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Research Article

Biosynthesis and characterization of silver nanoparticles (AgNPs) using marine bacteria against certain human pathogens

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Abstract

The present work investigates the synthesis of silver nanoparticles (AgNPs) by biological method using marine bacteria. The minimum inhibitory concentration (MIC) test was performed to find the inhibitory concentration of $AgNO_3$ against marine bacterial isolate. After MIC study, the biogenic AgNPs was prepared through marine bacterial isolate and characterized by using UV-visible spectroscopy; Scanning Electron Microscopy (SEM), X Ray Diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). From the UV-visible spectroscopy, the absorption peak was found at 420 nm. In SEM image, it is confirmed that the sample contains spherical shaped silver nanoparticles and most of the particles were below 100 nm in size. In XRD analysis, it was confirmed that the silver nanoparticles are crystalline in nature, which was confirmed by the FTIR peak at 518 cm^{-1} corresponding to the Ag vibration present in crystalline structure. The antimicrobial activity of silver nanoparticles was determined by disk diffusion method, and found that silver nanoparticles have significant antibacterial activity against most of the pathogens.

Keywords: Silver nanoparticles, Marine bacteria, Antimicrobial activity, Scanning Electron Microscopy.

1. Introduction

Nanoparticles (NPs) serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructured materials, play an ever increasing role in science, research and development as well as also in every day's life, as more and more products based on nanostructured materials are introduced to the market. Silver nanoparticles (AgNPs) are one of the promising products in the nanotechnology industry. Silver nanoparticles can be synthesized by several physical, chemical and biological methods, in which, one of such promising process is green synthesis. However, for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality. An increasingly application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, wound dressing, and biomedical devices contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria [1].

Bacteria are well known to produce inorganic materials either intracellularly or extracellularly. Marine bacteria are concluded as a potential biofactory for the synthesis of nanoparticles like gold, silver and cadmium sulphide. Some known examples of bacteria synthesizing inorganic materials include magnetotactic bacteria (synthesizing magnetic nanoparticles) and S layer bacteria which produce gypsum and calcium carbonate layers [2]. Silver nanoparticles shows very strong bactericidal activity against gram positive as well as gram negative bacteria including multi-resistant strains [3]. Some Athletic clothing companies have incorporated silver nanoparticles into their products mainly for reduce the bad smell. Many textile industries insert silver nanoparticles into its products to allow the particles to attach to the filaments. Once the silver nanoparticles encounter sweat from the human body, they naturally release a low concentration of silver ions into the moist environment [4].

This study aims to synthesis of silver nanoparticles through biological method by using of marine bacterial isolate and it was characterized by several methods. Further, the pharmacological applications such as antibacterial activity of AgNPs were studied against certain bacterial pathogens.

2. Materials and methods

2.1 Biofabrication of nanoparticles

The bacterial strain was isolated from sea water of Nagore during April 2016. The minimum inhibitory concentration (MIC) test was performed to find the inhibitory concentration of $AgNO_3$ against marine bacterial isolate. After MIC study, the biogenic AgNPs was prepared through marine bacterial isolate. For synthesis of silver nanoparticles, the isolated marine bacteria were inoculated in nutrient broth media along with 25 mM $AgNO_3$. The culture flask was kept on a shaker with speed of 150 rpm at room temperature for overnight incubation. After overnight Incubation the color was changed and turbid was occurred, that indicated the presence of silver nanoparticles in the culture.

2.2 Characterization of nanoparticles

2.2.1 UV-VIS spectroscopy

The Au nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behaviour of Au nanoparticles. The scanning range of the samples was 200-800 nm at a scan speed of 480 mm/min. Baseline correction of the spectrophotometer was carried out by using a blank reference.

2.2.2 Fourier transform-infra red (FT-IR) spectroscopy

The analysis of bio-reducing agent present in each of the extracts was measured by FT-IR. After the reaction, a small aliquot of the concentrated reaction mixture was measured in the transmittance mode at 400 to 4000 cm^{-1}. The spectra of the extracts taken after the biosynthesis of nanoparticles were analyzed.

2.2.3 Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS)

In this research work, Joel JSM-6480 LV SEM machine was used to characterize the mean particle size and morphology of nanoparticles. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDS analysis of Ag sample was done by the SEM (JEOLJSM 5800) machine. The EDS normally reveals the presence of phases.

2.2.4 X-ray diffraction method

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, Netherland) using Cu radiation. The generator voltage and current was set at 40 KV and 30 mA respectively. The Au sample was scanned in the range 10.0000 - 90.0000^o in continuous scan mode. The scan rate was 0.60/sec.

