

# Effect of Storage Period on Silver Nanoparticles Biosynthesized By *Pseudomonas Aeruginosa*

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## ABSTRACT

The present study demonstrates the effect of storage period on silver nanoparticles (AgNPs), which synthesized by *Pseudomonas aeruginosa* and their antibacterial activity. The result shows that the size of (AgNPs) which synthesis by *Pseudomonas aeruginosa* was 93.55nm after 4-72hour, and when storage about 2 years, we found that the size of AgNPs was stable and reduced to 69.0nm. Antibacterial activity against pathogenic microbes: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia sp*, *Streptococcus sp*, *Klebsiella sp*, *Candida albicans* was performed before and after storage and found that AgNPs have activity against this microbes.

**Keywords:** Antimicrobial activity, *Pseudomonas aeruginosa*, Silver nanoparticles, Storage period.

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## INTRODUCTION

Silver nanoparticles (AgNPs) have extensive applications in numerous fields as one of the nanomaterials of honorable metals. For plastics, earthenware manufacturing, glass, bond, elastic, greases, paints, balms, glues, colors, etc. they are usually used as an added substance. They can also be used for antibacterial materials, antistatic materials, superconductive cryogenic materials, and biosensor materials. Due to their optical irregularity,<sup>1</sup> photoelectrochemical,<sup>2</sup> the amalgamation of AgNPs has attracted considerable consideration. and electronic properties. The general techniques for blending AgNPs is by physical strategy, which yields low measures of item and creates loads of warmth and the compound conventions by and large connected for amalgamation of AgNPs, experience the ill effects of either confinement like high cost, utilization of poisonous chemicals, and so forth. Numerous microorganisms, for example, microscopic organisms, yeasts, growths, and actinomycetes have been utilized as a part of blend Nanoparticles of metal. An intracellular or extracellular microbial mixture of metal nanoparticles may happen.<sup>3-5</sup> Advances in downstream handling such as ultrasonics, chemical cell disruption to release.

AgNPs are needed if AgNPs should be intracellularly blended.<sup>6</sup> Then extracellular biosynthesis is shoddy again, and AgNPs can be efficiently isolated to explore their prospective applications, which promotes large-scale AgNP generation. As a consequence, countless research concentrated on extracellular strategies for amalgamation of metal

nanoparticles.<sup>7</sup> The approach to the use of distinct microscopic supernatants organisms, yeasts, growths, and actinomycetes for mixing silver nanoparticles is highly reported. Soundness is one of the obstacles in the amalgamation of AgNPs and its application. *P.aeruginosa's* biosynthesized AgNPs have been steady for a long time since late.

## MATERIALS AND METHODS

All secludes were obtained from Mustansiriyah University/ College of Science/Department of Biology; Isolates were fundamentally distinguished in our research facility, where they were recognized using the Vitek 2 GN ID and VITEK 2 framework (Biomerieux) at Al-Kindy Educational Hospital. *P. aeruginosa* seclude used as part of this inquiry to assess their capacity to biosynthesize silver nanoparticles, whereas *Pseudomonas aeruginosa*, *Streptococcus sp.*, *Klebsiella sp.*, *Staphylococcus aureus*, *Escherichia coli*, *Serratia sp.* were selected for antimicrobial action. What's more, *albicans* from *Candida*. The secluded bacteria were kept on the agar supplement. While on Sabouraud dextrose agar (SDA) yeast (*Candida albicans*) was maintained.

### Biosynthesis of silver nanoparticles

*Pseudomonas aeruginosa*, the bacterial strain, was separated from the wound source and refined in funnel-shaped cups at 100rpm in the nutrient soup at 37°C for 48 hours. The cell-free supernatant was acquired at 8000xg for 15 min by centrifugation. Used to amalgamate silver nanoparticles, *Pseudomonas aeruginosa* supernatant.

Silver nanoparticles biosynthesis was performed as shown in Kumar *et al.*<sup>8</sup> Quickly added watery silver nitrate (50 mL of 1 mM) to 50 mL of the supernatant culture of *Pseudomonas aeruginosa* (half, v/v) and kept at 37°C. By examining aliquots (2 mL) of the response blend, the bioreduction of silver particles was observed at consistent interims.

### Characterization of silver nanoparticles

The photograph of the Nuclear Force Microscopy was drawn using the XE-100 AFM of Park Systems. The AgNPs fluid was deposited on a new mica substratum. The instance aliquot was left with deionized water for 1-minute at that stage and left to dry for 15 minutes. The images were obtained by inspecting the mica in airin non-contact mode.<sup>9</sup>

### Antimicrobial Activity of AgNPs

Antibacterial activity of biosynthesized AgNPs by *P.aeruginosa* was tried against organisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus sp.*, *Serratia sp.*, *Klebseilla sp.* *Candida albicans*. A well dissemination strategy was utilized to decide the antibacterial action.<sup>10</sup> A small volume of sterile water was poured into a test tube that emulsified general settlements of microscopic test organisms taken directly from the plate, including refined sterile water, and acclimatized the suspension to 0.5 McFarland's Standard.

