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Bacterial Mediated Biosynthesis of Silver Nanoparticles (Ag NPs) and Evaluation of its Antimicrobial and antilarvicidal Efficacy

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A B S T R A C T

Silver nanoparticles (AgNPs) have been known for their inhibitory and bactericidal effects in the past decades. Silver Nanoparticles were synthesized by an eco-friendly biogenic approach mediated by using the bacterial culture (SP3). The antibacterial activity of the biogenic Ag nanoparticles was carried out by the conventional Kirby-Bauer well diffusion method against the selected Gram-positive (*Staphylococcus aureus, Bacillus cereus*) and Gram-negative (*Escherichia coli, Vibrio vulnificus*) bacterial strains. Silver nanoparticles exhibited maximum antagonistic activity on *Vibrio vulnificus* (14 mm) and *Bacillus cereus* (13 mm). The MIC of Ag NPs against *Bacillus cereus* and *Vibrio vulnificus* was found to be 200 µg/ml. Larvicidal activity gradually increased with the increase in the concentration of nanoparticles, exhibiting a maximum level (88.33 %) in the presence of 100 µg/ml of Ag NPs.

Introduction

Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction of synthetic and biological materials. Nanobiotechnology signifies an economic substitute for chemical and physical methods of nanoparticles formation (Ahmad *et al.*, 2005). Green nanotechnology in the development of material synthesis is of considerable importance to expand their biological applications (Tharanya *et al.*, 2015). The word "nano" is used to indicate one billionth of a meter or 10-9 (Priyadarshini *et al.*, 2013). Nanoparticles are clusters of atoms in the size range of 1–100 nm. Nanotechnology is presently engaged as a tool to explore the darkest opportunities of medical sciences to battle diseases caused by multidrug-resistant microbes (Singh *et al.*, 2014).

Microbial drug resistance has emerged as a global health concern, as microbes acquire resistance by changing their metabolic activities and genetic structure.

Nanotechnology is anticipated to open some new aspects to combat and inhibit diseases using atomic-scale tailoring of materials (Afreen *et al.,* 2011).

Silver nanoparticles (AgNPs) have been known for its inhibitory and bactericidal effects in the past decades (Cho *et al.,* 2005). Antibacterial activity of AgNPs can be applied in biomedical applications such as reduction of infections on the burn treatment, the treatment of various infectious diseases, prevention of bacterial colonization on catheters, and elimination of microorganisms on textile fabrics as well as disinfection in water treatment (Singh *et al.,* 2014; Karthika *et al.,* 2015). Silver ions have long been known to exercise robust inhibitory and bactericidal effects as well as to retain a comprehensive range of antimicrobial activities. Ag+ hinders phosphate uptake and interchange in bacterial cells and causes efflux of amassed phosphate as well as of mannitol, succinate, glutamine, and proline (Schreurs and Rosenberg, 1982).

Antibacterial activity of AgNPs can be explored in biomedical applications such as reduction of infections on the burn treatment, the treatment of various infectious diseases, preclusion of bacterial colonization on catheters, and eradication of microbes on textile textiles as well as disinfection in aquatic treatment (Karthika *et al.,* 2015). The present study emphasizes the biomedical applications of silver nanoparticles (Ag NPs) such as antibacterial, antifungal, and larvicidal activity.

Materials and Methods

Antibacterial activity of Ag Nanoparticles

The antibacterial effect of Ag nanoparticles was examined by disc diffusion method against food-borne pathogens such as *Staphylococcus aureus, Bacillus cereus, Escherichia coli, and Vibrio vulnificus* collected from lab stock. Muller Hinton agar was prepared and poured onto the sterile petriplates. After solidification, 2 wells were cut (for test and control) and each culture was swabbed individually on the respective plates. The synthesized Ag nanoparticles were diluted with distilled water (15µg/ml) and placed onto each well and incubated for 24 h. Following incubation, the zone of inhibition against nanoparticles was observed and measured (Yokeshbabu *et al.,* 2013; Karthika *et al.,* 2015).

