Electronic Supplementary Information

An approach towards continuous production of silver nanoparticle using *Bacillus thuringiensis*

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Methodology

To prove the approach as continuous process

To prove the current approach as a continuous process for synthesis of AgNP, we performed three successive batch cultures using the live cells from the early stationary phase of previous batch culture. Briefly, a loop full of bacterial culture was added to a 250 mL of nutrient broth and kept at 37 ^{o}C incubation with 220 rpm agitation for homogenous growth. At the mid log phage of growth kinetics, MIC concentration of $AgNO_3$ was added to the culture and kept in same conditions to reach the late log phase of the growth. The bacteria culture was collected, and centrifuged at 5000 rpm for 15 minutes. The pellet was collected and dispersed in 10 mL of autoclaved deionised water. The number of live cells in this suspension was evaluated using the Baclight viability assay, and the synthesis of AgNP was confirmed by analyzing the sample using UV-Vis and ATR-FTIR spectroscopes. Like first batch culture, the second and third batch culture were done by taking the 1 mL of cell suspended in deionised water for inoculation of next batch culture, followed by characterization of the samples as done for first batch culture to evaluate the number of live cells and production of AgNP.

Methodology for SDS-PAGE

For SDS-PAGE, the pellet of the *B. thuringiensis* culture was suspended in deionised water and sonicated after lysozyme (200 µg/ml) treatment. The lysate was centrifuged at 700 rpm for 15 minutes, and the supernatant harbouring the nanoparticle was used for SDS-PAGE analysis. For

SDS-PAGE, 25 µl of each supernatant, pellet of control cell lysates, and 0.15 mM $AgNO_3$ treated cell lysates were denatured in 2X SDS loading dye by boiling at 80 ^oC for 10 minutes. Electrophoresis was performed in 10 % polyacrylamide gel (tarsons vertical electrophoresis system) at a constant voltage of 90 volts for 2-3 hours, followed by staining with Commassie Brilliant Blue to check the presence of proteins with respect to the protein molecular weight marker (BIO-RAD Catalog -161-0394, BIO-RAD).

Internalization of Ag+

Initially, *B. thuringiensis* was allowed to grow till mid log phase of growth kinetics. Instead of adding $AgNO_3$ (MIC concentration), the culture was centrifuged at 5000 rpm for 30 mins at 4 ^oC, and the supernatant was separated from the pellet. The supernatant was further centrifuged for three times at same conditions to remove all the possible cells from suspension. The centrifugation was done at relatively lower speed and at 4 ^oC, to avoid cell disruption because of centrifugal force, which would have resulted in secretion of cytosolic content in supernatant. MIC concentration of $AgNO_3$ was added to the supernatant and kept it in incubator at 37 ^oC, 220 rpm agitation for 16 hrs. Additionally, to rule out the possibility of $AgNO_3$ reduction in presence of proteins and enzymes present in the nutrient broth, we also added $AgNO_3$ MIC concentration to the freshly autoclaved nutrient broth, and kept for 16 hrs in same conditions. The possibility of initial formation of AgNP was analyzed using UV-Vis spectroscope.

Result and discussion

The SDS-PAGE image shows the absence of some bands (black arrow) in the supernatant and pellet of 0.15 mM $AgNO_3$ treated cell compared to untreated cells, confirming the presence of respective proteins on nanoparticle surface. However, the bands are present in pellet and supernatant of *B. thuringiensis* culture. Hence, we confirmed that the proteins involved in capping of AgNP belong to the bacteria *B. thuringiensis*. However, in treated cells there are bands (shown by red arrow) which are absent in untreated bacterial culture. The bands can be attributed to the proteins expressed in stress conditions only.

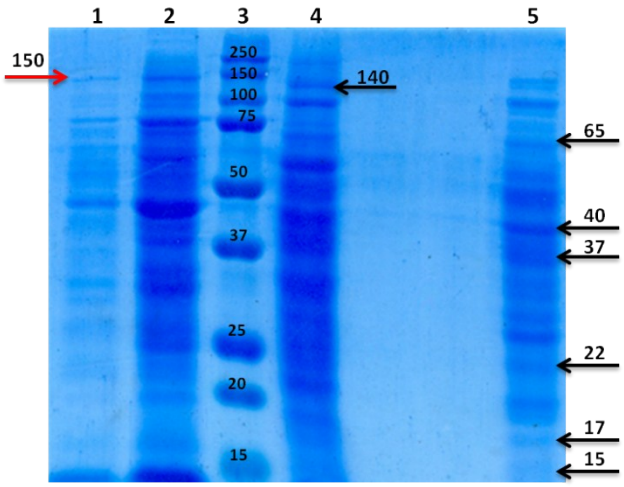

Fig. S1. SDS-PAGE image of (lane 1) supernatant of online fabricated AgNP, (lane 2) pellet of online fabricated AgNP, (lane 3) marker, (lane 4) pellet of *B. thuringiensis* culture, (lane 5) supernatant of *B. thuringiensis* culture.

