruginosa against E. coli (15mm), Staphylococcus aurous (17 mm).

Table (2) show the diameter of inhibition zone (mm) around each well with Ag NPs solution formed by environmental p. aeruginosa, that investigated highest antimicrobial activity against Staphylococcus aureus (19) mm followed by pathogenic Escherichia coli, Salmonella typhi and Staphylococcus epidermidis (17) mm. While, it investigated lower antibacterial activity against environmental Salmonella typhi ,E. coli and Enterococcus spp(15) mm.

The results demonstrated that the highest antimicrobial activity was formed by pathogenic p. aeruginosa, this due to the formation of more nanoparticles compared with environmental p. aeruginosa. which was observed by increasing intensity of brown color for test tube containing supernatant of pathogenic p. aeruginosa treated with solution of $AgNO_3$.

It was reported previously that E. coli being the model for Gram negative bacteria was found to be susceptible for silver nanoparticles thus confirming its antimicrobial property [21,23] , also opined that the AgNPs had inhibitory effect on Staphylococcus aureus and E. coli. It was clear from the experiment that Staphylococcus aureus being Gram positive showed most susceptibility against the nanoparticles in comparison to Salmonella typhi and Escherichia coli being Gram negative. The strongest reason about the susceptibility of nanoparticles agains Staphylococcus aureust may be due to their cell wall plasmolysis or separation of cytoplasm from their cell wall [24]. The antimicrobial mechanisms of bio-nanosilver particles may differ from species to species of bacteria and also on the size of the nanoparticles.

The antimicrobial mechanism of silver nanoparticales is related to the formation of free radicals and subsequent free radical induced membrane damage. These free radicals may be derived from the surface of silver nanoparticals and responsible for the antimicrobial activity [16]. Silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell wall , and also penetrate inside the bacteria. The bacterial membrane contains sulfur-containing proteins and the

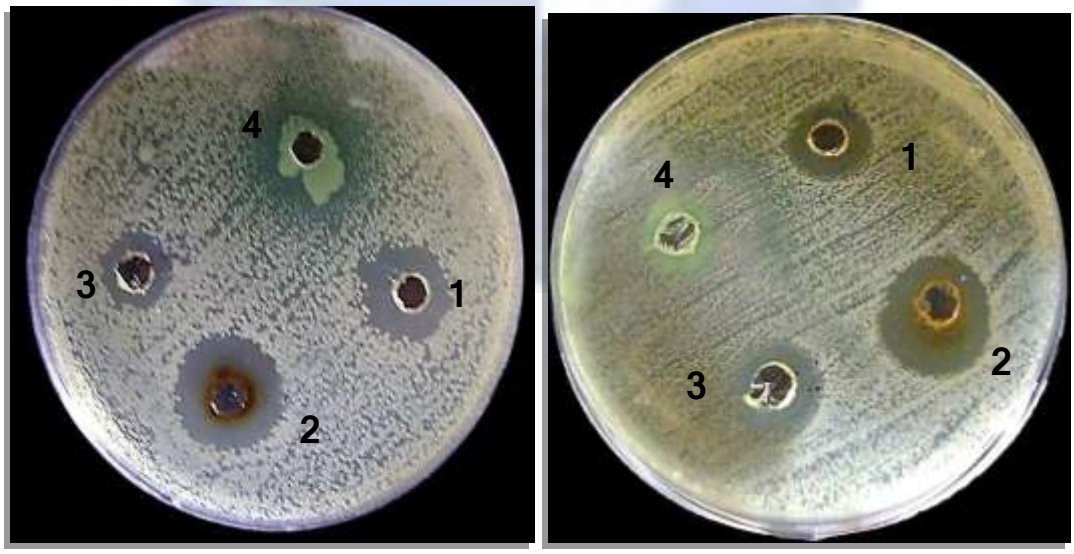
silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell, it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity [13,24].

Table (1) Antibacterial activities of silver nanoparticles synthesized by pathogenic p. aeruginosa against different bacteria

Pathogenic P. aeruginosa	Standard	Sediment	Supernatant	Bacteria Supernatant
Pathogenic E. coli	18	20	12	10
Environmental E. coli	13	18	12	6
Pathogenic S. typhi	15	17	14	6
EnvironmentalS.typhi	17	18	14	6
Staph. Aureus	20	22	14	27
Staph. Epidermides	19	22	9	31
Enterococcus spp.	12	14	11	6