Assaying the minimum inhibitory concentration of Ag NPs

The minimum inhibitory concentration (MIC) of Ag NPs was determined by using the standard plate count method. The powdered form of Ag NPs was sterilized with UV radiation for 1 h and weighed under aseptic conditions. Mueller-Hinton broth containing 105 CFU/ml of bacterial cells was used as a starter culture. Various concentrations of Ag NPs (0, 50, 100, 150, and 200 µg/ml) were inoculated onto the above-mentioned starter cultures and incubated in a shaking incubator at 37°C for 24 h. Following incubation, 100 µl of the test cultures were spread onto Muller-Hinton agar and incubated at 37° C for 24 h. After incubation, the number of colonies grown on the agar was counted (Wang *et al.,* 2006; Kim *et al.,* 2011).

Growth curve Determination of bacteria exposed to different concentrations of Ag NPs

To investigate the antibacterial efficacy of Ag NPs, the growth curve of bacterial cells exposed to different concentrations of Ag NPs was taken. Mueller-Hinton broth with different concentrations of Ag NPs powder (0, 50, 100, and 150 µg/ml) was prepared, and the test bacterial culture (105 CFU/ml) was inoculated and incubated in a shaking incubator at 37° C for 24 h. The growth curve of bacterial culture was attained through repeated measures of the optical density (O.D.) at 600 nm.

Larvicidal activity of Biogenic Ag NPs against *Culex* mosquito

Larvicidal activity of Biogenic Ag NPs against *Culex* mosquito was performed. Five containers

containing 25 ml of distilled water each were treated with 10 µg, 25 µg, 50 µg, 75 µg, and 100 µg/ml of Ag NPs respectively. The sixth container without the addition of Ag NPs was simultaneously kept as control. Totally, 20 late third and fourth instar larvae of the *Culex* mosquito were introduced into the containers (ISO, 2007). Larval mortality in both treated and control containers was recorded after 24 h and the percentage of mortality was calculated using the formula

Mortality percentage = No. of larvae killed / Total No. of Larvae tested X 100

Results and Discussion

Antibacterial activity of Silver nanoparticles against Foodborne Pathogens

The antibacterial activity of the biogenic Ag nanoparticles was carried out by the conventional Kirby-Bauer well diffusion method against Gram-positive (*Staphylococcus aureus, Bacillus cereus*) and Gram-negative (*Escherichia coli*, *Vibrio vulnificus*) bacterial strains. Silver nanoparticles exhibited maximum antagonistic activity on *Vibrio vulnificus* (14 mm) and *Bacillus cereus* (13 mm) (Table 1).

Growth curves of bacterial cells treated with different concentrations of Ag NPs

The growth curves of *Bacillus cereus* and *Vibrio vulnificus* cells treated with Ag NPs indicated the suppression of the bacterial growth and reproduction of bacterial cells. In the control group (cells not treated with Ag NPs), bacterial growth increased gradually with the increase in incubation time.

However, the cells treated with Ag NPs showed a gradual decline in their growth curve with an increase in the incubation time and an increase in the concentration of NPs (Fig 1 and 2).

Minimum inhibitory concentration of Ag NPs

The minimum inhibitory concentration (MIC) was evaluated to determine the lowest concentration of the Ag NPs that could completely inhibit the viability of the *Bacillus cereus* and *Vibrio vulnificus* cells. The viability of bacterial cells gradually decreased with the increase in the concentration of Ag NPs. The MIC of Ag NPs against *Bacillus cereus* and *Vibrio vulnificus* was found to be 200 µg/ml, at which the growth of both the bacterial strains was completely inhibited. The antibacterial activities of the Ag NPs against the Gram-positive *Bacillus cereus* and Gram-negative *Vibrio vulnificus* were almost identical (Fig 3 and 4).

Antifungal activity of Silver nanoparticles against fungal Pathogens

The antifungal activity of the biogenic Ag nanoparticles was carried out by conventional Kirby-Bauer well diffusion method against the selected fungal pathogens namely Penicillium sp., *Candida albicans*, *Aspergillus niger*, and *Rhizopus* sp. Silver nanoparticles exhibited maximum antagonistic activity on *Aspergillus niger* (16 mm) and *Rhizopus* sp. (14 mm) (Table 2).

