Production of Silver Nanoparticles by *Escherichia coli*: Green Approach

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**Abstract**

Physical and chemical approaches to synthesize nanoparticles are not suitable for safer medical usage. Thus, green method could be a suitable approach to produce safe and non-toxic nanoparticles. In this study, silver nanoparticles were synthesized by *Escherichia coli* and its biological activities were determined. The produced bacterial biomass (supernatant) was mixed with silver nitrate to produce silver nanoparticles. *Escherichia coli* acting as reducing agents to produce nanoparticles. The colour changes from colourless to brown was observed to indicate the formation of silver nanoparticles. It was further reconfirmed using UV-Vis Spectroscopy, where the peak at 300 nm confirmed the formation of AgNPs. The antimicrobial and cytotoxicity activity of synthesized nanoparticles was tested. At 1 mg/ml concentration, the nanoparticles were effective against all the micro-organisms tested, *Bacillus subtilis* (12.7±2.5 mm) *Staphylococcus aureus* (13.7±2.3 mm), *Pseudomonas aeruginosa* (14.3±2.3 mm) and *Aspergillus niger* (12.7±2.1 mm). With decreasing concentration, the antimicrobial activity also decreased. Similar results were obtained when the nanoparticles were tested for cytotoxicity ability on HeLa cells. With increasing concentration, more of the tumor cells were inhibited. Based on the result obtained, it can be concluded that *Escherichia coli* can be used in the production of silver nanoparticles. It was found that synthesized nanoparticles possess effective antimicrobial and cytotoxicity activities. Thus, these properties could make them an advancing field in the medical world.

**INTRODUCTION**

Research and development in the field of nanoscience and nanotechnology has caught large attention in the past decades (Keat et al., 2015). Nano sized particles are usually 1-100nm in size and these nanoparticles have various properties (Jaffat et al., 2017), such as very high surface area and volume ratio (Okafor et al., 2013). This property helps to increase their biological effectiveness in various applications, such as antimicrobial (Kushwaha et al., 2015), anti-tumour, anti-viral, anti-fungal (Thawadi et al., 2017) disinfectant (Poonam and Sanjiv Kumar, 2019) (Poonam and Sanjiv Kumar, 2019), biosensor materials (Kushwaha et al., 2015) and cosmetic products (Song and Kim, 2009). Among the various metals such as zinc, copper, gold and platinum, silver is a one of the most commonly used NPs (Singh et al., 2018).
tial environment and biological risk (Singh et al., 2018). Biosynthesis is an alternative route for nanoparticle synthesis. This uses plant or plant extracts (Sahni et al., 2015), micro-organisms such as bacteria (Jaffat et al., 2017) and fungi (Khan and Jameel, 2016) and enzyme (Lateef et al., 2015). These substances are abundant in nature and can be easily obtained. This method is suitable for large scale synthesis (Annamalai and Nallamuthu, 2016), faster synthesis rate (Iravani, 2014), pure particles (Koliparambil et al., 2016), sustainable to the environment and much less toxic. These particles can then be extracted and purified to be used.

In this project, Escherichia coli (E. coli), was used as a source to bio synthesis silver nanoparticles (AgNPs). E. coli is a gram-negative bacillus bacterium that is mostly used for laboratory purposes. The bacterium is expected to reduce silver ions to metal silver to produce silver NPs. The synthesized NPs was checked and analyzed for their antimicrobial activity against various strains and their cytotoxicity effect on cancer cells such as HeLa cells, to evaluate their effectiveness for safe therapy purposes.

MATERIALS AND METHODS

Production of Biomass

Master plate for E. coli was obtained from MIU laboratory, Department of Biotechnology. A single colony was taken from the master plate and streaked using 4-quadrat streaking on a nutrient agar plate and incubated at 37°C for 24 hours. For the biomass production a single colony of E. coli from the sub-cultured plate was inoculated in LB broth and incubated at 37°C for 24 hours. After 24 hours, the broth was centrifuged at 9000 rpm for 10 min and pellet and the supernatant were collected separately. The supernatant was used for the synthesis of AgNPs (Koliparambil et al., 2016).

