Advanced Research in Microbial Metabolites and Technology
ARMMT

Extracellular synthesis of silver nanoparticles by *Isoptericola variabilis* using rice bran

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Article Info

Document Type:
Research Paper

Received 27/03/2022
Received in revised form
10/04/2022
Accepted 19/04/2022

Keywords:
Green synthesis,
Silver nanoparticles,
Isoptericola variabilis,
antibacterial properties

Abstract

Green methods are environmentally friendly methods for the synthesis of nanoparticles. These methods use a wide range of biological and reducing agents produced by bacteria, fungi, yeasts, algae, and plants for making metal nanoparticles. The object of this study was the extracellular synthesis of silver nanoparticles using rice bran by *Isoptericola variabilis* and the subsequent comparison of the antibacterial activity of the synthesized optimized and non-optimized silver nanoparticles. Optimal conditions for producing silver nanoparticles were obtained using an experimental response surface methodology (RSM) design. Nanoparticles were characterized by SEM, FT-IR, and UV-visible spectroscopy. Antibacterial activity of the silver nanoparticles (AgNPs) was investigated using the disk diffusion method against *E.coli* on Mueller Hinton agar medium. The SEM images of the optimized AgNPs showed an increase in uniform generated spherical nanoparticles. In addition, optimizing the production conditions of nanoparticles not only developed their antibacterial activity but reduced their sensitivity threshold compared with synthetic nanoparticles in the initial conditions. The effective concentration of nanoparticles against *E. coli* decreased from 500 µg/mL to 100 µg/mL (a 5-fold reduction). In conclusion, silver nanoparticles can be produced by *Isoptericola variabilis*, and its optimization process not only led to increased productivity but also improved the antibacterial efficiency against *E. coli*.

1. Introduction

Metal nanoparticles have attracted attention in recent years due to their unique physical, chemical, and biological properties that may help solve various challenges in medicine, biology, and electronic fields. Bacterial resistance to antibiotics is one of the biggest challenges threatening the modern age of human health. Therefore, solutions such as controlling antibiotic use programs, improving overall health, and producing improved antimicrobial agents are required to limit increasing bacterial resistance. Accordingly, in recent years, the development of the production and application of nanoparticles as antimicrobial agents has been specifically considered (Neu, 1992). Although several chemical and physical

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DOI: 10.22104/ARMMT.2022.5619.1067

methods are primarily used for the production of nanoparticles, the use of toxic chemical compounds and hydrophobic solvents in the process of nanoparticle synthesis limits their application in clinical areas. Therefore, the development of biocompatible, safe, and efficient methods to produce nanoparticles is of particular importance. Environmentally friendly methods to synthesize nanoparticles are called green methods. These methods apply a wide range of redundant biological sources, such as bacteria, fungi, yeasts, algae, and plants, for making metal nanoparticles (Dhand et al., 2016; Gopinath et al., 2015; Nayak et al., 2016). These methods still face the challenge of finding optimal methods to produce uniform nanoparticles in a timely and cost-effective manner before they can become widely accepted.

Among the metal nanoparticles, silver nanoparticles (AgNPs) have attracted a lot of attention in various fields, including antimicrobial activity, treatment, biomolecular diagnostics, medical device coatings, and optical receivers. This is because AgNPs have strong antimicrobial activity against pathogens, while the free ions have very little toxicity to mammalian cells, foods, and tissues (Bosetti et al., 2002; Fernández et al., 2009).

Recently, a growing number of reports of green production of nanoparticles by various microorganisms, including *Fusarium oxysporum* (Gopinath et al., 2015), Soil Fungi (Devi & Joshi, 2012), *Neurospora intermedia* (Hamedi et al., 2014), *Pseudomonas aeruginosa* (Jeyaraj et al., 2013), *Klebsiella pneumoniae* (Kalpana & Lee, 2013), *Escherichia coli* (Gandhi & Khan, 2016), and *Bacillus marisflav* (Anthony et al., 2014) their antimicrobial effects have been published (Lara et al., 2010). The used culture media of these microorganisms were used as biological redundant for AgNPs production from Silver nitrate. However, the qualitative effect of optimizing the nanoparticle production process to better understand the characteristics and performance of synthesized nanoparticles, including their antibacterial properties, has been studied less. Therefore, the aim of this study was to evaluate the antibacterial effects of silver nanoparticles synthesized by biological method against *E. coli* before and after optimization process.

2. Materials and Methods

2.1. Chemical material and bacterial strains

Silver nitrate, chemicals, and culture media used other culture-producing nanoparticle reductant agents were purchased from Merck Co., Germany and Difco, America. Standard discs were purchased from the PATAN TEB Co., Iran. The supernatant of culture medium of IRSH1 and IDAH9 strains isolated from Iran's extreme areas and identified as *Isoptericola variabilis*, was used as redundant biological agents for extracellular synthesis of silver nanoparticles. The *E. coli* (PTCC1330*)* strain was obtained from the Persian Type Culture Collection (PTCC) at the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. the biosynthesis of silver nanoparticles was done using distilled water in all stages of the experiment.