Larvicidal activity of Silver NPs against C*ulex* mosquito

Different concentration of silver NPs synthesized ranging from 10 µg/ml – 100 µg/ml was investigated for their larvicidal activity against the C*ulex* mosquito. Larvicidal activity gradually increased with the increase in the concentration of nanoparticles, exhibiting a maximum level (88.33 %) in the presence of 100 µg/ml of Ag NPs. In contrast, 10 µg/ml of the silver nanoparticles exhibited only 26.66 % of larvicidal activity. In the presence of 25 µg/ml, 50 µg/ml, and 75 µg/ml of Ag NP s the larvicidal activity was found to be 41.66 %, 53.3 %, and 71.66 % respectively (Table 3).

Table.1 Antibacterial activity of Ag NPs against the selected bacterial pathogens

S. No.	Bacterial strains	Zone of Inhibition
1.	*Staphylococcus aureus*	**10 ± 0.3 mm**
2.	*Bacillus cereus*	**13 ± 0.3 mm**
3.	*Vibrio vulnificus*	**14 ± 0.4 mm**
4.	*Escherichia coli*	**11 ± 0.5 mm**

Table.2 Antifungal activity of Ag NPs against the selected fungal pathogens

S. No.	Bacterial strains	Zone of Inhibition
1.	*Candida albicans*	**12 ± 0.4 mm**
2.	*Aspergillus niger*	**16 ± 0.3 mm**
3.	*Rhizopus sp.*	**14 ± 0.2 mm**
4.	*Penicillium sp.*	**10 ± 0.3 mm**

Table.3 Larvicidal activity of Ag NPs synthesized using SP3 bacterial culture

Conc. of Ag NP s (µg/ml)	Mortality of larva after 24 h			Average	Mortality %
	Replica N=3				
	Trial 1	Trail 2	Trail 3		
Control	0	0	0	0	**0**
10 µg/ml	5	5	6	5.33	**26.66 %**
25 µg/ml	8	8	9	8.33	**41.66 %**
50 µg/ml	10	11	11	10.66	**53.3 %**
75 µg/ml	14	14	15	14.33	**71.66 %**
100 µg/ml	**18**	**18**	**17**	**17.66**	**88.33 %**

