Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 31, Issue 3, 2020 DOI: http://doi.org/10.23851/mjs.v31i3.361

Research Article **Open Access**

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

Layla Abdulhamid Said*

Department of Biology, College of Science, Mustansiriyah University, Baghdad, IRAQ.

*Contact email: laylahamid@uomustansiriyah.edu.iq

ArticleInfo

Received
22/11/2017

Accepted
07/06/2020

Published
20/08/2020

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 *Acinetobactor 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 *Enterobacteriacea*. This family considered a competent for rapid synthesis of AgNPs [1, 4-6].

P. agglomerns 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. agglumerans* 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 Vitek 2 System (Biomeriux) have been used to identify the isolates and to test their antibacterial susceptibility. *P. agglumerans* 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 $AgNO_3$ 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 $AgNO_3$)

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

Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 31, Issue 3, 2020 DOI: http://doi.org/10.23851/mjs.v31i3.361

completely at room temperature, then were inoculated with overnight grown culture of test bacteria and incubated for 24 hrs. at 37 ˚C.

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. agglumerans* 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 calcoacticus*. 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.

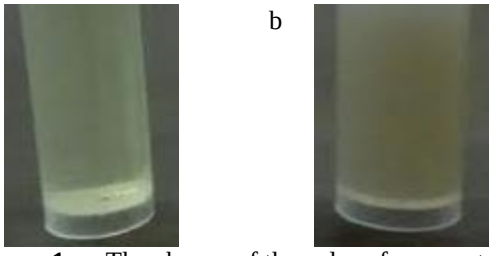

Figure 1. a- The change of the color of supernatant of *P. agglomerans* after the addition of $AgNO_3$ b-control without adding $AgNO_3$

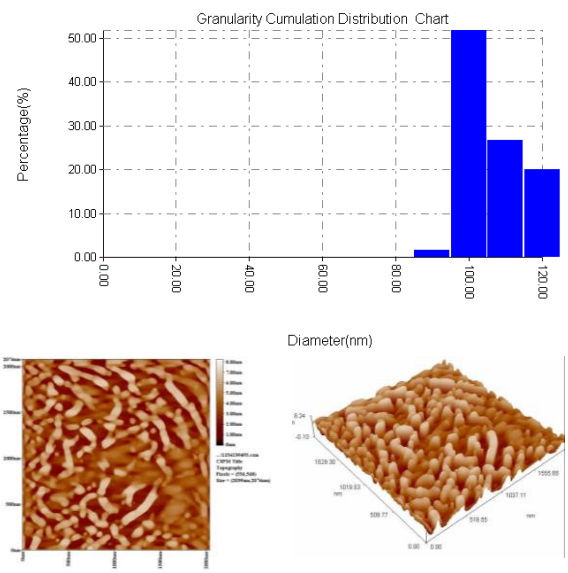

Figure 2. A- Granularity cumulation distribution chart of $AgNO_3$, B- AFM analysis of biosynthesized $AgNO_3$ from P. agglomerans.

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 *Pseudomanas 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 *Caltropis 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*.

ACKNOWLEDGMENT

The authors would like to express deep thanks for Mustansiriyah University, College of Science, Department of Biology, Microbiology Laboratory for their support to accomplish this research.

REFERENCES

[1] M. Ahmad, Sara M, R. S. Hamid, J Hossein, N. Ashraf-Asadat N, Rapid synthesis of silver nanoparticles using culture supernatant of Enterobacteria: a novel biological approach. Process Biochem. 42:919-923, 2007.

[2] S. He, Z. Guo, Y. Zhang, S. Zhang, J. Wang, G. Ning, Biosynthesis of gold nanoparticles using the bacteria *Rhodopseudomonas capsulata*. Materials Letter. 61: 3984-3987, 2007.

[3] G. Singaravelu, J. S. Arockiamarcy, V.G. Kumar, K. G. Govindaraju, A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, Sargassum wighlii Grevillc. Colloids and surface B. 57(1) 97-101, 2007.

[4] R. Y. Parikh, S. Singh, Prasad, and B LV. Patole, M. Sastry and Y. S. Schouche, Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from *Morganella sp*. Towards understanding biochemical synthesis mechanism. Chembiochem . 9: 1415 – 1422, 2008.

