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Bacterial enzyme-mediated synthesis of silver nanoparticles and antimicrobial activity

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Extracellular synthesis of silver nanoparticles (AgNPs) using bacteria has been explored for their unique physicochemical properties. Studies have shown that nitrate reductase enzyme catalyzes the bioreduction of Ag^+ to Ag^0 and formation of AgNPs. The objective of this study was to detect the presence/absence of nitrate reductase enzyme in selected bacteria and to study the formation of AgNPs. The antimicrobial activity of the biosynthesized AgNPs was also examined. *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* (confirmed clinical strain) and *Staphylococcus aureus* ATCC 25923 were cultured in Nutrient broth. After 72 h of incubation, $AgNO_3$ was added into the culture supernatant. AgNP formation was confirmed by Energy Dispersive X-ray analysis (EDX) and Transmission Electron Microscopy (TEM). For the nitrate reductase assay, heavy inocula of the above selected bacteria were inoculated in Nitrate broth and incubated at 37°C for 24 h. One dropper full of sulfanilic acid and α-naphthylamine were added to each tube and the colour change was observed. If no color change was observed a small amount of zinc (Zn) powder was added and the color change was observed. Well-diffusion method was performed to study the antimicrobial activity of the synthesized AgNPs against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 and selected clinical isolates of *P. aeruginosa*, *S. aureus* and *C. albicans*. Positive controls were 0.5% $AgNO_3$ and chemically synthesized AgNPs (0.436 mg/ml). All biosynthesized AgNPs were spherical in shape. The average sizes of the NPs were 11.14 ± 6.59 nm (*S. aureus*-NPs 0.435 mg/ml), 11.71 ± 2.73 nm (*P. aeruginosa*-NPs 0.45 mg/ml), 12.87 ± 2.95 nm (*E. coli*-NPs 0.99 mg/ml) and 12.22 ± 2.45 nm (*A. baumannii*-NPs 0.665 mg/ml). In general, zones of inhibition (ZOIs) given for chemically synthesized AgNPs, were higher than biosynthesized NPs. According to the well diffusion results, AgNPs produced by *S. aureus* resulted in the largest ZOI against the selected pathogens. Biosynthesized AgNPs were highly effective against Gram negative bacteria compared to Gram positive bacterial and fungal species, as well as *Candida albicans*, which were opportunistic pathogens. *A. baumannii*, *E. coli* and *S.aureus*, except *P. aeruginosa*, gave red colour after adding the two reagents and when Zn dust was added to *P. aeruginosa*, no colour change was observed. AgNP synthesis with a narrow size distribution was observed for all tested bacterial strains. AgNPs of *S. aureus* gave highest ZOI. Nitrate reduction was observed with all organisms. Further characterization of NPs is required to study the physical properties of silver NPs.

Keywords: Silver nanoparticles, TEM, Nitrate reductase

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