

Green synthesis and characterization of silver sulfide nanoparticles using *Bacillus safensis* strain GMS10 isolated from contaminated soil of gold mine

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Introduction

Nanotechnology creates enormous commercial possibilities on the production and usage of structures and systems by manipulation of individual atoms and molecules at the nanoscale. *Silver sulfide* (Ag_2S) nanoparticles are increasingly applied in various fields, including solar *cell batteries*, infrared detectors, novel *thermoelectric* temperature *sensors*, *and* antimicrobial therapy, due to their unique Optical, chemical, mechanical and electrical properties. This study aimed to isolate and identify the *silver-resistant bacteria and investigate on their potential in the biological synthesis of silver sulfide nanoparticles (Ag_2SNPs)*. Also, optimization of reaction parameters for obtaining Ag_2SNPs in nanometer size was *investigated*.

Materials & Methods

Soil samples were collected from contaminated soil of gold mine in the city of *Qorveh* in Kurdistan *Province* and were used as the source material for bacterial isolation. The samples were serially diluted in sterile 0.85 % NaCl and then plated onto Luria Bertani agar (LB) media supplemented with 0.5 mM *silver sulfate* (Ag_2SO_4) solution with shaking at 200 rpm and temperature 25° C. Tolerance pattern was *determined using the agar dilution method*. The bacterial isolate with the highest tolerance to silver sulfate was selected as the potent bacterial isolate for green synthesis of Ag_2SNPs under the resting cell strategy. *Preliminary* characterization of the Ag_2SNPs was *carried out* using visual observations and *UV-Visible spectroscopy*. *Scanning Electron Microscopy (SEM)* with Energy Dispersive X-Ray Analysis (EDX) was used to *determine size, morphology and elemental analysis of the nanoparticles*. *Fourier-transform infrared spectroscopy (FTIR) analysis* was performed to *determine the functional groups involved in the bioreduction of silver sulfate into Ag_2SNPs* . The bacterial isolate was identified using 16S rRNA gene sequence analysis, morphological and biochemical characteristics.

Results & discussion

Among the 18 bacterial isolates (GMS1-GMS10 and SW01-SW08) purified from soil samples using enrichment culture, *strain GMS10* showed the highest tolerance to the Ag_2SO_4 stock solution (5.25 mM) and was selected for the study and was found to have the ability to form Ag_2SNPs as observed by change in colour of the reaction from pale yellow to ***blackish-brown under resting cell culture***. The colour change observed for the extracellular biosynthesis was further confirmed by UV–Vis spectral analysis as part of primary confirmation. Ag_2SNPs are known to have an intense absorption peak in UV absorption spectra at around **332 nm** due to its surface plasmon excitation. The Selected Ag_2SNPs -producing strain GMS10 was further subjected to molecular identification by 16S rDNA sequencing-based method. The sequence data were subjected to BLAST analysis and the result showed its maximum identity of 99 % to various *Bacillus* sp. mainly *Bacillus safensis*. The 16S rDNA sequence of the isolate was submitted to NCBI under the accession number MW362307. Effect of reaction parameters on the yield, shape and size of the synthesized Ag_2SNPs , including initial Ag_2SO_4 concentration (0.5, 1, 1.5, 2, 2.5 mM), bacterium biomass concentration (5, 10, 15, 20, 25 g/l), and incubation time (12, 24, 36, 48, 60, 256 h) was assessed under resting cell strategy. The results showed *Bacillus safensis* strain GMS10 with highest tolerance to silver sulfate (50 mM) was able to synthesize ***spherical*** shape of Ag_2SNPs with an ***average size*** diameter of 22.2 nm ***under optimized conditions (1 mM silver sulfate, 15 g/L biomass) after 36 h incubation***. ***This study is the first report on the synthesis of Ag_2SNPs using *B. safensis*.***

Conclusion

The isolation of and characterization *silver sulfate* resistant *bacterial* strains from the contaminated soils of gold mine show that the new native ecosystems of Iran can have countless capabilities for microbial species. In this study, a new species of *Bacillus* was introduced, which *has* been considered to be potential candidate for synthesis of Ag_2SNPs . The Ag_2SNPs formed by the strain GMS10 was found to be stable with average size diameter 22.2 nm which indicate its potential applications.

Keywords: *B. safensis, Biosynthesis, Characterization, Silver sulfide nanoparticle, Soil*

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Declaration of conflict of interest:

The authors declare that there is no conflict of interest.