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Research Article

Biocompatible synthesis of silver nanoparticles (AgNPs) using marine *Vibrio* sp. and study their pharmacological applications

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Abstract

This report presents a rapid and a green biogenic approach for the biosynthesis of silver nanoparticles using marine bacteria such as *Vibrio* sp. The bioactive compounds of *Vibrio* sp. induced the reduction of Ag+ ions from $AgNO_3$, which resulted in the formation of bacterial mediated silver nanoparticles (AgNPs). The reduction of Ag+ ions to elemental silver was characterized by UV-vis spectroscopy, Fourier transform infra-red spectroscopy (FTIR), Scanning electron microscopy (SEM) and Dynamic light scattering (DLS). The nanoparticles show maximum absorbance at 421 nm on ultraviolet-visible spectra. The presences of biomolecules were identified by Fourier transform–infrared spectroscopy. The bioactive compounds and nanoparticles were found to direct different shape and sized and their size/ shape were varied. The silver nanoparticle synthesized was spherical, in the range of 40-80 nm in size. In antimicrobial screening, all the pathogens were fairly affected and nil effect was not observed in the test samples, In bacteria, the test sample was most effective against *Enterococcus faecalis* B2 and *Salmonella typhimurium* B5. In fungi, which was effective against *Candida albicans* F1, *Microsporum canis* F3 and *Trichophyton rubrum* F4.
Keywords: *Marine Vibrio* sp., Silver nanoparticles, Green synthesis, Antimicrobial activity.

1.Introduction

The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies [1]. The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticles show completely new or improved properties, such as size, distribution and morphology of the particles etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly on various fields [2]. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [3]. They are gaining the interest of scientist for their novel methods of synthesis. Over the past few years, the synthesis of metal nanoparticles is an important topic of research in modern material science. Recent years have seen remarkable progress in research and development of metal nanoparticles (NPs). In general, however, practical utilization of nanosized materials involves considerable difficulties since metal NPs are hard to handle directly, and easily aggregate to minimize their surface area [4]. The

inevitable aggregation of metal NPs often nullifies their unique functionalities, and eventually yields ordinary bulk metals. For that reason, an area of ongoing research has focused on effective immobilization of metal NPs on easily handled supports such as porous membranes and nanostructured inorganic sheets [5].

Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. However, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes[6]. Silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, antimicrobials, and biomaterial production. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution.

Silver has been known to be a disinfectant for several centuries and has been widely used in the treatment of clinical diseases, including newborn eye prophylaxis and topical burn wounds [5][7]. Silver serves as a potent antibacterial agent, acting against an exceptionally broad spectrum of bacteria while exhibiting low toxicity to mammalian cells. Since silver therapy is of significant clinical benefit in the control of bacterial infections, various forms of new agents medical, biological and pharmaceutical preparations containing the silver ions, such as creams, solutions, electrodes, ligatures, biological skin and catheters, have been developed over the past decades [6]. Therefore, not surprisingly, the antimicrobial properties of the silver ions have been extensively investigated, and many of the findings are well accepted universally. In medicines, silver and silver nanoparticles have an ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds [8], medical devices and implants prepared with silver-impregnated polymers [8].

Various approaches using biological materials have been used for the synthesis of metal nanoparticles. These approaches have many advantages over chemical, physical, and microbial syntheses because there is no need of the elaborated process of culturing and maintaining the cell, hazardous chemicals, high-energy requirements, and wasteful purifications [10]. In this study, the marine bacterial culture *Vibrio* sp. was used as a reducing agent for the synthesis of high valuable nanoparticle of silver nanoparticles.

2. Materials and methods

2.1 Preparation of culture medium

The sea water samples were collected from the Nagappattinam coast during February 2015 with 1000 mL of sterile plastic container. The seawater sample was kept in ice box and transport to laboratory. The sample was processing within 8 hours. The selective culture medium such as TCBS agar was used to isolate *Vibrio* sp. The biochemical tests were processed for identification of *Vibrio* isolates. Then, the isolates were subculture in nutrient broth. In this study, all the medium plates were prepared by the addition of old age sea water.

2.2 Synthesis of silver nanoparticles (AgNPs)

For the synthesis of silver nanoparticles, silver nitrate was prepared at the concentration of 10^{-3} M with pre-sterilized Milli-Q water. A quantity of 1.5 ml of bacterial culture was mixed with 30 ml of 10^{-3} M of silver nitrate for the synthesis of silver nanoparticles. Silver nitrate was taken in similar quantities of 1.5 ml without adding bacterial culture to act as control. The saline bottles were tightly covered with aluminum foil in order to avoid photo reduction of silver ions, incubated at room temperature under dark condition and observations were recorded at 15 m, 30 m, 1 and 2h.