Fig.1 Growth curve of *Vibrio vulnificus* in the presence of Ag nanoparticles

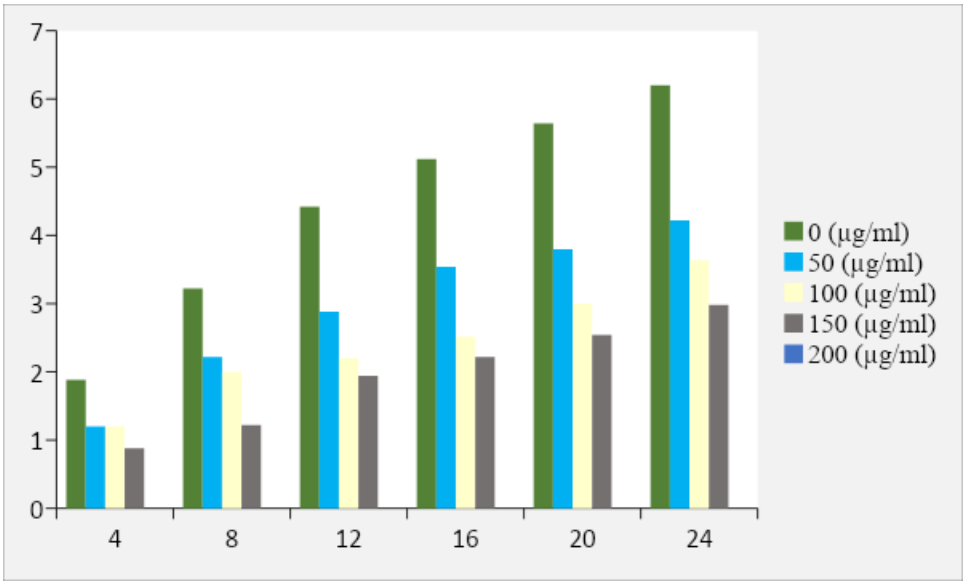


Fig.2 Growth curve of *Bacillus cereus* in the presence of Ag nanoparticles

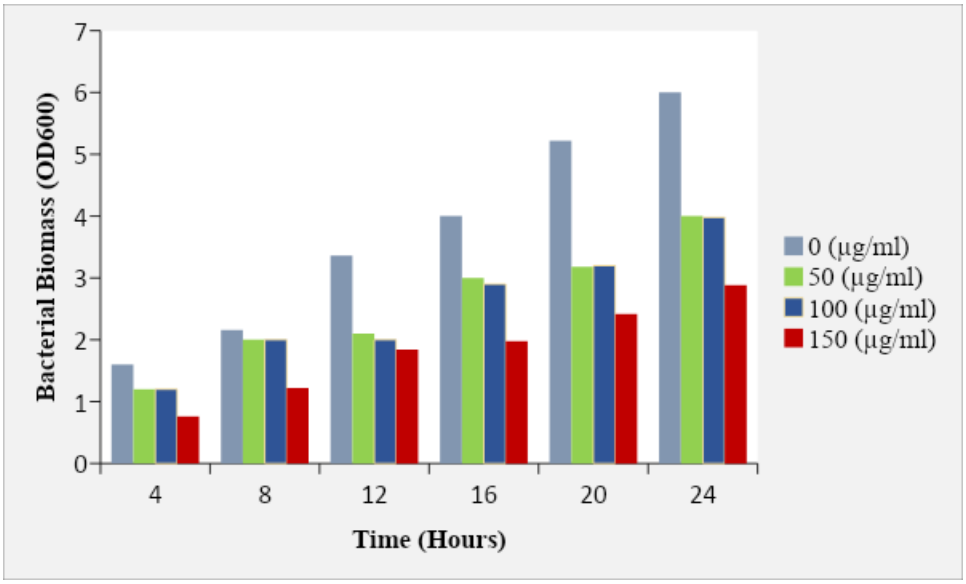


Fig.3 Minimum Inhibitory Concentration of Ag Ns on *Vibrio vulnificus*

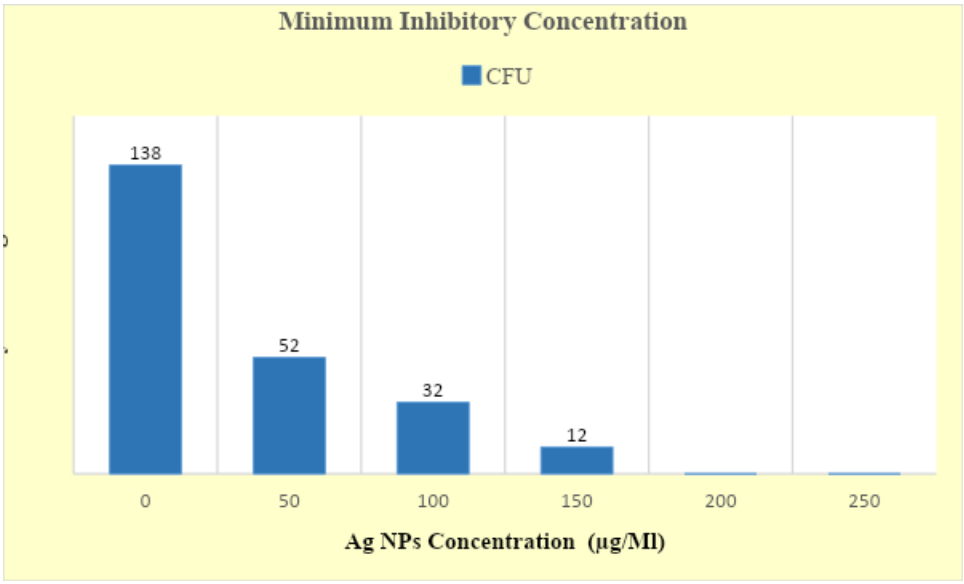
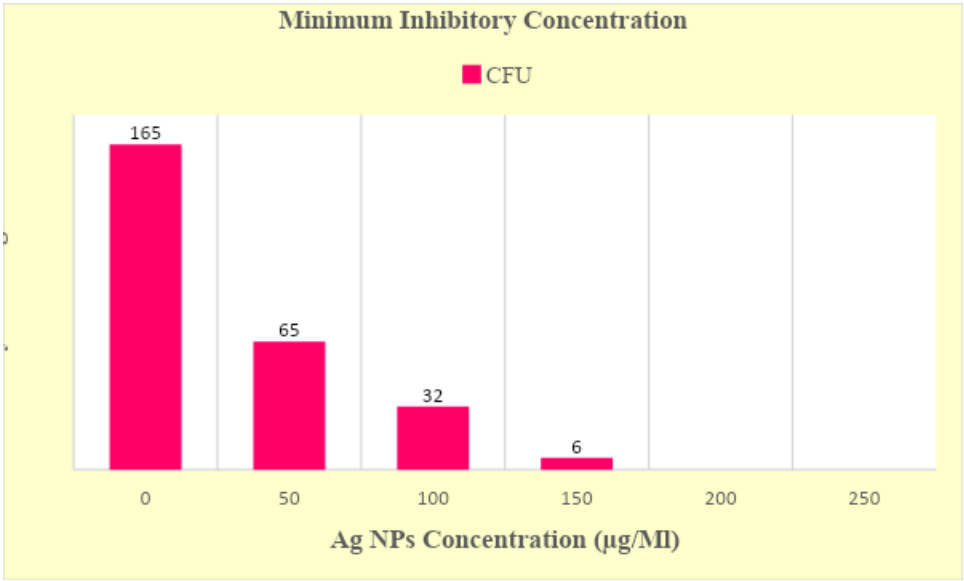


Fig.4 Minimum Inhibitory Concentration of Ag NPs on *Bacillus cereus*



Increasing bacterial resistance to antibiotics is a major problem in the field of medicine. The recent advances in research on metal nanoparticles appear to revive the use of silver nanoparticles (Ag NPs) for antimicrobial applications (Durairasu *et al.*, 2017). Ag NPs have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and viruses since ancient times (Lok *et al.*, 2006). The mechanism of inhibition by silver ions on microorganisms is partially known. According to Gupta *et al.*, (2008), it is understood that DNA loses its replication ability and cellular proteins become deactivated upon silver ion treatment. Furthermore, higher concentrations of

Ag+ ions have been shown to interact with cytoplasmic components and nucleic acids (Kim, 2007). Various mechanisms for the antibacterial activity of Ag NPs have been proposed. Firstly, Ag NPs adhere to the microbial cell membrane surface and interrupt its functions, such as permeability and respiration. The binding of the NPs depends on the interface of the accessible surface area. With a smaller particle size, a large surface area will have a stronger bactericidal effect. Secondly, Ag NPs are able to pierce the bacteria possibly by interacting with DNA and cause further damage (Gibbons and Warner, 2005). Thirdly, the silver nanoparticles release silver ions, which contribute to the bactericidal effect (Feng *et al.*, 2008; Durairasu *et al.*, 2017). The gram-negative bacteria have only a thin layer of peptidoglycan and a more complex cell wall with two cell membranes, an outer membrane, and a plasma membrane. The addition of the outer membrane of the gram-negative bacteria cells influences the permeability of many molecules (Logeswari *et al.*, 2012).

References

Afreen, V. Rathod and E. Ranganath (2011). Synthesis of monodispersed silver nanoparticles by *Rhizopus stolonifer* and its antibacterial activity against MDR strains of *Pseudomonas aeruginosa* from burnt patients. International Journal of Environmental Sciences. 1: 1583-1592.

