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Antimicrobial activity and MIC of microbial biosynthesized silver nanoparticles

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Abstract:

Since silver nanoparticles (AgNPs) have antiplasmodial, antibacterial, and antifungal properties, they offer promise as a therapeutic tool for the treatment of various illnesses and parasites. AgNO3 solution was employed in conjunction with cell-free supernatant from Levilactobacillus brevis cultivated on MRS medium to synthesize silver nanoparticles. Using the well diffusion method, samples' antimicrobial activity was evaluated in vitro against a variety of pathogens, such as filamentous fungi Aspergillus brasiliensis ATCC 16404, unicellular fungi Candida albicans ATCC 10231, and Gram positive bacteria Bacillus spizizenii ATCC 6633, Staphylococcus aureus ATCC 6538, and Gram negative bacteria Pseudomonas aeruginosa ATCC 9027 and Escherichia coli ATCC 8739. Furthermore, the minimum inhibitory concentration (MIC) of AgNPs against Staphylococcus aureus ATCC 6538 was determined using a range of concentrations (0, 5, 10, 15, 20, 25, 50, 75, and 100 µg). The growth of both Gram positive and Gram negative bacteria was inhibited by 100 µg AgNPs. It has been observed that filamentous and unicellular fungi are resistant to AgNPs. The MIC for 20 µg of AgNPs was found from the results. Given these encouraging therapeutic properties, the use of AgNPs in the control of infectious diseases is warranted.

Keywords: *Fungi, Bacteria, Silver nanoparticles.*

1. Introduction

The general term "nanobiotechnology" describes a branch of study centered on the manipulation of matter at the atomic and molecular level [1]. The scientific community is very interested in the synthesis of silver nanoparticles due to their extensive range of uses. Silver nanoparticles are also frequently employed in the detection and treatment of cancer [2,3]. Before the microbial synthesis, the nanoparticles were made using a range of costly, potentially environmentally harmful, chemical and physical processes that included the use of dangerous and poisonous compounds that pose a risk to human health. One significant area of nanotechnology is developing around the creation of experimental procedures for the synthesis of nanoparticles that are inspired by biology [4].

The microbial production of nanoparticles has numerous significant uses in medication delivery systems, filters, and biolabeling sensors. In addition to their novel physico-chemical characteristics, nanoparticles have antibacterial activity [5].

Since silver nanoparticles (AgNPs) have antiplasmodial, antibacterial, and antifungal properties, they offer promise as a therapeutic tool for the treatment of many illnesses and parasites [6]. As a result, the green synthesis of nanoparticles is a rapidly developing field of study that enables the biosynthesis of environmentally friendly, affordable, safe, and effective medications, frequently through the use of natural plant materials [7, 8].

Numerous illnesses that affect both humans and animals are brought on by bacteria, which frequently cause infections to prolong and postpone wound healing. Creating a fresh approach to investigating new antibiotic compounds is crucial in the fight against bacteria strains that are resistant to several drugs. In human systems, metal nanoparticles (NPs), particularly silver, are employed as antibacterial agents to control a range of bacteria and prevent infections from spreading to cuts, burns, and wounds [9–11]. In this study, investigation of microbial synthesis method employing Levilactobacillus brevis to generate AgNPs at room temperature was performed. Additionally, testing with six bacterial pathogens was done to assess the antimicrobial activity of AgNPs.

2. Materials and Methods:

2.1. Microorganism and cultivation growth conditions:

The Levilactobacillus brevis used in this study was obtained from pervious study. MRS broth [12] was used for growth at pH 6.2, 30°C for 24h. Cultures were stored in a refrigerator at 4°C.

2.2. Silver nanoparticles biosynthesis

Levilactobacillus brevis was grown for 24 h on MRS medium. The bacterial biomass was then separated using centrifugation, which was run for 10 minutes at 5000 rpm. The cell-free supernatant was mixed with 1 mM AgNO3 solution and left to incubate for the entire night at room temperature and in the dark. The production of AgNPs was ascertained by analyzing color changes in the mixture [8].

