Antimicrobial activity of biogenic silver nanoparticles synthesized by *Macrophomina phaseolina*

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Original Article

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Abstract

In the present study we investigated the anti-microbial effect of silver nanoparticles on *Bacillus subtilis*, *Klebsiella pneumoniae* *E.coli*, and *Pseudomonas aeruginosa*. Investigating method by using agar well diffusion technique in which the comparison between standard drug Streptomycin and synthesized silver nanoparticles produced by *Macrophomina phaseolina*. The present study indicates polydisperse spherical and 1-15 nm sized silver nanoparticles have considerable anti-microbial activity comparison with the standard anti-microbial drug. The zone of inhibition seems in both gram positive and gram negative bacterial culture. The result shows that the anti-microbial activity against different pathogen so it deserving further investigation for clinical application.

Key words: Anti-microbial effect, Silver nanoparticles, *Bacillus subtilis*, *Klebsiella pneumoniae* *E.coli*, *Pseudomonas aeruginosa*, *Macrophomina phaseolina*.

1. INTRODUCTION:

Public health protection is the major problem and need has been crated due to the many diseases resistant microorganism. These microorganism exhibit strong anti-microbial activities of anti-biotics. Many researchers have tried to develop new, effective, anti-microbial agent which are free from disease resistant and the prevention of deleterious infections. The antibacterial effect of silver salt has been noticed since antiquity [1]. It is well known that silver ions and silver base compounds are highly toxic to micro-organism which showing strong biocidal effect on many bacteria [2]. Thus silver ions used as anti-bacterial components mainly in the formulation of dental resin composites [3,4] and in coating of medical devices [5,6,7]. Silver nanoparticles have anti-bacterial activity against *Salmonella typhi*, *Streptococcus epidermis*, *Staphylococcus sps.*, *Proteus vulgar* [8]. Biological synthesized silver nanoparticles when incorporated in materials, they providing sterile property [9]. The gold ion and gold based components also effect on Gram positive and Gram negative bacteria [10]. Anti-microbial agents are play and important protective role in the field of biology, silver nanoparticles have consequential application. Due to their good antimicrobial activity silver nanoparticles used in water purification techniques [11], creams and ointment is used to prevent infection of burns and open wounds [12]. Chemical, physical and biological methods are used for the synthesis of metal nanoparticles [13]. Chemical and Physical methods are widely applied because the large quantity of nanoparticles are produced within a short period but they require toxic, environmentally damaging chemicals which are not safe. At that time novel biological method has been emerged out which are non-toxic, safe and ecofriendly for the production of nanoparticles. These biogenic nanoparticles are water soluble and have anti-microbial activities against gram positive and gram negative bacteria which are very important for many applications. Thus the aim of study is to check the antibacterial activity of biogenic silver nanoparticles. The present investigation worked out with the isolation, synthesis and characterization of silver nanoparticles from *Macrophomina phaseolina* and their antibacterial activity was used to produce novel drug to overcome drug resistance ability of bacteria.

2. MATERIAL AND METHODS:

2.1 Collection of Materials: *M. phaseolina* was isolated from soil and maintained on potato dextrose agar (PDA) medium at 28°C. The isolated fungus was identified using morphological characteristics. The four (*B.subtilis*, *E.coli*, *K.pneumoniae* and *P.aeruginosa*) kind of bacteria’s were tested which are obtained from National Chemical Laboratory (NCL) Pune.

2.2 Biomass Preparation: To prepare biomass for biosynthesis of *M.phaseolina* was...
grown in glucose nutrient broth medium (GNB). The flask was inoculated with shaker (120 rpm) for 4 days. The biomass was harvested by filtration through whatman filter paper no.1 and then washed with distilled water to remove any components of the medium. 15 gm. biomass was placed in individual flask containing 100 ml distilled water. The flask was incubated 72 hr. The biomass was filtered and the crude cell filtrate was collected for experiment.

2.3 Biosynthesis of silver nanoparticles:
Silver nanoparticles were synthesized using 15 ml of crude cell filtrate mix with 15 ml of 1mM AgNO3 solution in 250 ml Erlenmeyer flask incubated at 28°C in dark for 24 hours. AgNO3 was used as control.

2.4 Characterization of silver nano-particles:
The reduction of silver ions was confirmed by qualitative testing of supernatant by UV-visible spectrophotometer. 1 ml of sample supernatant was withdrawn after 24 hours and absorbance was measured by using UV-visible spectrophotometer between 300 to 600 nm. TEM technique was used for the study of the detailed structure of nanoparticle i.e. size and shape. Characterization of silver nanoparticle was done by TEM Morgagni268D AIIMS, New Delhi. It is the confirmationary test of presence of silver nanoparticles.

