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POTENTIAL OF SILVER NANO PARTICLES (AGNPS) PRODUCING BACTERIUM

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ABSTRACT

The present study of trustworthy processes for the synthesis of Nanosized materials is of countless importance in the field of nanotechnology. Biosynthesis of Silver Nanoparticles (AgNPs) using bacteria has arriving weighty interest because of their potential to synthesize nanoparticles. In the current study, synthesis of silver nanoparticles by a bacterial strain (DA-12) isolated from soil is reported. The bacterium was isolated, screened and characterized by morphological, biochemical and 16S rRNA analyses. Molecular identification of the isolate was done which showed a strain is *Bacillus shackletonii DA-12*. When treating the isolated bacteria with 1mm Silver nitrate ($AgNO_3$), it was found to have the proficiency to form silver nanoparticles at 37^0 C within 24 hours of incubation. This was confirmed by the visual observation and FT-IR analysis. Therefore, the current study is a much of an effective synthesis of silver nanoparticle by the present Bacillus strain. Silver nanoparticles were also tested against antibacterial potential of some human pathogens.

KEYWORDS: *Bacillus shackletonii DA-12*.

INTRODUCTION

Silver Nanoparticles are evolving as a new generation of antibacterial agent, which has been used in medicinal applications, hygiene and antibacterial water filter. Silver Nanoparticles have proved to be the most effective as it has good antibacterial efficiency against bacteria, other eukaryotic micro-organisms and viruses. Microorganisms affecting the mobility and reactivity of metals can be used for remediation and detoxification. Many organisms both eukaryotes and prokaryotes are known to produce many inorganic materials either extracellular or intracellular (Ahmad *et al*., 2003).Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in Biomedical, Optical and Electronic fields. Nanotechnology ("nanotech") is manipulation of matter on an atomic, molecular, and supra molecular scale. The synthesis of silver Nanomaterials/Nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. Based on their enormous biotechnological applications, microorganism such as bacteria, fungi, and yeast are now regarded as possible eco-friendly “Nano- Factories”.

MATERIALS AND METHODS

1. Chemicals and Reagents

Analytical grade silver nitrate ($AgNO_3$) used in the project was procured from laboratory.

2. Collection of soil sample

The agricultural soil sample was collected from the root zone region of the different plants from Dr. D. Y. Patil Arts, Commerce & Science College, Pimpri, Pune Area.

3. Isolation and Characterizations of the Bacteria

The serially diluted soil sample is streaked on nutrient agar medium and colony characterization is done.

4. Detection of Silver Nanoparticle production by bacteria using $AgNO_3$

Biosynthesis of Silver Nanoparticles was investigated by reducing sliver nitrate with the isolate at room temperature using $AgNO_3$.

5. Identification of Silver Nanoparticle producing bacteria

The Silver Nanoparticle producing bacterium is identified on the basis of its colony characterizations, biochemical characterizations and 16s rRNA Analysis.

6. Confirmation of Silver Nanoparticle production by FT-IR Analysis

The synthesized nanoparticles characterized by the FT-IR analysis.

7. *In Vitro* Evaluation of Antimicrobial Activity of Silver Nanoparticles

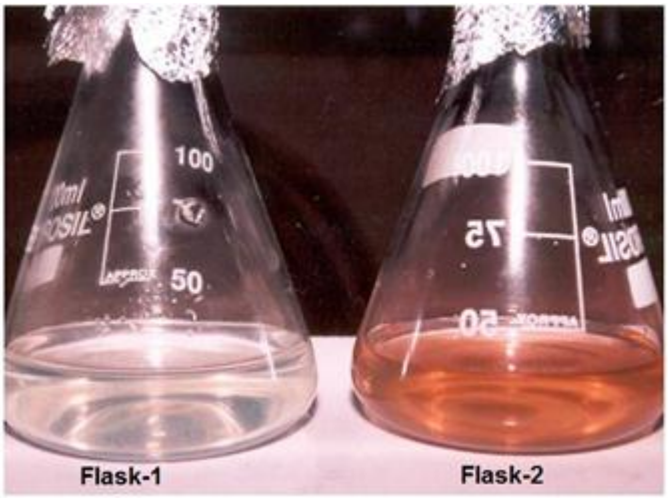
Invitro profiling of antibacterial activity of the Silver Nanoparticles was studied by plate assay, against the isolated bacterial strains *Viz. Escherichia Coli*, *Klebsiella spp., and Staphylococcus aureus*.

RESULTS

In all total 05 organisms were obtained. Out of these, *DA-12* was efficient Nanoparticles producing organism. The potential strain was phylogenetically identified as *Bacillus shackletonii DA-12* on the basis of its colony characterizations (Table 1), biochemicals characterizations (Table 2) and 16s rRNA gene sequence (Figure 1).

Detection of Silver Nanoparticle production by bacteria using $AgNO_3$

Silver Nanoparticles were biologically synthesized by the cultural supernatant. The appearance and color change from yellow to brown in the flask indicated that the formation of Silver Nanoparticles, whereas no color change was observed in the cultural supernatant without silver nitrate (Photograph 1).