2.3. Antimicrobial Activity Assay

The antimicrobial activity of the samples was tested in vitro against a number of pathogens using the well diffusion method [13, 14]. These pathogens included unicellular fungi like Candida albicans ATCC 10231, filamentous fungi like Aspergillus brasiliensis ATCC 16404, and Gram positive bacteria like Bacillus spizizenii ATCC 6633 and Staphylococcus aureus ATCC 6538, and Gram negative bacteria like Pseudomonas aeruginosa ATCC 9027 and Escherichia coli ATCC 8739. Fresh overnight cultures of the bacteria were inoculated using Mueller-Hinton liquid medium, shaken, and incubated at 30°C. After incubation, 1 mL of each sample was swabbed onto Mueller Hinton agar plates for bacteria and fungi, and Sabouraud Dextrose Agar plates for Candida. After obtaining 106 CFU/mL, the optical density was set to 0.5 MacFarlane recommendations, and it was let dry for five minutes. Samples were added to 5.0 mm diameter wells, which were then UV-sterilized for two hours. After that, wells on the agar were filled using sterile forceps. The petri dishes were placed in the refrigerator for one hour in order to give the antibacterial ingredient time to spread evenly. Fungzol was the standard antifungal agent and streptomycin (S10) the usual antibacterial agent. Inhibition zones were measured following a 24-h incubation period for bacteria and a 3-day incubation period for fungi at 37°C on the plates, in compliance with CLSI recommendations [15]. In parallel, sterile borer holes were punched into the wells, and varying amounts of nanoparticles (0 to 100 µg) were introduced. The plate was incubated at 37 °C for 24 h, and the diameter of the inhibition zone was measured [16]. The experimental data were recorded from the mean of three replicates.

3. Results and Discussion

3.1. Antimicrobial Activity of AgNPs

The 100 µg of AgNPs inhibit the growth of Gram positive bacteria, Bacillus spizizenii ATCC 6633, Gram negative bacteria, Pseudomonas aeruginosa ATCC 9027, and Escherichia coli ATCC 8739, and an inhibition zone was measured. AgNP resistance was observed in filamentous fungi, Aspergillus brasiliensis ATCC 16404, and unicellular fungi,

Candida albicans ATCC 10231 (Figure 1). Furthermore, the minimum inhibitory concentration (MIC) of AgNPs against Staphylococcus aureus ATCC 6538 was determined using a range of concentrations (0, 5, 10, 15, 20, 25, 50, 75, and 100 µg). The inhibitory zone diameter measured at 18 mm for 20 µg of AgNPs was found to be MIC, as shown in figure 2. Ag ions with metabolites cling to cell membranes, changing the lipid bilayer and causing damage and eventual cell death [17]. This is why cells become more permeable. When using relatively smaller nanoparticles, this potent antibacterial activity seems to be more noticeable.