Half ml of the volume of the suspension was spread over the plates containing Muller–Hinton agar using a sterile cotton swab to achieve a consistent microbial development. Well of 6 mm measurement using sterile plug-borer on the Muller–Hinton agar plate. 50 µL AgNP. The arrangement was poured on each well on all plates using a micropipette, a similar amount of supernatant was taken as control without AgNO<sub>3</sub>. The plates were brooded for 24 hours at 37°C; the distance across the obstacle zone was estimated in mm

## RESULT AND DISCUSSION

### Biosynthesized of AgNPs

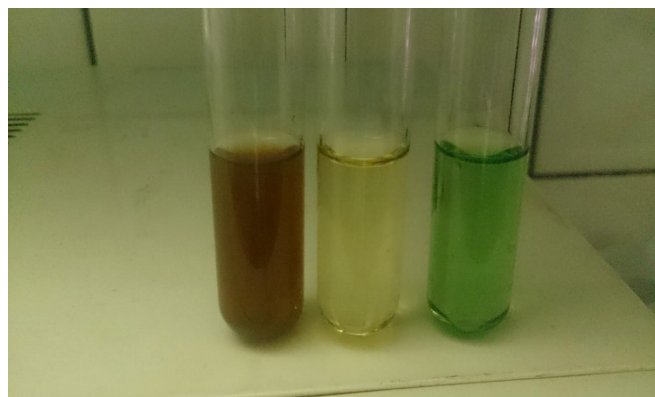
The culture supernatant of *P. aeruginosa* studied extracellular biosynthesis of silver nanoparticles. Visual observation showed a color shift in supernatant from yellow or green bluish (pyocyanin generated for isolate) to brown (Figure 1). In contrast, no color change was noted in supernatant culture without silver nitrate or in silver nitrate media alone. The release of brown color in silver nitrate treated culture supernatant has

proposed the fashioning of silver nanoparticles.<sup>11</sup> A comparable observation was produced by Duran *et al.*<sup>9</sup> In the biosynthesis of Ag-NPs by *P. aeruginosa* strain through an extracellular method, the excitation of Ag-NPs surface Plasmon vibration could lead in a medium-to-brown color change.<sup>12</sup> The Ag-NP biosynthesis mechanism is well established, but it was presumed that silver ions in their extracellular environment wanted the NADPH-dependent nitrate reductase enzyme to reduce it.<sup>13</sup> In the present study, a change of color from yellow to brown was observed after 4h of incubation. Nitrate reductase is known for shutting electron to the metal group from nitrate. These findings cited the role of the enzyme of nitrate reductase in silver nanoparticles biosynthesis.<sup>14</sup>

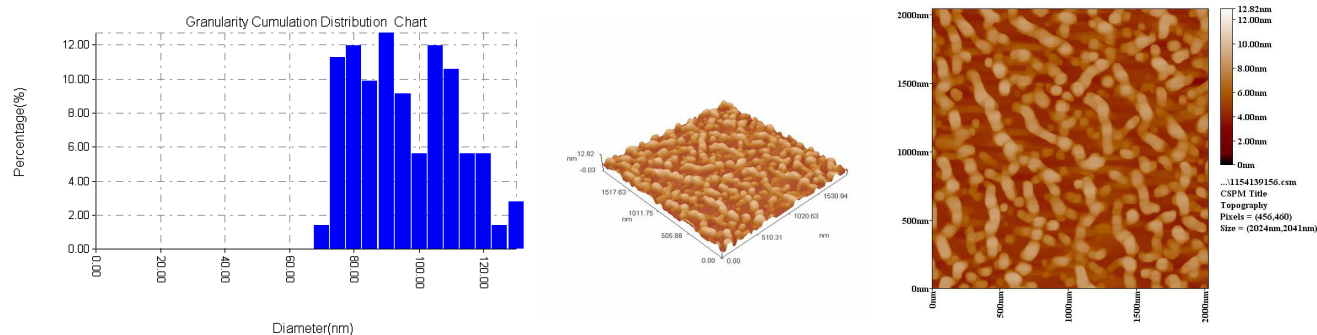
The reduction of Ag<sup>+</sup> to Ag<sup>0</sup> is reported to occur through the enzyme of nitrate reductase, these enzymes secreted in the solution can reduce silver nitrate to silver nanoparticles through proteins as capping agents.<sup>15</sup>