Conical flask containing the filter of the *Bacillus shackletonii DA-12* in aqueous solution of $AgNO_3$ at the beginning of the reaction (flask-1) and after 2 days of reaction (flask-2), silver nanoparticle obtained in flask-2.

Photograph 1: Detection of biosynthesis of Silver Nanoparticles *Bacillus shackletonii DA-12.* Identification of Silver Nanoparticle producing bacteria.

Table 1: Colony Characterizations of the isolates on nutrient agar medium incubated at room temperature after 24 hours.

Sr. No.	Culture Code	Size in mm	Shape	Margin	Elevation	Consistency	Opacity	Colour
1.	DA-1	1	Circular	Entire	Slightly convex	Moist	Transparent	Creamy
2.	DA-2	2	Irregular	Irregular	Flat	Moist	Semi Transparent	Creamy White
3.	DA-3	1	Circular	Regular	Raised	Moist	Opaque	White
4.	DA-4	1	Circular	Slightly irregular	Raised	Moist	Opaque	Yellowish White
5.	DA-12	2	Irregular	Entire	Flat	Moist	Opaque	Creamy White

Gram nature	Motility
Gram positive Short rods	Highly motile
Gram positive Short rods	Motile
Gram positive Short rods	Motile
Gram positive Short rods	Motile
Gram positive Short rods	Motile

Table 2: Biochemical Characterizations of the isolates.

Name of Biochemical Tests	Results				
	DA--1	DA--2	DA--3	DA--4	DA--12
Fermentation of-					
Glucose	A, G	A	A, G	A	A, G
Fructose	A, G	A	A	A, G	A
Lactose	A	A, G	A	A	A
Maltose	A, G	A	A	A	A, G
Sucrose	A	A	A, G	A	A
Hydrolysis of-					
Starch	+	+	+	+	+
Casein	+	+	-	+	-
Nitrate Reduction	+	+	+	+	+
Enzyme Activity-					
Amylase	+	+	+	+	+
Catalase	+	+	+	+	+

Where, **A, G** = Production of acid and gas

A = Production of acid only

+ = Positive test

- = Negative test

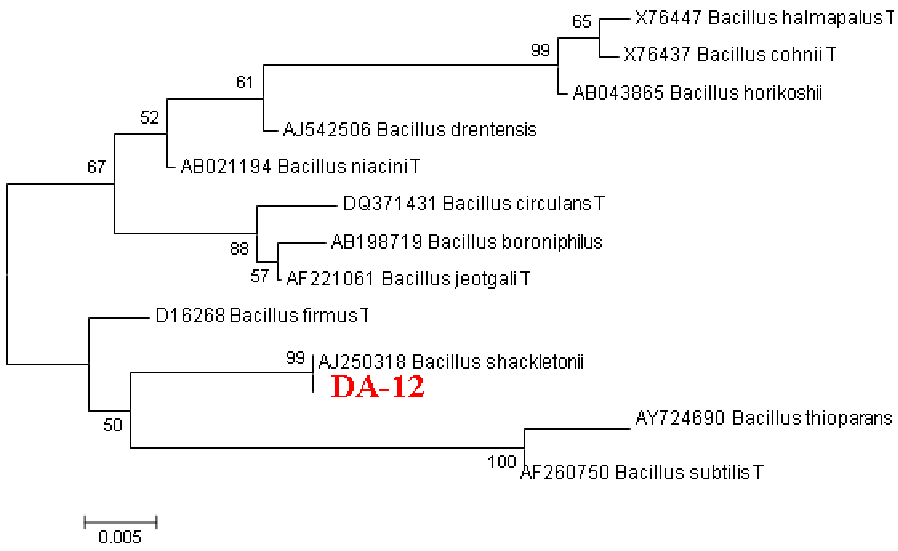


Figure 1: Phylogenetic Identification of the isolate DP-12.

Confirmation of Synthesis of Silver Nanoparticles by FTIR Analysis

The synthesized nanoparticles characterized by the FTIR analysis (**Figure 2 and 3**), revealed the presence of Silver Nanoparticles by exhibiting the bending vibration between 1640-1540 cm^{-1} wave number. A band at 1640 -1540 cm^{-1} indicates presence of AgNPs.

Fourier Transform Infrared (FT-IR) Spectrum recorded with synthesized Silver Nanoparticles.

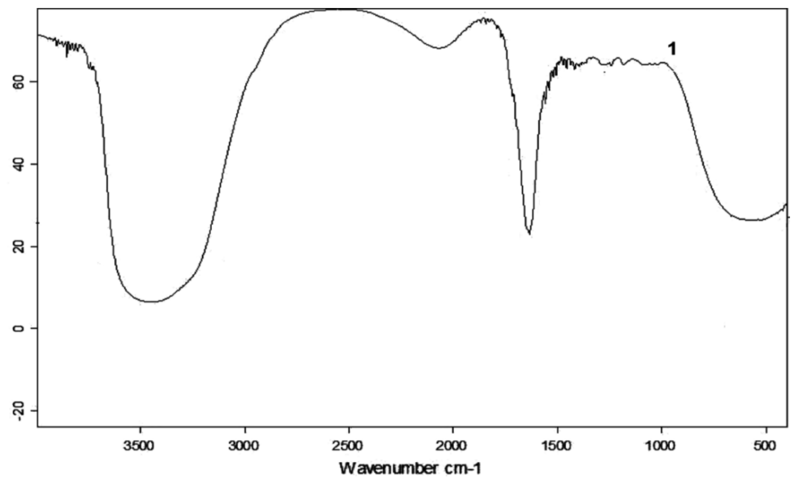


Figure 2: Standard FTIR spectrum of AgNPs.