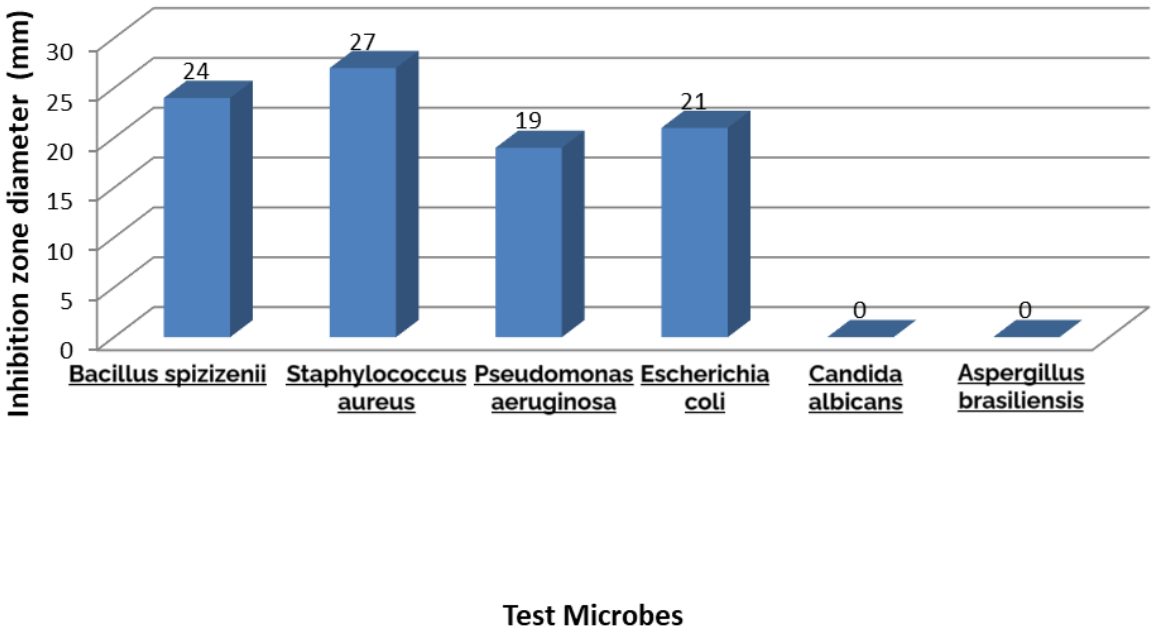

Figure 1: Antimicrobial assay of synthesized silver nanoparticles against Bacillus spizizenii ATCC 6633, Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027 , Escherichia coli ATCC 8739, Candida albicans ATCC 10231 and Aspergillus brasiliensis ATCC 16404.

It was shown that AgNPs adhered to and accumulated on the cell surface of Gram-negative bacteria in particular. AgNPs have the ability to completely penetrate prions, which are water-filled channels found in the outer layer of Gram-negative bacteria. The passive translocation of hydrophilic molecules with varying sizes and charges across membranes is mostly facilitated by porins. Gram-negative bacteria exhibited a more marked activity of

AgNPs than Gram-positive bacteria; this is likely due to the latter's stronger cell wall, which allows silver ions to flow into the cytoplasm [18]. It's also possible that lipopolysaccharides make Gram-negative bacteria's cell walls more morphologically intact, which makes the bacterium more vulnerable to silver nanoparticles since the lipopolysaccharides' negative charge encourages AgNP adherence [19]. Due to the electrostatic interaction between positively charged silver ions and the negatively charged surface of the cell membrane caused by the phosphate, carboxyl, and amino groups, the metabolite present in the supernatant and the silver ions attached to the bacterial cell wall work effectively to pass through the cell wall, modifying its structure and permeability in the process. Membrane degradation then results from the loss of the proton motive force [20, 21]. AgNPs may also function as a more ingenious means of delivering Ag+ to bacterial cells, where its proton motive force lowers the ambient pH and increases the release of Ag+ [22]. Combining the available data, the theory is that when silver nanoparticles interact with bacteria, they produce free radicals that rupture the cell membrane and cause it to become porous [23].

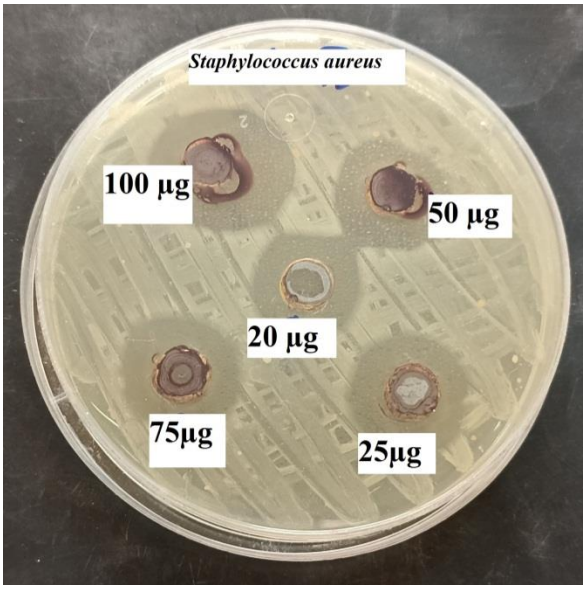

Figure 2: Determination of AgNPs MIC against Staphylococcus aureus ATCC 6538.

4. Conclusions

In summary, this study showed that the supernatant for the bacterially mediated generation of AgNPs may be successfully generated. AgNPs that were biosynthesized showed promise as bactericidal agents against infections in humans. Given these encouraging therapeutic properties, the use of AgNPs in the control of infectious diseases is warranted. It's also necessary to elucidate the mechanism underlying the biosynthesized AgNPs' antibacterial inhibitory actions.

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