2.3 Testing of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the plate diffusion method [5]-[9]. The antibacterial and antifungal activities of test samples were analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively [10][11]. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The 15 and 30 µL of test solutions were poured in each disc, separately. One separate disc was used for control study by taking sterile triple 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/ antibiotic zone scale-C [12][13]. 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. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

3. Result and discussion

3.1 Biofabrication of AgNPs

Marine bacteria are one of the important sources for the drug production and some of the marine bacteria are involved in the drug synthesis [14]. Now a day, many biological living things are involved in the nanoparticles synthesis. Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of change in local electronic structure of the surface due to smaller sizes. The effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surface.

3.2 UV-Vis spectroscopy analysis

The UV-VIS spectroscopic studies revealed the presence of beard peaks at 420 nm Figure 1. The plasmon resonance of the silver nanoparticles was recorded. When the precursor silver nitrate solution has mixed with the marine bacteria extracts they were reduced into silver (Ag) nanoparticles. When the marine bacteria extract was mixed with silver nitrate ($AgNO_3$) aqueous solutions, the solutions changed

their color from white to brown for silver nanoparticles. The absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 421 nm. A remarkable broadening of peak at around 350 nm to 480 nm indicates that the particles are polydispersed. Similar study was carried by Huang *et al*[15] in 2007, reported that the formation of silver nanoparticles when constant aqueous AgNo3 at 50 ml, 1 mM with 0.1 g bio-mass produced silver nanoparticles as indicated by sharp absorbance at around 440 nm in *Cinnamomum camphora*.

3.3 FTIR and SEM analysis

The Figure 2 shows the FTIR image of silver nanoparticles synthesized from Marine bacteria. FT-IR analysis revealed the strong bands at 3383, 2352, 1601, and 1404, 1113, 675, 518 cm^{-1}. The band at 2352 for O-H stretching corresponds to carboxylic acid, 1601 cm^{-1} for stretching C=C corresponds to aromatic amino groups. The band at 675 cm^{-1} corresponds to C-H stretching of phenyl ring of substitution band, whereas the stretch for Ag-NPs was found around 518 cm^{-1}. The morphological features of synthesized silver nanoparticles were studied by SEM analysis shown in Figure 3. SEM analysis suggested that most of the particles are spherical in shape.

3.4 XRD analysis

Silver nanoparticles were synthesized using marine bacteria and X-ray diffraction (XRD) pattern of synthesized particles were analyzed and found peak profile of relevant particles (Figure 4). In this result, peaks were observed at 2θ of 38, 44, 65 and 77 are corresponding to the Bragg's reflections such as (111), (200), (220) and (311). Other peaks were also observed along with the main peaks. This may be due to the crude nature of the extracts containing other metabolites and salts. These components would have reacted with the ionic silver during the synthesis reaction. These compounds might be reason for the formation of other peaks.

3.5 Antimicrobial activity

The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test. The results of the antimicrobial activities are summarized. The two tested concentrations such as 15 and 30 µL /disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (30 µL/disc) concentration of sample got greater sensitivity than (15 µL/disc) 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 *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Vibrio cholerae* (B6) (Table 1). In fungi, which was effective against *Cryptococcus sp*. MTCC 7076 (F2) whereas smaller effect was observed in *Candida albicans* (F1). All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. Silver nanoparticles have a great bactericidal effect against several bacteria including multi resistant strains; can be considered as potential antifungal agent [16]. Anitha *et al*[17] in 2011 found that the Ag nanoparticles have exhibited considerable activity against some human pathogens. The antimicrobial property of silver is found to be the best among different metals in the following order Ag >Hg > Cu >Cd>Cr>Pb>Co>Au>Zn>Fe>Mn>Mo>Sn [18]. It is disc known that Ag ions and Ag-based compounds have strong antimicrobial effects [19], and many investigators are interested in using other inorganic nanoparticles as antibacterial agents [20]. This study enlighten that the marine bacteria is act as a metal reducing agent and it also involved in the biogenic synthesis of metal nanoparticles with less cost effect.