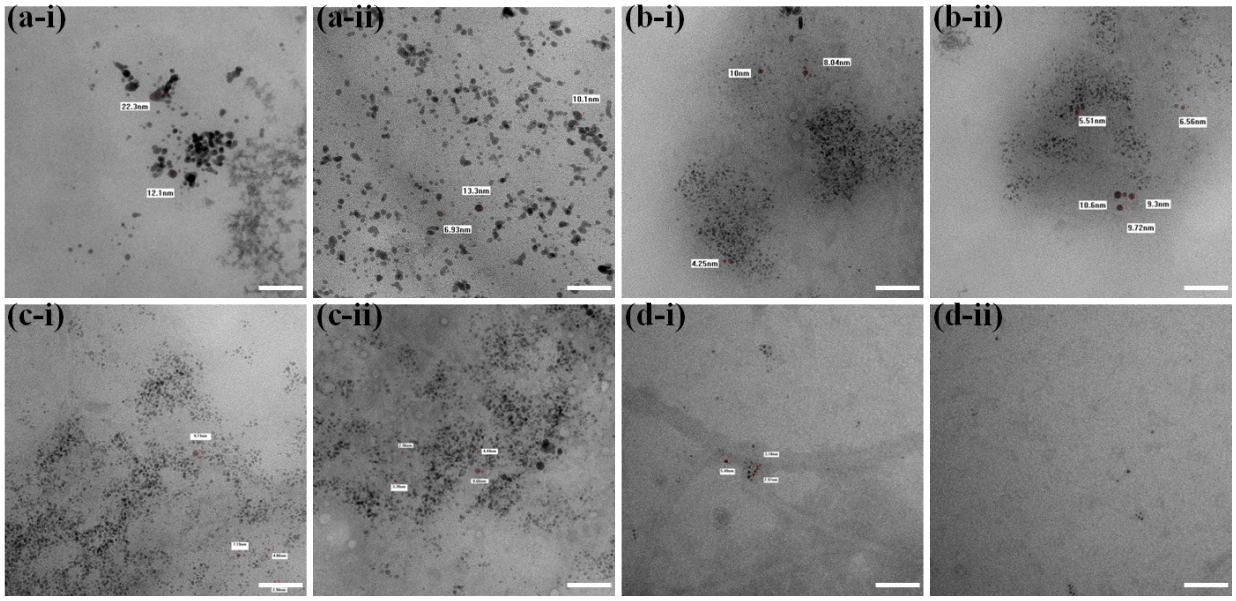

Fig. S2. TEM image of AgNP obtained for SEC load (a-i & a-ii), elution 1 (b-i & b-ii), elution 2 (c-i & c-ii), and elution 3 (d-i & d-ii). The scale bar represents 50 nm.

The statistical analysis using ImageJ software (N.I.H., U.S.A.) reveals the presence of 55 % green cells and 45 % red cells, evaluating the antimicrobial potential of commercially available AgNP (Fig. S3). Hence, presence of 9.2 µg/mL Sigma AgNP in the bacterial culture reduces the viability of the bacteria to 55 %, which is relatively higher than the viability found in presence of the fabricated nanoparticle.

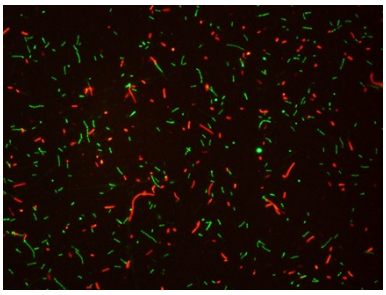

Fig. S3. The fluorescence microscopic image of *E. coli* cells in presence of commercially available AgNP from Sigma Aldrich.

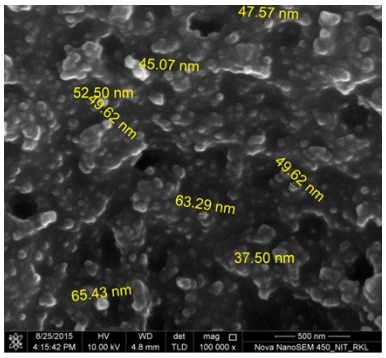

Fig. S4. FE-SEM image of fabricated AgNP dispersed in deionised water with 10 mM SHMP.

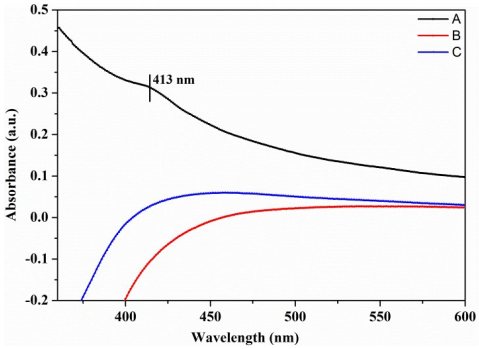

Fig. S5. UV-Vis spectra showing the synthesis of AgNP. (A) $AgNO_3$ added in the *B. thuringiensis* culture at the mid log phase of growth, (B) $AgNO_3$ added in the supernatant obtained upon centrifugation of the *B. thuringiensis* culture, and (C) $AgNO_3$ added in the autoclaved nutrient broth.

As expected, when $AgNO_3$ was added at the mid log phase of *B. thuringiensis* culture, synthesis of AgNP was observed (Fig. S5 A). However, when we added $AgNO_3$ in the supernatant and nutrient broth, no UV-Vis peaks corresponding to 413 nm were observed (Fig. S5 B & C). Hence, this study confirmed the internalization of Ag(I) is essential for reduction into Ag element, which will further assemble into silver nanocrystals, AgNP.