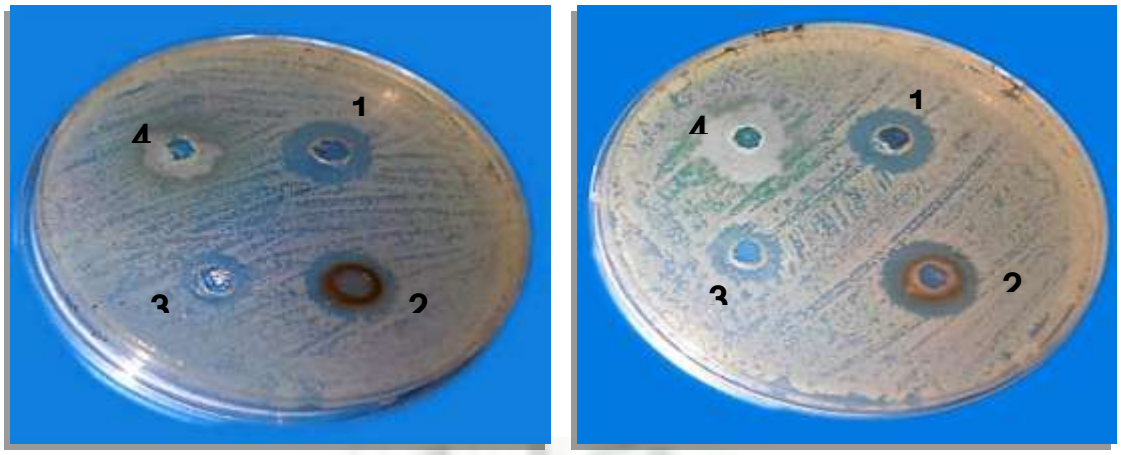
Table(2) Antibacterial activities of silver nanoparticles synthesized by Environmental p. aeruginosa against different bacteria

Environmental P. aeruginosa	Standard	Sediment	Supernatant	Bacteria Supernatant
Pathogenic E. coli	14	17	12	6
Environmental E. coli	15	15	11	6
Pathogenic S .typhi	15	17	14	6
Environmental S .typhi	13	15	12	6
Staph. Aureus	14	19	13	34
Staph. Epidermides	15	17	12	30
Enterococcus spp.	12	15	12	6



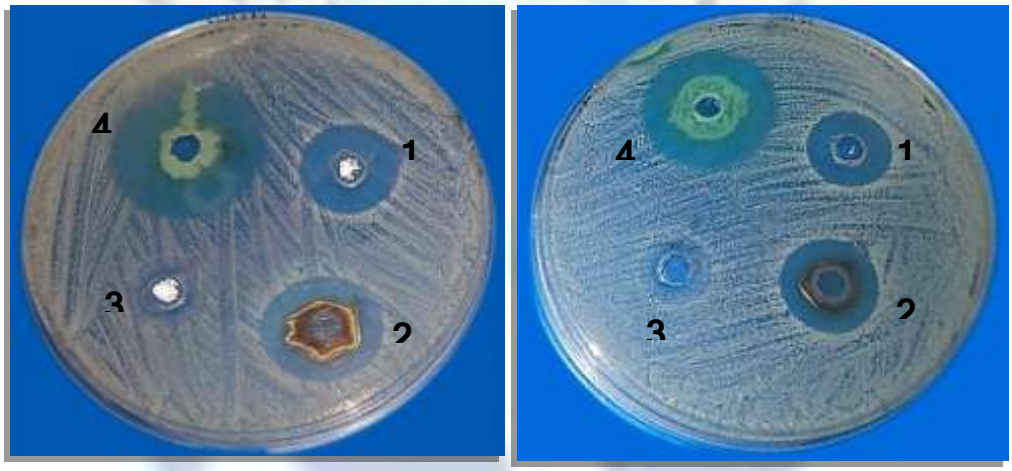
Pathogenic E. coli **Environmental E. coli**

Figure (3): Antimicrobial activities of silver nanoparticles synthesized by pathogenic p. aeruginosa (1) standard (2)Sediment (3) Supernatant (4) Bacteria Supernatant



EnvironmentalE. coli **pathogenic E. coli**

Figure(4): Antimicrobial activities of silver nanoparticles Environmental p. aeruginosa (1)standard (2) Sediment (3) Supernatant (4) Bacteria Supernatant



Pathogenic p. aeruginosa **Environmental p. aeruginosa**

Figure (5): Antimicrobial activities of silver nanoparticles against staph epidermis (1) standard (2) Sediment (3) Supernatant (4) Bacteria Supernata

Pathogenic p. aeruginosa **Environmental p. aeruginosa**

Figure(6) Antimicrobial activities of silver nanoparticles against staph aureus (1) standard (2) Sediment (3) Supernatant (4) Bacteria Supernatant

Conclusion

The bacterium p. aeruginosa can be used to synthesize bioactive nanoparticles efficiently using inexpensive substances in an ecofriendly and nontoxic manner. UV-VIS spectrophotometer technique have confirmed the reduction of AgNO3 to Ag NPs by p. aeruginosa. The zone of inhibition formed in the antimicrobial screening test indicated that the AgNPs synthesized in this process have efficient antimicrobial activity against environmental and pathogenic bacteria. It showed that silver nanoparticles synthesized by this process find use in various fields.

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