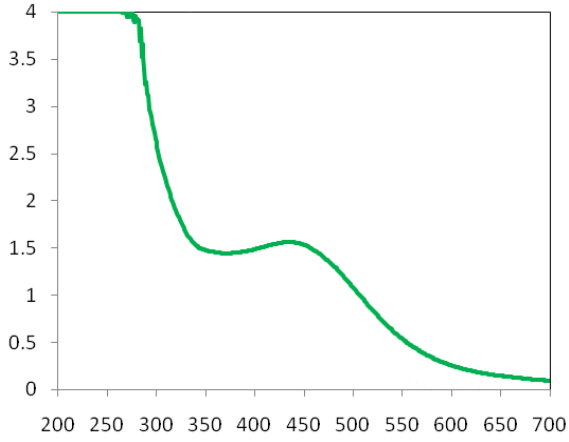

Figure 1: UV-Vis spectra of silver nanoparticles synthesized by marine bacteria

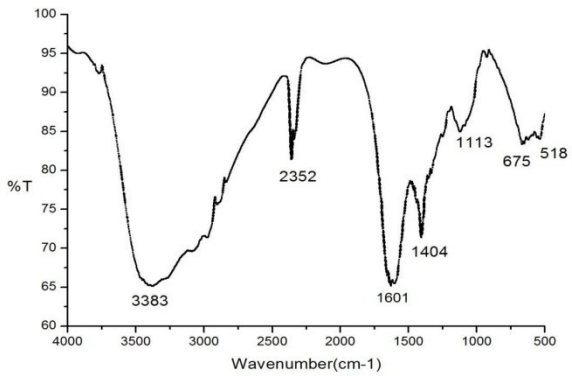

Figure 2: FTIR spectra of AgNPs

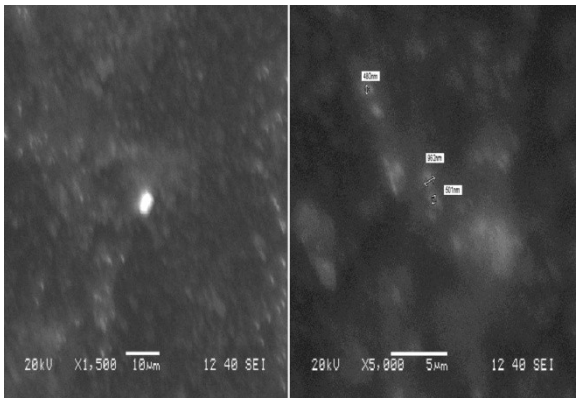

Figure 3: SEM image of AgNPs

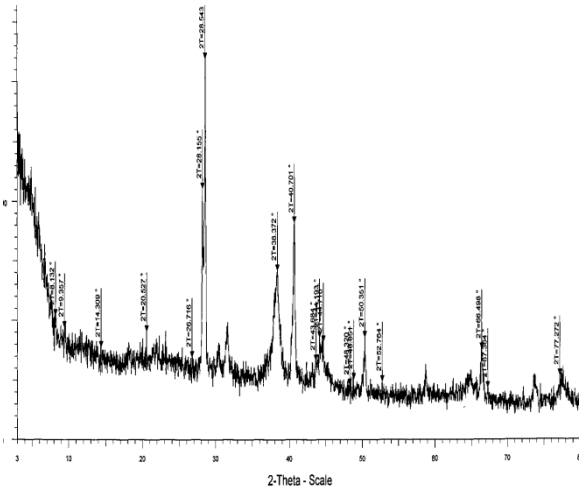

Figure 4: XRD spectra of AgNPs

Table 1: Antimicrobial activity of AgNPs

S. No	Test Microorganisms	Zone of inhibition (mm) Sample (15 & 30) µL / disc			
Bacteria		15 µL	30 µL	PC	Remarks
1.	*Aeromonas liquefaciens* B1	14	15	14	> PC
2.	*Enterococcus fecalis* B2	13	15	8	> PC
3.	*Klebsiella pneumoniae* B3	12	14	28	< *PC*
4.	*Micrococcus luteus* B4	12	16	38	< *PC*
5.	*Salmonella typhimurium* B5	15	18	0	> PC
6.	*Vibrio cholarae* B6	10	12	16	< *PC*
Fungi					
7.	*Candida albicans* F1	11	13	10	> PC
8.	*Cryptococcus* sp. F2	12	16	9	> PC
9.	*Microsporum canis F3*	11	14	9	> PC
10.	*Trichophyton rubrum* F4	12	14	7	> PC

PC - Positive Control
Bacteria – Methicillin (10mcg/disc);
Fungi – Itraconazole (10mcg/disc);
> PC – greater than positive control;
< PC – less than positive control

4. Conclusion

Silver nanoparticle was successfully synthesizing by using of marine bacteria through biological method. The Surface Plasmon Resonance (SPR) property of synthesized nanoparticle was studied by UV-Vis spectroscopy and the peak of the spectra was found to be at 421 nm. The morphological study of AgNPs using SEM suggests that the nanoparticles are spherical in shape with diameter around 450 nm to 1000 nm. The physiochemical properties of silver nanoparticles using FTIR, XRD conclude that the nanoparticle form in the process is crystalline with miller index of 011 and angle of diffraction of 2© = 32^{O}. The antibacterial activity of silver nanoparticles concludes that the silver nanoparticles shows significant antibacterial activity against *Salmonella typhimurium* whereas less activity against *Vibrio cholerae.*

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References

[1] Vignesh S, Karthikeyan B, Udayabhaskar R, Arjunan V, Muthukumar K, Ashok M, Narayana Kalkura S, Arthur James R. Antimicrobial activity of biological green synthesized silver nanoparticles. *Asian Journal of Physics*. 2014; 23(6): 1025-1030.