[5] S. Gurunathan, K. Kalishwaralal, R. Vaidyanthan, D. Venkataraman, S. R. K. Pandian, J. Muniyandi N, Hariharan and S.H. Eom. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids and Surfaces B . 74(1), 323–335, 2009.

[6] N. Samadi, D. Golkaran, A. Eslamifar, M. R. Fazeli, F. A. Mohseni, Intra/extracellular biosynthesis of silver nanoparticles by an autochthonous strain of *Proteus mirabilis* isolated from photographic waste. J Biomed Nanotechnol. 5 (3): 247-253, 2009.

[7] E. K. E Elbeshehy, A. M. Elazzaz, and G. Aggelis, Silver nanoparticles synthesis mediated by new isolates of *Bacillus spp*., nanoparticles characterization and their activity against Bean Yellow mosaic Virus and human pathogens Front. Microbiol. 6:453, 2015.

[8] A. M. Elgorban , A. N., Al- Rahman , S. R. Sayed, A. Hirad , A. A. Mostafa, and A. H. Bachkali, Antimicrobial activity and green synthesis of silver nanoparticles using *Trichoderma viride*. Biotechnology & Biotechnological Equipment. 30 (2): 299-304, 2016.

[9] N. Prabhusaran, A. R. Susethira, L. Radhakrishna, P. Revathi, S. T.Jeyasselan and PID Joesph, Extracellular biosynthesis of silver nanoparticles using bacterial sources and its pathogenic inhibition assay. Int. j. pharm. res. health sci..4 (2): 1080-85, 2016.

[10] H. V. Rostenberghe, W. Ibrahim , R. Norada and H. Maimunah, The clinical picture of neonatal infection with *Pantoea species*. JPN J INFECT DIS. 2006, 59(2): 120-1, 2006.

[11] V. Mahar, D. Yadav, J. Sanghvi, N. Gupta, and K. Singh, Pantoea dispersa an unusual cause of neonatal sepsis. Braz J Infect Dis, 17:726-8, 2013.

[12] F. Otsuka and H. Hagiya. Pantoea dispersa bacteremia caused by central line-associated bloodstream infection. Braz J Infect Dis 18 (6), 2014.

[13] A. Cheng, CY. Liu, HY. Tsai, MS Hsu, CJ Yang, YT. Huang, CH. Liao and PR. Hsueh, Bacteremia caused by *Pantoea agglomerans* at a medical center in Taiwan, 2000-2010. J Microbiol Immunol Infect 46:187-94, 2012.

[14] S. B. Almasaudi. *Acinetobacter spp*. as nosocomial pathogen: epidemiology and resistance features. Saudi J. Biol. Sci. 25(3): 586-596, 2018.

[15] S. L. Krein, C. P. Kowalski, T. P. Hofer, S. Saint, Preventing catheter-associated urinary tract infection in the United States: a national Survey of Practices Reported by United States Hospitals in 2005 and 2009. Int. J. Gen. Med 27 (7): 773-779, 2013.

[16] A. Khanna, M. Khanna, A. Aggarwal, Serratia marcescens- A rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients. Clin Diag Res. 7 (2): 243-246, 2013.

[17] H. Gossans. Susceptibility of multi-drug resistant Pseudomonas aeruginosa in intensive care units: results from European MYSTIC study group. Clin Microbiol Infect .9 (9): 980- 983. (2003)

[18] R. Singh, S. Singh, and M. S. Smitha, The role of nanotechnology in combating multi drug resistant bacteria. . Nanosci. Nanotechnol. 14(7): 4745-56, 2016.

[19] D. Moradiagara, C. T. Boinett, V. Martin, S. J. Peacock and J. Parkhill, Recent independent emergence of multidrug resistant Serratia marcescens clones within the United Kingdom and Ireland. Genome –c ship-org on July 19, 2016-published by Cold Spring Harbor Laboratory Press, 2016.

[20] H. H. Lara, N. V. Ayala – Nunez, L. C. I. Turrent and C.R. Padilla, Bactericidal effect of silver nanoparticles against multidrug resistant bacteria, World J Microbiol, 26:615 -621, 2010.