### Characterization of silver nanoparticles

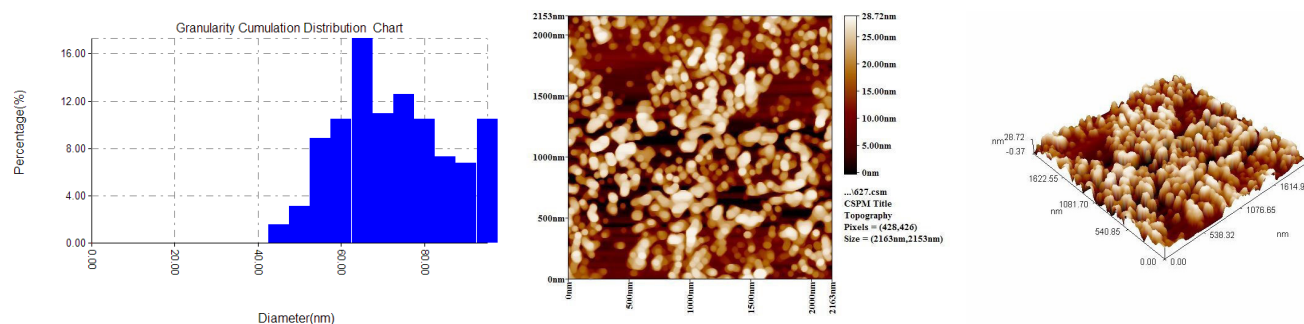
In this examination, the microscopy of nuclear power has altered over the investigation of molecule measurement. AFM was used to show the nanoparticles ' surface and three-dimensional perspective (Figure 2) and discovered 93.55 nm of ordinary particle size. The image reveals *P. aeruginosa*'s sensible shape and size of the AgNPs, while Jeevan *et al.*<sup>12</sup> found that the estimated particles ranged from 20-100 nm while the span of AgNPs 69.0 nm was found in Figure (3) after capacity for 2 years.



**Figure 1:** Supernatant of *P.aeruginosa* : (1) Supernatant without AgNO<sub>3</sub>, (2) Nutrient broth before culturing and (3) Supernatant With AgNO<sub>3</sub>



**Figure 2:** Atomic Force Microscopy image of silver nanoparticles synthesized by *P.aeruginosa* Before storage



**Figure 3:** Atomic Force Microscopy of P.aeruginosa synthesized silver nanoparticles after storage.

**Table 1:** Antimicrobial activity of AgNPs Biosynthesized by P.aeruginosa

Microorganism	Zone of inhibition (mm)		
	Supernatant without AgNO <sub>3</sub>	AgNPs Before storage	AgNPs After storage
<i>Staphylococcus aureus</i>	-	9	8
<i>Streptococcus sp.</i>	-	11	9
<i>E. coli</i>	-	12	9
<i>Klebsiella sp.</i>	-	14	11
<i>P.aeruginosa</i>	-	14	11
<i>Serratia sp.</i>	-	12	10
<i>Candida albicans</i>	-	11	8

### Antimicrobial assay

AgNP's antimicrobial activity combined by *P. aeruginosa* before and after capacity was tried against pathogenic organisms, *Staphylococcus aureus*, *Serratia sp.*, *P. aeruginosa*, *E. coli*, *Klebsiella sp.*, *Candida albicans* by utilizing agar well dissemination test strategy.

Restraint zone was dictated by estimating the distance across of microorganism freedom after 24 hours, the most extreme activity of silver nanoparticles is found for by *p. aeruginosa* and *Klebsiella sp.* 14 mm zone of inhibition. The measurements of the hindrance zones against, *E. coli*, *Streptococcus .sp* and *Serratia.sp* and *staphylococcus aureus* were observed to be 12,11,12,9 mm, separately, while if there should be an occurrence of *C. albicans* were 11 mm. (Table 1). Then again, when contrasted and AgNPs following 2 years, we found that the antimicrobial action of AgNPs was bring down against every single pathogenic organism aside from *Kleb. Sp* and *p.aeruginosa* (11)mm, while *Staph. aureus*, *E. coli*, *Strep. spp.*, *Ser.spp.*, and *C. Albicans* were 8,9,9,10,8 mm separately. The outcome in our examination demonstrates that AgNPs was remained action following 2 years stockpiling while Pandian *et al.*<sup>16</sup> demonstrated that the AgNPs combined by *P.aeruginosa* were steady for 24days. The results of this research were spent with their findings by (Hamzia Ali Ajah *et al.*, 2018). It has a strong antimicrobial activity against *Pseudomonas aeruginosa*, *Streptococcus spp.*, *Klebsiella spp.*, *Staphylococcus aureus*, *Escherichia coli*, *Serratia spp.* and *Candida albicans*.<sup>17</sup>

### CONCLUSION

In our investigation we found that the span of particles of AgNPs which created by *P.aeruginosa* was diminished after capacity along period and spared were activity

