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Dabhi BK
Department of Microbiology and Biofertilizers, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Jambukiya J
Department of chemical Engineering, Dharmsinh Desai University, Nadiad, Gujarat, India

RV Vyas
Department of Microbiology and Biofertilizers, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Correspondence
Dabhi BK
Department of Microbiology and Biofertilizers, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

Anti-bacterial action of Silver Nanoparticles

Dabhi BK, Jambukiya J and RV Vyas

Abstract
The antibacterial activities of Silver nanoparticles and compare them on both Gram negative and Gram positive bacteria in this investigation. The activities of Silver nanoparticles synthesized in round bottom flask. The antimicrobial activity more in Gram (+) bacteria than Gram (-) bacteria These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

Keywords: Silver nanoparticles, Antibacterial effect

Introduction

For centuries, people have used silver for its antibacterial qualities. Nanoparticles usually have better or different qualities than the bulk material of the same element. In the case of silver nanoparticles the antibacterial effect is greatly enhanced and because of their tiny size. Nanoparticles have immense surface area relative to volume. Therefore minuscule amounts of Silver Nanoparticles can lend antimicrobial effects to hundreds of square meters of its host material. Nanomaterials are the leading requirement of the rapidly developing field of nanomedicine, bio nanotechnology. Nanoparticles are being utilized as therapeutic tools in infections, against microbes thus understanding the properties of nanoparticles and their effect on microbes is essential to clinical application [1]. Among noble metal nanoparticles, silver nanoparticles have received considerable attention owing to their attractive physicochemical properties. Ag-nanoparticles have already been tested in various field of biological science, drug delivery, water treatment and an antibacterial compound against both Gram (+) and Gram (-) bacteria by various researchers. Most of the bacteria have yet developed resistance to antibiotics and in this view in future it is need to develop a substitute for antibiotics. Ag-nanoparticles are attractive as these are non-toxic to human body at low concentration and having broad-spectrum antibacterial nature. Ag nanoparticles inhibit the bacterial growth at very low concentration than antibiotics and as of now no side effects are reported.

The nano silver when in contact with bacteria and fungus will adversely affect cellular metabolism and inhibit cell growth. The nano silver suppresses respiration, basal metabolism of electron transfer system, and transport of substrate in the microbial cell membrane. The nano silver inhibits multiplication and growth of those bacteria and fungi which cause infection, odour, itchiness and sores.

Nano silver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains [2]. Gram-negative bacteria include genera such as *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella*, and *Vibrio*. *Acinetobacter* species are associated with nosocomial infections, i.e., infections that are the result of treatment in a hospital or a healthcare service unit, but secondary to the patient's original condition. Gram-positive bacteria include many well-known genera such as *Bacillus*, *Clostridium*, *Enterococcus*, *Listeria*, *Staphylococcus*, and *Streptococcus*. Antibiotic-resistant bacteria include strains such as methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*, and *Enterococcus faecium*. Nano Silver will combine with the cell walls of pathogenic bacteria, will then directly get inside the bacteria and quickly combine with sulphydryl (-SH) of oxygenic metabolic enzyme to deactivate them, to block inhalation and metabolism and suffocate the bacteria

Materials and Methods

For the synthesis of Silver Nanoparticles, Silver Nitrate (99.8%) was purchased from Qualigens Fine Chemicals, Mumbai.

Sodium Dodecyl Sulfate (SDS) was purchased from S.D. Fine Chemicals, Mumbai. The Sodium Citrate (99%) was pursached from Merck Specialities Private Limited, Mumbai. The Lauryl Alcohol (98%) was purchased from S.D. Fine Chemicals, Mumbai. All the solutions were prepared from type-1(millipore) water.

Method for preparation of Silver Nanoparticles

Preparation of Silver Nanoparticles was carried out in 3-neck Round Bottom Flask (RBF). The RBF was dipped into heating medium. The paraffin heavy oil was used as a heating medium. The RBF was equipped with condenser to condense vapour generated from heating mixture. The Silver Nitrate (0.5 ml) was used with a different concentration (50mM, 100 mM, 200 mM). The Sodium Citrate (2%) was used as a reducing agent [4]. The Sodium Dodecly Sulfate (50 ml) was used with different concentration (5mM, 25mM, 35mM, 50mM, 100mM, 200mM). The mixture of Silver Nitrate, Sodium Citrate and Sodium Dodecyl Sulfate was put in RBF. The reaction mixture was heated at constant temperature of 90 °C for 1 hour. The reaction mixture was stirred with the help of magnetic stirrer. Mechanism of reaction could be expressed as follows:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$

The experimental set up is shown in figure below.