[21] N. K. Palanisamy, N. Ferina, A. N. Amirulhusni, Z. Nohd-Zain, L. J. Ping, and R. Durairaj, Antibiofilm properties of chemically synthesized silver nanoparticles found against *Pseudomonas aeruginosa* J. Nanobiotechnology.(12) 2, 2014.

[22] S. Sanyasi. Polysaccharide – capped silver nanoparticles inhibit formation and eliminate multidrug resistant bacteria by disrupting bacteria cytoskeleton with reduced cytotoxicity. Sci. Rep. 66 ,: 24929, 2016.

[23] K. Chaloupka, Y. Malam, and A. M. Seifalian. Nanosilver as a new generation of nanoproduct in biomedical application. Trend. Biotechnol. 28: 580-8, 2010.

[24] W. Salem, D. R. Leitner, F. G. Zingl, G. Schatter, R. Prassl, W. Goessler, J. Reid and S. Schild. Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxigenic *Escherichia coli*. Int J Med Microbiol 305, 85-95, 2015.

[25] J. P. O'Gara, and H. Humapheys, *Staphylococcus epidermidis* biofilms: Importance and applications, J Med Microbiol. 50:582-7, 2001.

[26] M. R. Donald and W. Costeron, Biofilms: survival mechanisms of clinically relevant microorganisms, Clin Mircobiol Rev.15:167-193, 2002.

[27] RLE. Castrillon, R. A. Palma and DMC. Padilla Interferencia de Las biopeliculas en el proceso de curacion de heridas. Dermatologia. Rev Mex 55: 127-139, 2011.

[28] E. Ranganth, V. Rathod and A. Banu A, Screening of Lactobacillus spp. for the mediating of biosynthesis of silver nanoparticlesfrom silver nitrate. Journal of Pharmacy. 2(2);237-241, 2012.

[29] S. C. G. Kiruba Daniel, K. Nehru and M. Sivakumar, Rapid biosynthesis of silver nanoparticles using *Eichornia crassipes* and its antibacterial activity. Current Nanoscience 8:125-129, 2012.

[30] J. M. Andrew. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 49 (6) 1049, 2002.

[31] G. D. Christensen, W. A. Simpson, and E. H. Bisno Aland Beachey Adherance of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun. 37:318-26, 1982.

[32] C.Malarkodi, K.M. Chitra and G. Annadurai. Novel eco-friendly synthesis of titanium oxide nanoparticles by using *Planomicrobium sp.* and its antimicrobial evaluation. Der Pharmacia Sinica. 4(3):59-66, 2013.

[33] M. Saravanan, A.K. Vemu and S.K. Barik. Rapid biosynthesis of silver nanoparticles from *Bacillus megaterium* (NCIM 2326) and their antibacterial activity on multidrug resistant clinical pathogens. Coll Surf B. 88:325-331, 2011.

[34] N. Duran, P.D. Marcato, O. Alves and G. Souza. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J. Nanotechnol, 3:8, 2005.

[35] R. Singh. Synthesis, optimization, and characterization of silver nano -particles from *Acinetobacter calcoaceticus* and their enhanced antibacterial activity when combined with antibiotics'. Int. J. Nanomedicine, 8: 4277-90, 2013.

[36] V.L Das, R. Thomas, R.T. Varghese, E.V. Soniya, J. Mathew and E.K. Radhakrishnan Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrial area. 3 Biotech 4:121-126, 2014.

[37] M.A.Raza, Z. Kanwal, A. Rauf, A.N. Sabri, S.Riaz and S. Naseem. Size and Shape-Dependent antibacterial studies of silver nanoparticles synthesized by wet. Chemical routes. Nanomaterials 6,74,2, 2016.

[38] K. Kalishwarall, S. Barathmanikanth, S. Pandian, V. Deepak, and S.Guruanthan Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus aureus. Colloids and Surfaces B: Biointerfaces. 79(2): 340-344, 2010.

[39] S. Hendiai, A. Ahy Abdi, P. Mohammedi and S. Kharrazi Synthesis of silver nanoparticles and its synergistic effects in combination with imipenem and two biocidales against biofilm producing Acinetobacter baumannii. Nanomed J 2, 291-298, 2015.

[40] W. Salem Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. Int J Med Microbiol. 305(1): 85–95, 2015.