### REFERENCES

1. K. Kalimuthu, R. Suresh Babu, D. Venkataraman, M. Bilal, and S. Gurunathan, "Biosynthesis of silver nanocrystals by *Bacillus licheniformis*," *Colloids and Surfaces B: Biointerfaces*, vol. 65, pp. 150-153, 2008.
2. Khawlah Jebur Khalaf. Detection of Periplasmic Nitrate Reductase Enzyme in Proteolytic *Pseudomonas aeruginosa*. Iraqi Journal of Biotechnology, 2015, Vol. 14, No. 2 , 91-98
3. Layla A. H. Said, Khawlah Jebur Khalaf and Manal Khalid Muhammad. Biosynthesis of silver nanoparticles from *Enterobacter cloacae*, *Raoultella terrigena*, and some other Enterobacteria and their antibacterial activity. *ejbps*, 2016, Volume 3, Issue 4, 86-92.
4. Selvi, V. T. and K. V. Radha.(2013). "Synthesis of silver nanoparticles from *Pseudomonas putida* NCIM 2650 in silver nitrate supplemented growth medium and optimization using Response Surface Methodology," *Digest Journal of Nanomaterials and Biostructures* vol. 8, pp. 1101 – 1111.
5. N. Jain, A. Bhargava, S. Majumdar, J. C. Tarafdar, and J. Panwar, "Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: A mechanism perspective," *Nanoscale*, vol. 3, pp. 635-641, 2011.
6. Abdulkadir M. N. Jassim<sup>1</sup>, Safanah A. Farhan, Jehan A. S. Salman, Khawla J. Khalaf, Mohammed F. Al Marjani, Mustafa T. Mohammed.(2015). Study the Antibacterial Effect of Bismuth Oxide and Tellurium Nanoparticles International Journal of Chemical and Biomolecular Science Vol. 1, No. 3, pp. 81-84
7. Hamzia Ali Ajah, Khawlah Jebur Khalaf, Ali Murtatha Hasan and Hassan Ali Aja. Extracellular biosynthesis of silver nanoparticles using *Sphingomonas paucimobilis*, *Serratia sp.* And *Pseudomonas aeruginosa* and their antimicrobial activities. *ejbps*, 2016, Volume 3, Issue 6, 144-148
8. A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar, and M. Sastry, "Extracellular biosynthesis of silver

- nanoparticles using the fungus *Fusarium oxysporum*,” *Colloids and Surfaces B: Biointerfaces*, vol. 28, pp. 313-318, 2003.
9. Duran, N.; Marcato, P. D.; Alves, O. L.; Souza, G.I. and Esposito, E.(2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. *Journal of Nanobiotechnol.*, 3(8):1-7
  10. R. Thomas, A. Viswan, J. Mathew, and R. E. K, *Evaluation of antibacterial activity of silver nanoparticles synthesized by a novel strain of marine Pseudomonas sp*, 2012.
  11. Sastry, M.; Ahmad, A.; Khan, M. I. and Kumar, R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycetes, *Curr. Sci.*, 85:162-170.
  12. Jeevan, P.; Ramya, K. and Edith Rena, A. (2012). Extracellular biosynthesis of silver nanoparticles by supernatant of *Pseudomonas aeruginosa*. *Indian Journal of Biology*, 11(1): 72-76.
  13. Minaecian, S. ; Shahverdi, A. R. ; Nohi, A. S. and Shahverdi, H. R. (2008). Extracellular biosynthesis of silver nanoparticles by some bacteria. *J. Sci. I. A . U. (JSIAU)*., 17( 66):1-4.
  14. Ranganath, E.; Rathod, V. and Banu, A. (2012). Screening of *Lactobacillus spp*, for mediating the biosynthesis of silver nanoparticles from silver nitrate. *Journal of Pharmacy.*, 2(2) : 237-241.
  15. Natarajan, K.; Selvaraj , J. and Amachandra ,V. (2010). Microbial production of silver nanoparticles. *Digest Journal of Nanomaterials and Biostructures .*, 5( 1): 135 – 140.
  16. Pandian,S.S.; G, Mariaamalraj S, Achary, A.(2014). Influence of Biosurfactant in Biosynthesis of Silver Nanoparticles by *Pseudomonas Aeruginosa* AMB AS7. *International Journal of Innovative Research in Science, Engineering and Technology Volume 3, Special Issue 3*.
  17. Hamzia Ali Ajah, Khawlah Jebur Khalaf, Ashraf Sami Hassan1, and Hassan Ali Aja. (2018). Extracellular biosynthesis of silver nanoparticles by *Haemophilus influenzae* and their antimicrobial activity . *J. Pharm. Sci. & Res.* Vol. 10(1), 175-179.