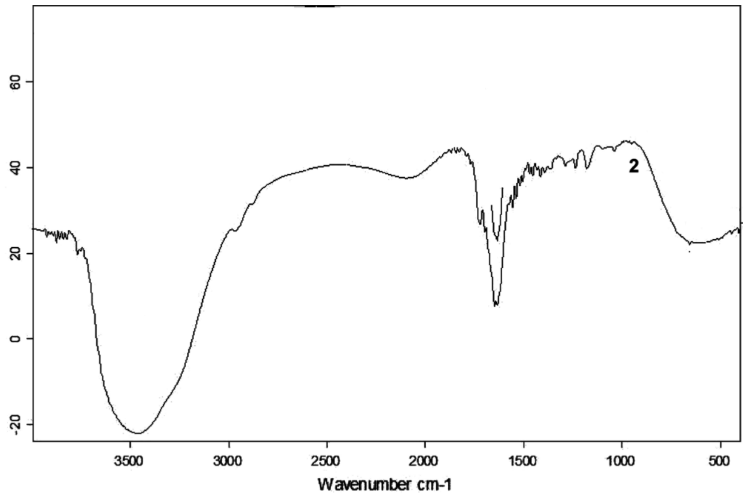


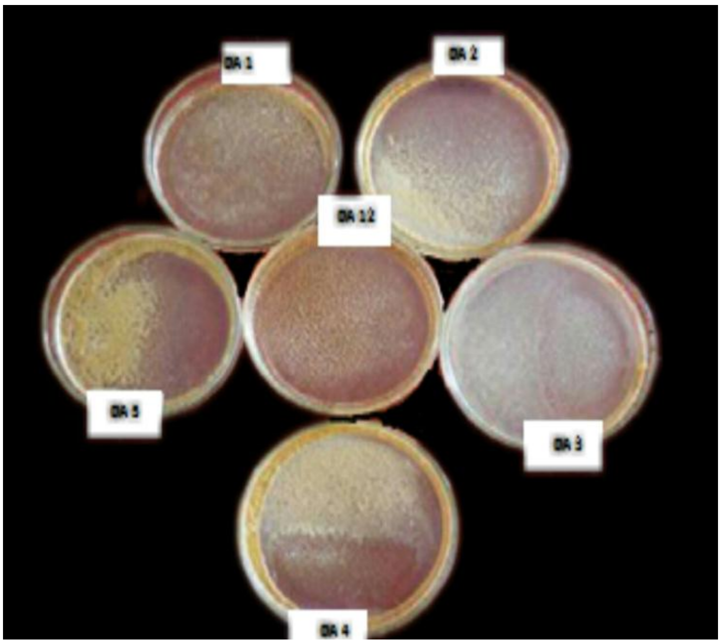
Figure 3: FTIR spectrum of Test Sample.

In Vitro Evaluation of Antimicrobial Activity of Silver Nanoparticles

In vitro evaluation of antibacterial activity of Silver Nanoparticles was also studied against *Escherichia coli, Klebsiella sp.* and *Staphylococcus aureus* (**Photographs 2**).

The biological synthesized AgNPs inhibited different pathogenic microorganisms. The resulting zones of inhibition formed were mainly due to the destabilization of the outer membrane, collapse of the plasma membrane and depletion of intracellular ATP by the Silver Nanoparticles. *Escherichia Coli, Klebsiella spp., Staphylococcus aureus* were effectively inhibited by the Silver Nanoparticles.

Photograph 2: _In Vitro_ Evaluation of Antimicrobial Activity of Silver Nanoparticles



Crystals of Silver Nanoparticles

CONCLUSIONS

1. The Silver Nanoparticles causes more damages to the Gram Positive and Gram negative cells.
2. Silver nanoparticles used in this work exhibit a broad size distribution and morphologies with highly reactive facets.
3. We have identified that silver nanoparticles act primarily in three ways against Gram-negative bacteria:[1] nanoparticles mainly in the range of 1–10 nm attach to the surface of the cell membrane and drastically disturb its proper function, like permeability and respiration;[2] they are able to penetrate inside the bacteria and cause further damage by possibly interacting with sulfur- and phosphorus-containing compounds such as DNA;[3] nanoparticles release silver ions, which will have an additional contribution to the bactericidal effect of the silver nanoparticles.
4. We have found it to be very useful in the study of bactericidal effects of silver particles, and it can be extended to other related research.

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