Ahmad, A., S. Senapati, M. I. Khan, R. Kumar and M. Sastry (2005). Extra -/ intracellular, biosynthesis of gold nanoparticles by an alkalotolerant fungus, Trichothecium. J. Biomed. Nanotechnol. 1: 47-53.

Cho, K. H., J. E. Park, T. Osaka and S. G. Park (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta., 51:956–960.

Durairasu, M., V. Indra, N. Arunagirinathan, J. Hemapriya and Vijayanand, S (2017). Biosynthesis of Silver Nanoparticles using *Bacillus* Sp. and Evaluation of its Antibacterial Activity. Int. J. Curr. Microbiol. App. Sci. 6(3): 2496-2503.

Feng, Q., J.Wu, G. Q. Chen, F. Z. Cui, T. N. Kim and J. O. Kim (2008). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res., 52:662-668.

Gibbins, B. and L. Warner (2005). The role of antimicrobial silver nanotechnology. Med Device Diagnostic Indust Mag., 1: 1–2.

Gupta, P., M. Bajpai, S. K. Bajpai (2008). Investigation of antibacterial properties of silver nanoparticle-loaded poly (acrylamide-co-itaconic acid)-grafted cotton fabric. J Cotton Sci., 12: 280– 286.

Karthika, D., K. Vadakkan, R. Ashwini, A. Shyamala, J. Hemapriya and S.Vijayanand (2015). Prodigiosin mediated biosynthesis of silver nanoparticles and evaluation of its antibacterial activity. Int. J. Curr. Microbiol. Appl. Sci., 3(10): 868-874.

Kim, J. S (2007). Antibacterial activity of Ag+ ion-containing silver nanoparticles prepared using the alcohol reduction method. J Ind Eng Chem., 13: 718–722.

Kim, S. Hwan, H. S. Lee, D. S. Ryu, S. J. Choi and D. S. Lee (2011). Antibacterial Activity of Silver nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. Korean J. Microbiol. Biotechnol. 39(1): 77–85.

Logeswari, P., S. Silambarasan and J. Abraham (2012). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. J Saudi Chem Soc., 4(3): 439-447.

Lok, C. N, C. M. Ho, R. Chen, Q. Y. He, W. Y. Yu, H. Sun, P. K. Tam, J. F. Chiu and C. M. Chen (2006). Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res., 5:916–924.

Priyadarshini, S., V. Gopinath, N. M. Priyadharsshini, D. Mubarak Ali and P. Velusamy (2013). Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. Colloids Surf B Biointerfaces., 102:232–237.

Schreurs, W. J. A. and H. Rosenberg (1982). Effect of silver ions on transport and retention of phosphate by *Escherichia coli*. J Bacteriol., 152:7–13.

Singh, K., M. Panghal, S. Kadyan, U. Chaudhary and J. P. Yadav (2014). Antibacterial Activity of Synthesized Silver Nanoparticles from *Tinospora cordifolia* against Multi Drug-Resistant Strains of *Pseudomonas aeruginosa* Isolated from Burn Patients. J. Nanomed. Nanotechnol., 5: 192.

Tharanya, P., K. Vadakkan, J. Hemapriya, V. Rajeshkannan and S. Vijayanand (2015). Biogenic approach for the synthesis of Titanium dioxide nanoparticles using a halo bacterial isolate *Chromohalobacter salexigens* strain PMT-1. Int. J. Curr. Res. Aca. Rev., 3(10): 334-342.

Wang, J. X., L. X. Wen, Z. H. Wang, and J. F. Chen (2006). Immobilization of silver on hollow silica nanospheres and nanotubes and their antibacterial effects. Mater. Chem. Phys., 96: 90-97.

Yokesh Babu, M., V. J. Devi, C. M. Ramakritinan, R. Umarani, N. Nagarani, A. K. Kumaraguru (2013). Biosynthesis of silver nanoparticles from seaweed-associated marine bacterium and its antimicrobial activity against UTI pathogens. Int. J. Curr. Microbiol. Appl. Sci., 2(8): 155–168.

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