2.3 Characterization of silver nanoparticles

2.3.1 UV-vis analysis

The optical property of AgNPs was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35, Germany). After the addition of $AgNO_3$ to the plant extract, the spectra's were taken in different time intervals up to 24 hrs between 350 nm to 500 nm. Then the spectrum was taken after 24 hrs of $AgNO_3$ addition.

2.3.2 FTIR analysis

The chemical composition of the synthesized AgNPs was studied by using FTIR (Perkin-Elmer LS-55-Luminescence spectrometer). The solutions were dried at 75 ^{o}C and were characterized in the range 4000–400 cm^{-1} using KBr pellet method.

2.3.3 SEM analysis

The morphological features of synthesized silver nanoparticles from *Vibrio* sp. were studied by Scanning Electron Microscope (JSM-6480 LV). After 24 hrs of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV

2.3.4 DLS and Zeta potential analysis

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min at 25^0C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries, Malvern instrument Nano Zs).

2.4. Screening of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* B1, *Enterococcus faecalis* B2, *Klebsiella pneumoniae* B3, *Micrococcus luteus* B4, *Salmonella typhimurium* B5, *Vibrio cholerae* B6, *Candida albicans* F1, *Cryptococcus* sp. F2, *Microsporum canis* F3 and *Trichophyton rubrum* F4. Microbial strains were tested for antimicrobial sensitivity using the well diffusion method [11-13]. This method was used to evaluate *in vitro* antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on Muller Hinton agar (MHA) and potato dextrose agar (PDA), respectively [14][15]. A sterile cotton swab was used to inoculate the standardized bacterial suspension on surface of agar plate for even growth. The 2.5, 5 and 10 µL of test solutions (prepared with sterile double distilled water) were poured in each well (6 mm diameter), separately. One separate well was used for control study by taking of double distilled water (without test sample). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungi). After incubation, the zone of inhibition was measured with ruler/ HiAntibiotic ZoneScale-C. The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control, and sterile double distilled water used as a negative control) [16]. All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

3. Result and Discussion

Nano materials are seen as solution to many technological and environmental challenges in the field of solar energy conversion, catalysis, medicine, and water treatment. Reduction of silver ions into silver nanoparticles during exposure to culture was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance (SPR) phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 421 nm in case of *Vibrio* sp. From different literatures it was found that the silver nanoparticles show SPR peak at around 420 nm. From our studies we found the SPR peak for *Vibrio* sp. at 421 nm. So, we confirmed that *Vibrio* sp. has more potential to reduce Ag ions into Ag nanoparticles, which lead us for further research on synthesis of silver nanoparticles from *Vibrio* sp. The intensity of absorption peak increases with increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles.

The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag+ ions is complete within 2 hrs after addition of the metal ions to the culture medium. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticles solutions with time. On the behalf of UV-vis data it was cleared that reduces metal ions. So, the further characterizations were carried out with *Vibrio* sp. (Figure 1). The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions [17]. Huang *et al*[18] in 2007 reported formation of silver nanoparticles when constant aqueous AgNO3 at 50 ml, 1 mM with 0.1 g biomass produced silver nanoparticles as indicated by sharp absorbance at around 440 nm in *Cinnamomum camphora*.

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles (Figure 2) wherein some pronounced absorbance were recorded in the region between 4000 and 400 cm^{-1}. They include 3432 (secondary amine, free, N-H

asymmetric stretching), 2922 (Diazo, RCH=N=N Stretching), 1634 (etartiN, O-NO_2 Stretching asymm), 1384 (Alkanes, CH_3 symmetric bending, R-CH_3), and 667 (C-S, R-C-CH_3 stretching for sulphur compounds), cm^{-1}. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