Synthesis of Silver Nanoparticles (AgNO₃)

Collected supernatant was added separately to the reaction flask containing 1 mM silver nitrate (AgNO₃). The flask was completely covered with aluminum foil to prevent light penetration and left in the incubator at 37°C for 48 hours. AgNO₃ was used as control. The change in colour from yellow to brown showed the formation of silver nanoparticles. in the range of 200-700 nm. After the OD was measured, the solution was centrifuged at 9000 rpm for 15 min. The supernatant was discarded and the pellet containing the silver nanoparticles was allowed to dry at 70°C until no moisture was left. The NPs were scrapped off and collected in powder form (Koliparambil et al., 2016). The formation of silver nanoparticles was further confirmed using UV-Vis Spectroscopy. 1 ml of reaction mixture was taken in a quartz cuvette and the corresponding spectra were measured in the range of 200-700 nm.

Antimicrobial Activity

Synthesized silver nanoparticles by E. coli was tested for its antimicrobial properties against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus niger via disc diffusion method. The pure cultures of organism were subcultured on nutrient broth and incubated at 37°C for 24 hours. After 24 hours the growth of microorganisms was monitored every 4 intervals using UV spectrometer until the optical density reached 0.8-0.1 at 595nm. This represents 1x10⁸ CFU/ml. After inoculation and cultivation of different target bacteria on top of nutrient agar, discs were placed in selected area on different plates. For the antifungal assay, Aspergillus niger from the master plate was swabbed using a sterile cotton swap into a PDA plates and discs were placed in selected area on different plates. 10 µl of AgNPs with different concentrations (1mg/ml, 0.1mg/ml, 0.01mg/ml) were loaded on the top of discs. Ampicillin and distill water were used as positive and negative control respectively. All the plates were sealed with parafilm and incubated at 37°C for 48 hours. The diameter of the clear zone (mm) was measured and recorded to evaluate antimicrobial activity (Baluiri et al., 2016).

Cytotoxicity Test

The cytotoxicity test was carried using MTT assay. In a 96 titer well plate of 200μl capacity, pre-cultured cell suspension was added to each well along with different concentration (1 mg/ml, 0.1 mg/ml, 0.01 mg/ml) of AgNPs. The cells with AgNPs were then incubated at 37°C in 5% CO₂ incubator for 24 hours. 1 Mm of silver nitrate was used as positive meanwhile Dimethyl Sulfoxide (DMSO) was used as negative control (Kaba and Egorova, 2015). After 24 hours of incubation, 10μl of MTT solution was added to each well and again incubated at 37°C in 5% CO₂ incubator for 4 hours. The plates were then taken out followed by removal of spent medium and replacement with DMSO. The wells were then read at 620 nm (cell line/sample) absorbance using ELISA reader. This reading is the optical density of the wells containing cells that determines the presence of living cells (Bahuguna et al., 2017).
RESULTS AND DISCUSSION

Visual observation and UV-visible spectroscopy of Synthesized AgNPs

Green synthesis is an alternative and sustainable route for synthesis of NPs since the biological components such as microorganisms and plant extracts are abundant and easy to obtain. This allows to produce large number of pure particles that can be used safely (Singh et al., 2018; Prabhu and Poulose, 2012).

In this experiment, the yellow bacterial supernatant solution with AgNO$_3$ turned slightly brown after 48 hours with brown deposits at the bottom of the flask (Figure 1) due to the reduction of silver metal ions Ag$^+$ into silver nanoparticles Ag$^0$. The colour change in this experiment was explained clearly due to the excitation of electrons. Accordingly, similar results were obtained by Koliparambil et al. (2016) and Kushwaha et al. (2015) where the colour change indicates the formation of AgNPs. The exact mechanism of the reduction process is still never understood yet where Iravani (2014) proposed that the exo-chemicals released from the bacteria may be present in the supernatant that acts as reducing agents to reduce the ions. The formation of AgNPs was further confirmed using UV-Vis Spectroscopy in the range of 200 to 700 nm.

Figure 2 shows the absorption spectra of AgNPs obtained by reaction of E. coli and AgNO$_3$. The result showed maximum absorption was observed at 300 nm. Considering similar protocols by Koliparambil et al. (2016) and Kushwaha et al. (2015), the peak was expected to be 380nm - 400 nm.