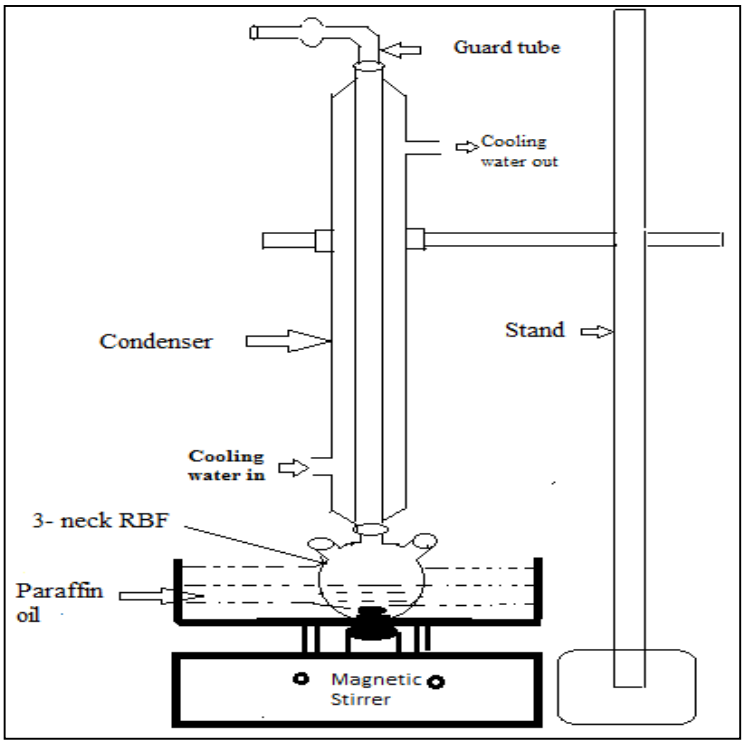


Fig 1: Experiment set-up for Silver Nanoparticle formation.

Method to determine Antibacterial activity

Prepare a sterile N-agar plate Streak a gm +ve & gm –ve bacteria into sterile N-agar plate. Spread the culture suspension of gm +ve (*B. subtilis*) & gm –ve (*E. coli*) culture on N-agar plate. Pore the test culture on a well then transfer the test culture.Incubate the plate for 48 hrs at 37° temperature & measure the zone diameter.

Results and Discussion

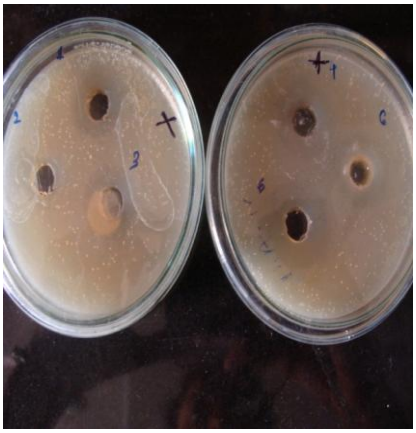
Silver Nanoparticles & Its Antibacterial Activities

Silver, a naturally occurring element, is non-toxic, hypoallergenic, does not accumulate in the body to cause harm and is considered safe for the environment. Many manufactured goods like washing machines, air conditioners and refrigerators are using linings of silver nanoparticles for their antimicrobial qualities. Sportswear, toys and baby articles, food storage containers, HEPA filters, laundry detergent etc. are made with silver nanoparticles. The medical field also is using products with silver nanoparticles, such as heart valves & other implants, medical face masks, wound dressings and bandages.

Nanomaterials are the leading in the field of nanomedicine, bio nanotechnology and in that respect nano toxicology research is gaining great importance. Silver exhibits the strong toxicity in various chemical forms to a wide range of microorganism is very well known and silver nanoparticles have recently been shown to be a promising antimicrobial material [3]. Analysis of bacterial growth showed that the toxicity of silver nano spheres is higher than that of gold nanospheres. In addition, no research has discovered any bacteria able to develop immunity to silver as they often do with antibiotics.

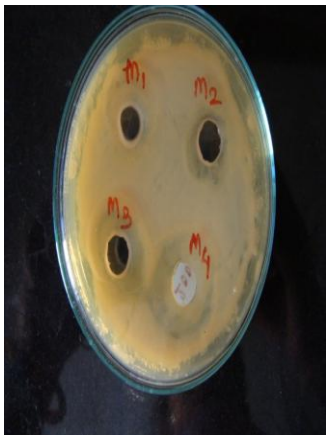
Antibacterial Properties of Silver Nanoparticles on Gram Positive (*Bacillus subtilis*) bacteria:

The images of Zone of inhibition of Gram Positive bacteria (*Bacillus subtilis*) obtained by Silver Nanoparticles produced from various SDS concentration and 100 mM $AgNO_3$, 100mM $AgNO_3$ with addition of 10 mol% Laurly Alcohol and 200 mM $AgNO_3$ is shown below.



100 mM $AgNO_3$

100mM $AgNO_3$ with addition of 10 mol% Laurly Alcohol



200 mM $AgNO_3$.

Zone of inhibition Gram Positive bacteria (*Bacillus Subtilis*) obtained by Silver Nanoparticles produced from various SDS concentration

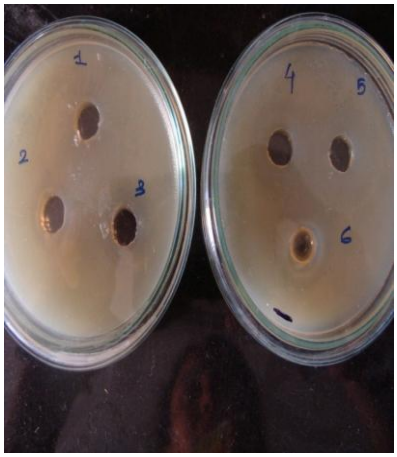
The zone diameter (zone of inhibition) of Gram Positive bacteria (*Bacillus subtilis*) obtained by Silver Nanoparticles prepared from various $AgNO_3$ and SDS concentration is shown below Table.