Figure 1: UV-vis spectral analysis of Ag nanoparticles

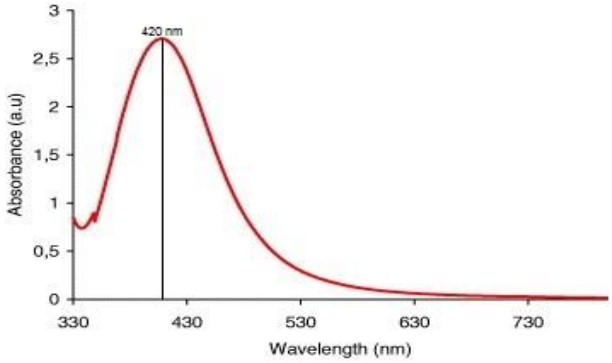
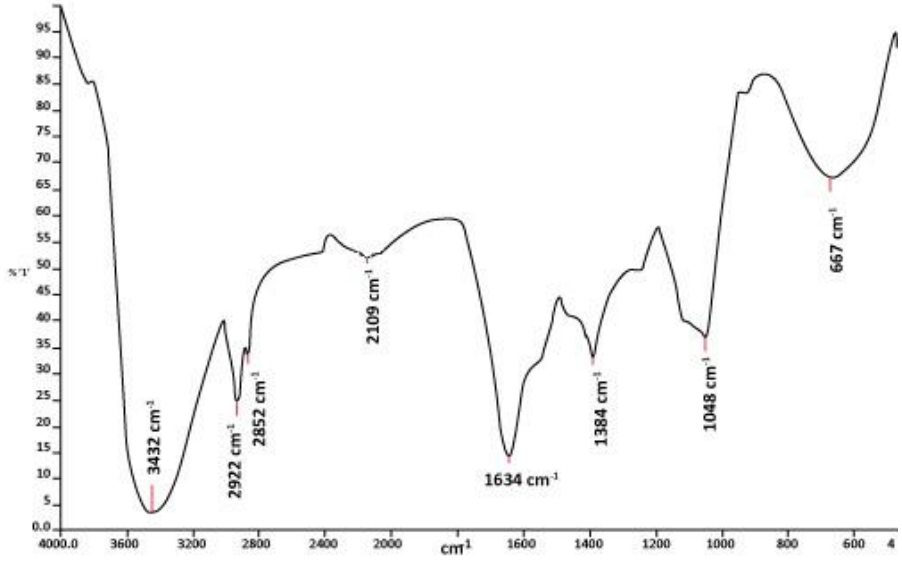


Figure 2: FTIR analysis of vibration modes and function groups of AgNPs



SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes several nm. i.e. between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles (Figure 3). The particle size distribution (PSD) of synthesized silver nanoparticles, it was found that Ag nanoparticles size were in the range of 80-120nm. However, beyond 100 nm range the percentage of nanoparticles present is very less. The highest fraction of Ag NP present in the solution was of 75 nm (Figure 4) is very appropriate since it gives lowest average size of nanoparticles. The Figure 4 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and is related to the electrophoretic mobility and stability of the suspension of nanoparticles of the nano silver. The overall absorbance of Zeta potential revealed the energetically very unstable. Therefore the particles undergo agglomeration/ aggregation to stabilize themselves. So there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis. Zeta potential (Surface potential) has direct relation with the stability of a form/ structure as mentioned below (Figure 5).