Antimicrobial Activity

The antimicrobial properties of synthesized AgNPs was tested against 3 bacteria and 1 fungus using different concentration (Table 1). The antimicrobial activity of AgNPs clearly increasing with increasing concentrations. AgNPs with 1 mg/ml, showed high antimicrobial activity against all tested strains, where zone of inhibition of B. subtilis, S. aureus, P. aeruginosa and A. niger was 12.7 ± 2.5, 13.7 ± 2.3, 14.3 ± 2.3 and 12.7 ± 2.1 respectively. At 0.1 mg/ml only P. aeruginosa and A. niger showed positive response. The diameter clearly decreased, and at 0.01 mg/ml concentration, the AgNPs were not effective at all against any microorganisms.

It was found that gram negative bacteria displayed better zone of inhibition compare to gram positive. Being a gram-negative bacterium, P. aeruginosa has a higher tolerance of external pressure due to their outer membrane. Yet this was explained by Slavin et al. (2017) the outer membrane layer in gram negative bacteria makes it easy for silver to bind, since they have a higher affinity than the peptidoglycan monomers. Therefore, gives an easy opportunity to destroy the bacteria. This makes the gram-negative bacteria susceptible to be destroyed even at lower concentration than gram positive bacteria (Jung et al., 2008). The fungi have a cell wall made of chitin molecules. Chitin has similar affinity to that of peptidoglycan to bind to the cell wall and enter the fungi. The antimicrobial mechanism of AgNPs has various theories. One mechanism is
Table 1: Antimicrobial activity of silver nanoparticle synthesized from *Escherichia coli*

| Organism                  | Mean Diameter of the clear zone/mm |
|---------------------------|------------------------------------|
|                           | Concentration of AgNPs (mg/ml)     |
|                           | 1 mg/ml | 0.1 mg/ml | 0.01 mg/ml | Positive control | Negative control |
| *Bacillus subtilis*       | 12.7 ± 2.5 | 0 ± 0  | 0 ± 0 | 28 ± 10.6 | 0 ± 0 |
| *Staphylococcus aureus*   | 13.7 ± 2.3 | 0 ± 0  | 0 ± 0 | 42.3 ± 4.6 | 0 ± 0 |
| *Pseudomonas aeruginosa*  | 14.3 ± 2.3 | 6 ± 5.3 | 0 ± 0 | 27.0 ± 20.0 | 0 ± 0 |
| *Aspergillus niger*       | 12.7 ± 2.1 | 6 ± 1  | 0 ± 0 | 0 ± 0 | 0 ± 0 |

that silver is toxic and has high affinity to bind to any component in the microbial cell wall. Once bound to the cell wall, silver can enter the cell and cause disruption of DNA during synthesis phase in the cell cycle (Jaffat et al., 2017).

**Cytotoxicity Activity**

Cytotoxicity activity of synthesized AgNPs on Hela cells are displayed in Figure 3. The result showed that the silver nanoparticles were able to inhibit the growth of Hela cells. In a dose dependent manner where, as the concentration increased the inhibition ability of AgNPs also increasing. Hence, AgNPs synthesized by *E. coli* can kill cancer cell. According to Praetorius and Mandal (2007), AgNPs are particles that are very small and can pass through the blood vessels to reach the cancer site. Once AgNPs reach the cancer cells it induces alterations in the cell morphology. Besides that’s, AgNPs decrease the metabolic activity and increase oxidative stress leading to DNA damage that eventually leads to apoptosis (Verma and Maheshwari, 2019).

**CONCLUSION**

Herein, we concluded that on the successful synthesis of silver nanoparticles by using *Escherichia coli* which is free from harmful reducing agents. The result of visual observation and UV-visible spectroscopy has confirmed the formation of AgNPs formation. Synthesized AgNPs showed excellent antimicrobial activity against gram positive, gram negative and fungi as well as excellent cytotoxic effects against Hela cell line. Thus, silver nanoparticles synthesized by using *Escherichia coli* could be a suitable antimicrobial and anticancer drug.

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**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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