Biosynthesis and Characterization of Silver Nanoparticles from *Pantoea agglomerans* and Some of Their Antibacterial Activities

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**ABSTRACT**

Recently, the biosynthesis of nanoparticles from bacteria have attracted attention, this study has been made for biosynthesize and characterizes silver nanoparticles (AgNPs) from local clinical isolate *Pantoea agglomerans*. The ability of those particles to inhibit the virulence factors biofilm and hemolysin produced by some local clinical multidrug-resistant human pathogens including Acinetobacter *haemolyticus*, Escherichia *coli*, Serratia *marcescens* and *Staphylococcus aureus* were investigated by treating all of the test isolates with sub-MIC (16 mg/ml) AgNPs. The AgNPs produced were characterized using Atomic Force Microscopy (AFM). *Pantoea agglomerans* were found to have the ability to synthesize AgNPs at room temperature within 24hrs and were spherical in shape as depicted by AFM. The AgNPs produced exhibited a potential antibiofilm and hemolysin inhibition activities against tested pathogens.

**KEYWORDS:** *Pantoea agglomerans*; silver nanoparticles; antibiofilm; hemolysin inhibition.

**INTRODUCTION**

In recent years, nanotechnology has opened new aspects to fight infectious diseases by nanoparticles. Using microorganisms is one of the options to develop a nontoxic method for the synthesis of nanoparticles. Researchers have mentioned that many microorganisms can produce different shapes and sizes metal nanoparticles through either extracellular or intracellular route [1-9]. *P. agglomerans* bacteria belong to the family *Enterobacteriaceae*. This family considered a competent for rapid synthesis of AgNPs [1, 4-6].

*P. agglomerans* is a rare common opportunistic pathogen causing systematic infection [10]. Recently *Pantoea* species is being frequently isolated from nosocomial environment [11-13]. *P. agglomerans* is the most common *Pantoea* species recovered from such environment [13]. Many researches mentioned that Acinetobacter species, *E. coli*, *S. marcescens* and *S. aureus* are important opportunistic pathogens capable of causing nosocomial infections responsible of morbidity and mortality at high rates. [14] Reported nosocomial bacteremia caused by *Acinetobacter* species, [15] mentioned the nosocomial infection caused by *E. coli* and *S. aureus*. [16] Reported the nosocomial outbreak caused by *S. marcescens*. The resistance of those pathogens to commonly used antibiotics treatment has been reported by [17-19]. Resistance to current medication by those multidrug resistant pathogens has been a serious problem in public health; therefore, researchers start developing novel bactericides to overcome this problem [20]. AgNPs are considered as an effective broad-spectrum antimicrobial agent against various multidrug resistant pathogens because microbial pathogen cannot mutate to avoid its biocide effect [18, 21], which makes them pharmaceutical product that could be used to prevent the transmission of those pathogens in the environment [22]. [23] Reported many applications of AgNPs such as reduce the nosocomial infections coating of catheters, component of wound dressing, water purification agricultural uses. Silver toxicity is due to thiol group that renders inactive inhibiting DNA replication and translation of crucial proteins [24].
The cause of 80% of microbial infections is biofilms [25]. Bacterial biofilms are defined as bacterial communities that colonize and attach to living tissues or surfaces, they are cell clusters embedded in self-producing exopolysaccharide slime, biofilm plays a major role in development of infectious diseases i.e.: otitis media, endocarditis and nosocomial infections, it has been mentioned as a problem in medical field. Different approaches have been used to prevent formation of biofilm and one of them is using AgNPs [21, 25-27]. This work provides the extracellular biosynthesis of AgNPs using *P. agglomerans* bacteria, the helpful use of biosynthesized AgNPs as anti-biofilm and to prevent the hemolysin production by clinical multidrug resistant human pathogenic microorganisms.

**MATERIAL AND METHODS**

**Isolation and Identification of Bacteria**

Isolates of bacteria were obtained from different clinical samples from Baghdad Teaching Hospital, Baghdad, Iraq, the Vitel 2 System (Biomeriux) have been used to identify the isolates and to test their antibacterial susceptibility. *P. agglomerans* isolate were chosen for the AgNPs production. The antibiofilm formation and hemolytic activity of AgNPs produced were examined in several other representatives of the multidrug resistant isolated bacteria, *A. hemolyticus*, *E. coli*, *S. marcescens* represent the Gram-negative microorganisms, while *S. aureus* represent the Gram-positive bacteria.

**Biosynthesis of AgNPs**

To produce the biomass for the synthesis of AgNPs, *P. agglomerans* was cultured in nutrient broth medium; after incubating the culture at 37 °C for 24 hrs., the growth was centrifuged at 100 rpm for 10 minutes. The supernatant appears pale yellow. To 5 ml of each sample supernatant taken in a test tube, 1 mg of AgNO₃ was added in the laboratory under ambient conditions [28]. A brown mass gets deposited at the bottom of each test tube after 24- hours. Experimental control was run along with experimental test tubes (without adding AgNO₃).