Figure 3: SEM –microscopic view of *Vibrio* sp. reduced silver nanoparticles

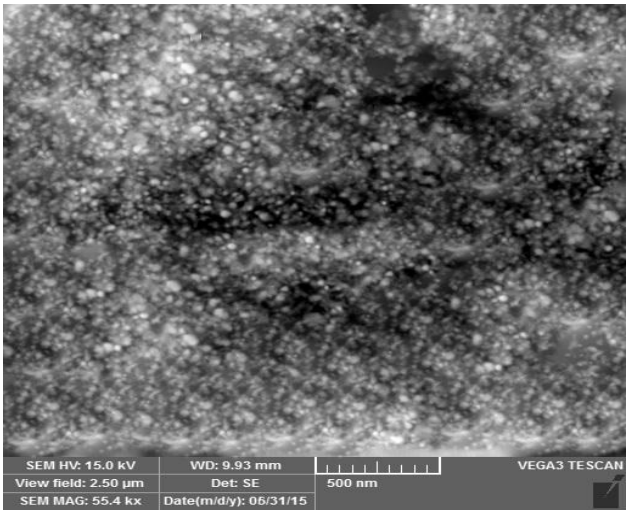


Figure 4: Dynamic light scattering of particle size analyzer of Ag nanoparticles

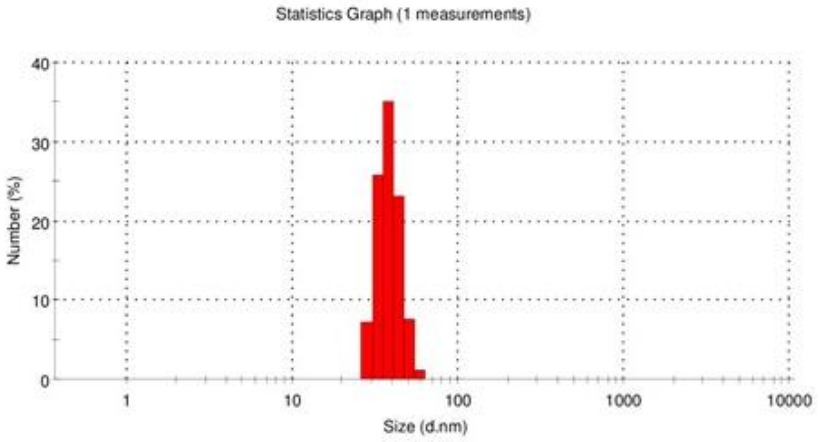
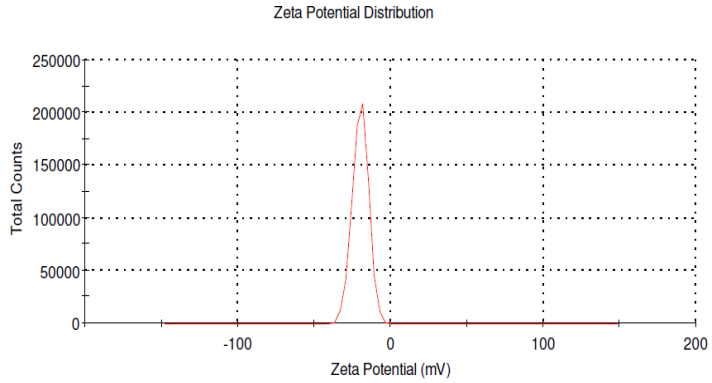


Figure 5: Dynamic light scattering of zeta potential measurement of AgNPs



Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -17.7	Peak 1:	-17.7	100.0	5.22
Zeta Deviation (mV): 5.99	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.828	Peak 3:	0.00	0.0	0.00
Result quality : Good				

Zeta Potential (mV): from ±10 to ±30 = Incipient instability

The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) well diffusion test. The results of the antimicrobial activities are summarized in Table 1.

Table 1: Antimicrobial activity of biologically synthesized nanoparticles by *Vibrio* sp

S.No	Test Microorganisms		AgNPs (µL/well)			PC*	Diseases	Route of Transmission
	Bacteria		2.5	5.0	10.0	10 mcg		
1.	*Aeromonas liquefaciens*	B1	9	11	13	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus faecalis*	B2	11	14	18	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae*	B3	9	11	12	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus*	B4	10	15	16	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium*	B5	10	11	14	0	Typhoid	Water / Food
6.	*Vibrio cholerae*	B6	11	13	14	16	Cholera	Water / Food
	Fungi							
7.	*Candida albicans*	F1	10	12	17	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp.	F2	9	10	12	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis*	F3	11	14	18	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum*	F4	10	14	18	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC* - Positive control

The two tested concentrations such as 2.5, 5 and 10 µL /well produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (10 µL/ well) concentration of sample got greater sensitivity than (2.5 and 5 µL/ well) lower concentration in all the tested microorganisms. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples, In bacteria, the test sample was most effective against B2 & B5. While moderate effect was noticed from B3, B4 & B6. In fungi, which was effective against F1, F3 & F4 whereas average effect was observed in F2. All the microbial strains depict higher sensitivity to the higher concentration (10 µL) for the test samples. Gram-positive, Gram-negative bacteria and fungal strains were challenged against test samples. The Gram-positive were highly sensitive than Gram-negative bacteria. Selected microorganisms were showed significant sensitivity against the biosynthesized nanoparticles. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (distilled water), reflecting that antimicrobial activity was directly related to the sample. The present study showed the more or less equal results to the previous reports. Hemanth and co-workers [19] (2010) when test bacteria were treated with highly reactive metal oxide nanoparticles. A bacterial membrane with this morphology exhibits a significant increase in permeability, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane and, finally, causing cell death. It is well known that the outer membrane of gram negative cells is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provide an effective permeability barrier [20].

4. Conclusion

The rapid biological synthesis of silver nanoparticles using *Vibrio sp.* culture provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The *Vibrio* strain mediated AgNPs was characterized by using UV-vis spectrophotometer, SEM, DLS, Zeta analyzer, XRD, and FTIR techniques. All these techniques proved that the bacterial culture reduced the metal ion into nanoparticles and were played an important role in the shape determination of the nanoparticles. The biosynthesized silver nanoparticles using marine bacteria *Vibrio* sp. proved excellent antimicrobial activity. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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