Table 1: The Zone of inhibition of Gram Postive bacteria (*Bacillus Subtilis*) obtained by Silver Nanoparticles produced from various SDS concentration and 100 mM $AgNO_3$, 100mM $AgNO_3$ with addition of 10 mol% Laurly Alcohol and 200 mM $AgNO_3$.

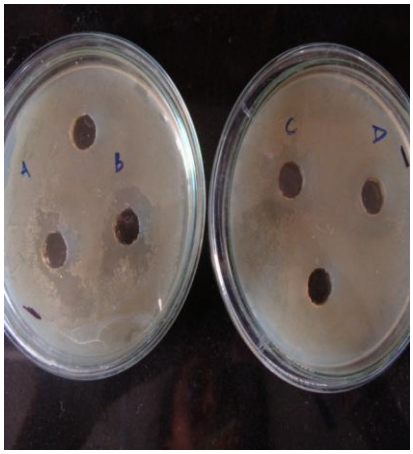
SDS concentration (mM)	Zone	diameter (cm)	
	100 mM $AgNO_3$	**100 mM $AgNO_3$ +10 mol% Laurly Alcohol**	**200 mM $AgNO_3$**
25	2.5	2.8	-
35	2.3	2.5	2.6
50	3	2.7	2.8
100	3	3	3.0
200	3.5	-	3.3

Antibacterial Properties of Silver Nanoparticles on Gram Negative (*Ps. fluroscence)* bacteria

The images of Zone of inhibition of Gram Negative bacteria (*Ps. fluroscence*) obtained by Silver Nanoparticles produced from various SDS concentration and 100 mM $AgNO_3$, 100mM $AgNO_3$ with addition of 10 mol% Laurly Alcohol and 200 mM $AgNO_3$ is shown below.



100 mM $AgNO_3$



100mM $AgNO_3$ with addition of 10 mol% Laurly Alcohol

200 mM $AgNO_3$

Zone of inhibition Gram Negative bacteria (*Ps. fluroscence*) obtained by Silver Nanoparticles produced from various SDS concentration.

The zone diameter (zone of inhibition) of Gram Negative bacteria (*Ps. fluroscence*) obtained by Silver Nanoparticles prepared from various $AgNO_3$ and SDS concentration is shown below.

Table 2: The Zone of inhibition of Gram Negative bacteria (*Ps. fluroscence*) obtained by Silver Nanoparticles produced from various SDS concentration and 100 mM $AgNO_3$, 100mM $AgNO_3$ with addition of 10 mol% Laurly Alcohol and 200 mM $AgNO_3$.

SDS concentration (mM)	Zone	Diameter (cm)	
	100 mM $AgNO_3$	100 mM $AgNO_3$ +10 mol% Laurly Alcohol	200 mM $AgNO_3$
25	2.3	2.5	-
35	2.2	2.6	2.5
50	2.7	3.0	2.8
100	2.8	2.8	3.2
200	3.0	-	3.5

The mechanism of the growth-inhibitory effects of Ag nanoparticles on microorganisms has not been well understood. One possibility is that the growth inhibition may be related to the formation of free radicals from the surface of Ag. Uncontrolled generation of free radicals can attack membrane lipids and then lead to a breakdown of membrane function. The positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles. The antimicrobial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticle, and was closely associated with the formation of pits in the cell wall of bacteria. Then, Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. In general, Ag ions from nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death. Another proposed mechanism involves the association of silver with oxygen and its reaction with sulfhydryl (–S–H) groups on the cell wall to form R–S–S–R bonds, thereby blocking respiration and causing cell death. Silver nanoparticles interact with sulfur-containing proteins of the bacterial membrane as well as with the phosphorus containing compounds like DNA to inhibit replication.

Antimicrobial effects of silver nano particles can be associated with different characteristics of the membrane structure, in order to the considerable differences between the membrane structures of Gram+ve and Gram-ve. These differences mainly rely on the peptidoglycan layer thickness, the rigidity and extended cross linking that makes the penetration of nanoparticles very difficult. The bacteria exposed to the silver nanoparticles formed holes on the bacteria cell wall. The holes were caused by a significant accumulation of silver nanoparticles. The ionic silver strongly interacts with thiol group of vital enzymes and inactivate the enzyme activity. DNA loses its replication ability once the bacteria have been treated with silver ions.

Conclusion

We have come for conclusion that Silver nanoparticles synthesized in 3 round nacked flask are showing antibacterial activities against both Gram (-) and Gram (+) bacteria but the antibacterial activity of the Silver Nanoparticles is higher for Gram Positive bacteria than Gram Negative bacteria. The antibacterial activity of Silver Nanoparticles increases with increase in precursor's concentration ($AgNO_3$) and also with increase in addition of Lauryl alcohol.

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