**Characterization of AgNPs**

Using XE-100 AFM from Park Systems Atomic Force Microscopy the image was taken. The aqueous AgNPs was deposited onto a freshly cleaved mica substrate. After leaving the sample aliquot for 1 min it was washed with deionized water and left to dry for 15 min. By scanning the mica in the air in non-contact mode the images were obtained [29].

**Antibacterial Activity**

On the basis of minimum inhibitory concentration (MIC) values (lowest concentration of an antimicrobial that will inhibit the visible growth of a bacterium after overnight incubation) antibacterial activity of AgNPs was determined. MIC was determined for *A. hemolyticus*, *E. coli*, *S. marcescens*, *S. aureus* by broth dilution method as described by [30]. Briefly a stock solution of AgNPs from *P. agglomerans* in distilled water were diluted to concentrated ranging (2, 4, 8, 16, 32, 640) mg/ml.

**Effect of AgNPs on Bacterial Biofilm Using Tube Method**

As described by [31] qualitative assessment of biofilm formation was determined. Brain–heart infusion broth (BHI) supplemented with 25% sucrose 0.5mL+0.5mL sub MIC AgNPs (16mg/ml) was inoculated with one loopful of microorganisms from overnight culture, in the other hand 1 ml BHI/sucrose was incubated with loopful of test bacteria as a control and incubated for 24 hours at 37 °C. After decanting and washing the tubes with distilled water the tubes were left to dry then were stained with (0.1%) crystal violet, after removing the excess stain the tubes were washed with deionized water and were left to dry in inverted position to observe the biofilm formation, when a visible film lined the wall and bottom of the tube positive results is considered while ring formation at the liquid interface was considered negative results.

**Effect of AgNPs on Bacterial Hemolysin Production**

Hemolysin production by the test bacteria was studied by blood agar method as described by [32], 1 ml of sub MIC AgNPs was poured on blood agar medium. Plates were left to dry
RESULTS AND DISCUSSION

Bacteria are considered an ideal candidate for the synthesis of AgNPs. In the current study the selected *P. agglomerans* local isolate showed the ability to synthesize AgNPs. The biosynthesized AgNPs was observed by the change of color of silver nitrate solution from pale yellow to dark yellow within 24-h of incubation at room temperature as shown in Figure 1. At the same temperature as time experimental conditions control showed no color changes. Observation of color change is a method generally used by previous researchers to screen microbial isolates for AgNPs synthesis [7]. The result indicated that microorganisms could indeed be used for the synthesis of AgNPs. The change of color is due to the reduction of aqueous silver ions to AgNPs [33], one of the mechanism behind the synthesis of nanoparticles is an enzyme like nitrate reductase secreted by microbes, nitrate reductase is responsible for the bio reduction of metal ions to metal nanoparticles [34]. AFM investigation was conducted to characterize the biosynthesized AgNPs by *P. agglomerans* local isolate (Figure 1).

The shape of AgNs obtained was mostly spherical with average diameter: 101.15 nm. AgNPs of different shapes and sizes were synthesized by different bacteria some of which belonging to the Family Enterobacteriaceae, [6] reported the production of spherical shape of AgNPs by *Proteus mirabilis*, [35] had mentioned the production of spherical shape of AgNPs by *Acinetobacter calcoaceticus*. A study by [5] conducted that AgNPs biosynthesized by *E. coli* were in size range (1-100 nm), another study by [36] reported the synthesis of AgNPs with size range 42-92 nm by Bacillus strain CS. The topic that how the shapes and sizes of AgNPs influence their antimicrobial performance is still ongoing, a research [37] conducted that anisotropic shapes of AgNPs, ie: nanoplates, triangular AgNPs demonstrated less antimicrobial activity as compared to spherical shaped AgNPs.

In the current study, the AgNPs produced by *P. agglomerans* show antibiofilm activity against all test bacteria. Numerous literatures have reported the use of AgNPs to impede the formation of bacterial biofilms. The results obtained during this study are similar to the result obtained by [38] who mentioned that treating *Pseudomonas aeruginosa* and *S. aureus* with AgNPs resulted in more than 95% inhibition in biofilm formation, similar result had been reported by [39] who documented that AgNPs impede the formation of biofilms of *Acinetobacter sp.*. [40] had concluded the effectiveness of AgNPs synthesized by *Caltripis procera* flower against the formation of biofilm of enterotoxic *E. coli*.

A great effect against bacterial hemolysin activity of all test isolates was exhibited by the AgNPs produced by *P. agglomerans* when all isolates become non hemolytic on blood agar medium.
CONCLUSIONS
In the current study an easy approach for biosynthesis of AgNPs by P. agglomerans bacteria have been reported, the AgNPs formed where characterized by AFM. The AgNPs exhibited a potential antibiofilm and hemolysin inhibition activity against multidrug resistant pathogenic test isolates, including A. hemolyticus, E. coli, S. marcescens, S. aureus.

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