2.5 Anti-bacterial Analysis:
The anti-bacterial activities of isolated fungal silver based nanoparticle solution were tested by standard agar well diffusion method. The test bacteria’s B.subtilis, E.coli, K .pneumoniae and P.aeruginosa were included in the present study. 0.9 % saline solution was used for bacterial suspension which spread on nutrient agar with the help of cotton swab. The AgNPs solution was loaded on marked wells with the help of micropipette and plate was incubated at 27°C for 24 hours for observing inhibition rate.

3. RESULT AND DISCUSSION:
3.1 Visual Analysis of Silver Nano-particles:
It is the preliminary test of appearance of dark brown colour solution, after the addition of silver nitrate solution. In Figure-1(A) shows clearly pale yellow of cell free extract of Macrophomina phaseolina before immersion in AgNO3. In Figure-1(B) shows different shade of brown colour changes periodically after the exposure to 1 mM aqueous solution of AgNO3 for 72 hours. It is observe that the colour of silver nanoparticles periodically change from pale yellow to different shade of brown (Table1). It clearly indicates the synthesis of silver nanoparticles.

<table>
<thead>
<tr>
<th>Time</th>
<th>Colour of AgNPs</th>
</tr>
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<tbody>
<tr>
<td>1 hr.</td>
<td>-</td>
</tr>
<tr>
<td>12 hr.</td>
<td>+</td>
</tr>
<tr>
<td>24 hr.</td>
<td>+</td>
</tr>
<tr>
<td>36 hr.</td>
<td>++</td>
</tr>
<tr>
<td>48 hr.</td>
<td>++</td>
</tr>
<tr>
<td>60 hr.</td>
<td>+++</td>
</tr>
<tr>
<td>72 hr.</td>
<td>+++</td>
</tr>
</tbody>
</table>

3.2 Photometric Analysis of Silver Nano-particles: In photometric method the UV-Visible spectrophotometry was used. The UV visible spectra of silver nanoparticles recorded a strong broad peak at 450 nm after the different time interval like12, 24, and 36hr respectively (Fig-2).The spectra clearly show that as the time interval increases the colour intensity of silver nanoparticles also increases, indicating the formation of increased number of silver nanoparticles in brown with 1mM silver nitrate in the solution.

3.3 TEM Analysis of Silver Nanoparticles:
After reduction the AgNPs were analyzed by TEM. The samples were prepared by placing a drop of AgNPs solution on a copper grid of TEM. Copper grid is the platform of sticking the nanoparticles. After 2 minutes copper grid allows to dry and then observe the images under TEM. Figure-3 shows typical TEM images of AgNPs synthesized by M. phaseolina. AgNPs are polydisperse spherical in shape and size range in between 1-50 nm

3.4 Anti-microbial activity:
Silver nitrate has powerful and natural anti-biotic and anti-bacterial agents which are exhibited anti-bacterial properties against a gram positive and gram negative bacteria. Anti-bacterialactivities of the synthesized silver nanoparticles have been investigated against B.subtilis, E.coli, K. pneumoniae and P.aeruginosa. M. phaseolina synthesized AgNPs shows very strong inhibitory action against Bacillus subtilis (21 mm zone of inhibition) while as streptomycin
Fig-3 TEM micrograph of silver nanoparticles synthesized by M. phseolina.

Fig-4 Antibacterial activity of silver nanoparticles against (A) B.subtilis (B) E. coli (C) K. pneumoniae (D) P. aeruginosa with (1) Silver nanoparticles (2) Streptomycin. shows less (14 mm) zone of inhibiton. Gram negative bacterias like E. Coli (13 mm zone of inhibition), K. pneumoniae (14 mm zone of inhibition), P. aeruginosa (19 mm zone of inhibition) shows less zone of inhibition against streptomycin drug (Fig-4).

4. CONCLUSION: Anti-bacterial activity of Macrophomina phseolina synthesized silver nanoparticles against Bacillus subtilis, E.coli, K.pneumoniae and P.aeruginosa were reported. Silver nanoparticles shows higher toxicity in gram positive bacteria comparision with gram negative bacteria. The bio-synthesized nano silver are further employed for various purposes like medical, agricultural, hospital etc. The characterization of silver nanoparticles was made by UV visible spectroscopy, which shows broad peak at 450 nm. The TEM shown the synthesis of polydisperse spherical AgNPs in the range 1-50 nm with no agglomeration. Production of AgNPs is ecofriendly, cost effective, safe and without toxic chemical process. Fungi are easily available for large scale production. Fungi synthesized nanoparticles have good stability even after prolonged storage. There is not much agglomeration of AgNPs even after preserving solution.

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5. REFERENCES:


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