[2] Jain N, Bhargava A, Majumdar S, Tarafdar JC, and Panwar J. "Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus Flavus NjP08: a mechanism perspective. *Nanoscale,* 2011; 3(2): 635–641.

[3] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan, M.I. Kumar. and Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloids. *Surf. B Biointerf*, 2006; 28: 313–318.

[4] Duran N. Marcato P.D., Alves O.L., De Souza G.I.H. and Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strain. S. *Nanobiotechnology*. 2006; 3; 14.

[5] Pandiyarajan T, Udaybhaskar R, Vignesh S, Arthur James R and Karthikeyan B. Concentration dependent antimicrobial activities of CuO

nanoflakes. *Material science and engineering C*. 2013; 33(4): 2020 – 2024.

[6] Muthukumar K, Vignesh S, Dahms HU, Gokul MS, Palanichamy S, Subramanian G, Arthur James R. Antifouling assesments on biogenic nanoparticles: A filed study from polluted offshore platform. *Marine Pollution Bulletin*. 2015 http://dx.doi.org/10.1016/j.mar.bul.2015.08.033.

[7] Vignesh G, Arunachalam S, Vignesh S, Arthur James R. BSA binding and antimicrobial studies of branched polyethyleneimine - copper (II) bipyridine / phenanthroline complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2012; 96: 108 - 116.

[8] Vignesh S, Muthukumar K, James RA. Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India. *Marine Pollution Bulletin*. 2012; 64:790–800.

[9] Vignesh G, Sugumar K, Arunachalam S, Vignesh S and Arthur James R. A comparative study on the binding of single and double chain surfactant–cobalt (III) complexes with bovine serum albumin. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013; 113: 415 – 422.

[10] Vignesh G, Pradeep I, Arunachalam S, Vignesh S, Arthur James R, Arun R and Premkumar K. Biological and protein-binding studies of newly synthesized polymer–cobalt (III) complexes. *Luminescence*. 2015 DOI 10.1002/bio.2992.

[11] Vignesh G, Sugumar K, Arunachalam S, Vignesh S, Arthur James R, Arun R and Premkumar K. Studies on the synthesis, characterization, human serum albumin binding and biological activity of single chain surfactant–cobalt (III) complexes. *Luminescence*. 2015 DOI 10.1002/bio.2991.

[12] Beevi, M.H., Vignesh, S, Pandiyarajan, T, Jegatheesan, P, Arthur James, R, Giridharan, N.V., Karthikeyan, B. Synthesis and antifungal studies on CuO nanostructures. *Advanced Materials Research*. 2012; 488-489: 666 - 670.

[13] Lakshmi praba J, Arunachalam S, Riyazuddin R, Divya R, Vignesh S, Akbarsha A and Arthur James R. DNA/ RNA binding and anticancer/ antimicrobial activities of polymer-copper (II) complexes. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2013; 109:23 – 31.

[14] Vignesh S, Raja A, Arthur James R. Marine Drugs: Implication and Future Studies. *International Journal of Pharmacology*. 2011; 7: 22 – 30.

[15] Huang J Li Q Sun D Lu Y Su Y Yang X Wang H Wang Y Shao W He N Hong J and Chen C. 2007. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum campora leaf. *Nanotechnology*. 18:104-105.

[16] Zeng F, C. Hou C., Wu SZ, Liu, XX, Tong and S. N. Yu. Silver nanoparticlesdirectly formed on natural macroporous matrix and their anti-microbial activities. *Nanotechnology,* 2007; 18: 1-8.

[17] Anitha R, Karthikeyan B, Pandiyarajan P, Vignesh S, Arthur Jam. R, Vishwanathan. K and Murari. B.M, Antifungal studies on bio-compatible polymer encapsulated silver nanoparticles. *International Journal of Nanoscience* 2011; 10: 1-5.

[18] Petica A, Gavriliu S, Lungua M, Burunteaa N, Panzarub C. Colloidal silver solutions with antimicrobial properties. *Mater Sci Eng B*. 2008; 152:22-7.

[19] Furno F, Morley KS, Wong B, Sharp BL, Arnold PL, Howdle SM, Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection. *J Antimicrob Chemother*, 2004; 54:1019- 24.

[20] Hamouda T, Myc A, Donovan B, Shih A, Reuter JD, Baker Jr JR, A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. *Microbiol Res*, 2000; 156:1-7.