2.2. Extracellular synthesis of silver nanoparticles and its optimization

The supernatant of the culture medium of the strain *Isoptericola variabilis* IRSH1 and IDAH9 were used for the extracellular synthesis of silver nanoparticles. For this purpose, the bacteria were cultured in a 250 ml Erlenmeyer flask containing the previously described liquid medium (Azizi et al., 2015). First, the Erlenmeyer flask containing the bacteria culture medium was incubated for 72 hours in the shaker incubator at 150 rpm and 50 °C. Then, bacterial supernatants were separated from the biomass by centrifugation at 4750 g for 15 minutes and used as a redundant biological

agent for the extracellular synthesis of silver nanoparticles.

To synthesize the initial silver nanoparticles, a 0.1 M concentration silver nitrate solution in a 250 ml Erlenmeyer flasks was first added to the bacterial supernatant until the final concentration of silver ions in the reaction solution reached 5 mM. Then, the Erlen flask containing the reaction solution was incubated for 48 hours in a shaker incubator at 110 rpm to obtain the initial colloid silver nanoparticles (Gurunathan et al., 2009). Nex, UV-Vis spectroscopies of the synthesized silver nanoparticles were done among 300-700 nm. To characterize the synthesized nanoparticle silver, the nanoparticles' average size and polydispersity index of the silver nanoparticles were determined using a dynamic light scattering instrument (Malvern, U.K.). The measurement was performed in the range of 0.1 nm to 104 n at 25 °C. In order to evaluate the appearance of the NPAgs, a scanning electron microscope SEM (VEGA3.Tescan, Czech) was used. To prepare the sample, a droplet of silver nanoparticle colloid was poured on an aluminum base and dried at room temperature; then, it was covered with a thin layer of gold and finally studied with an electron microscope at 20 kV (Hamedi et al., 2014).

Minitab 17 and Design Expert7 were used to perform optimization with Placket-Burman designs and response surface design methods. First, the factors that significantly affect the biosynthesis of silver nanoparticles were screened using the Placket–Burman method. Then, after the screening and identifying the factors affecting the construction of silver nanoparticles, nanoparticle synthesis was optimized using the response surface methodology (Fathima & Balakrishnan, 2014; Nyakundi & Padmanabhan, 2015).

2.3. Antibacterial effect of synthesized silver nanoparticles against microorganisms

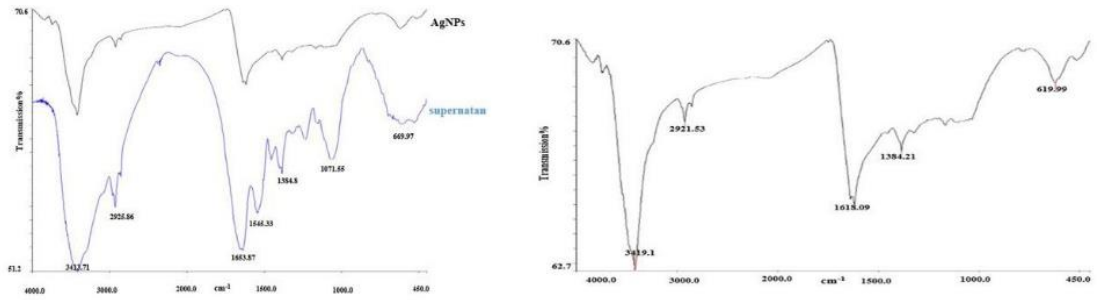
The antibacterial activity of the initial and optimized silver nanoparticles was investigated using the disk diffusion method (Kirby–Bauer) against *E.coli* bacteria using a suitable dilution of standard 0.5 McFarland (10^5-10^6 CFU/mL) on plates containing agar Mueller-Hinton medium incubated for 24 h at 35 °C. Then, on each of the discs 25 µl solution of silver nanoparticles with appropriate dilution was poured and placed on a Mueller-Hinton agar medium, while one disc containing the supernatant and AgNO3 solution at a concentration of 5mM was used as a control; the plates were incubated finally for 18 h at 35 °c (Priyadarshini et al., 2013; Reller et al., 2009; Shahverdi et al., 2007). All experiments to determine the diameter of the inhibition zone were performed three times. All results were reported as mean ± standard error. Data were analyzed using the Minitab17, Excel 2013 software, and statistical One-way ANOVA and Tukey tests.

3. Results and discussion

UV-Vis spectroscopy of the silver nanoparticles produced by the used isolates showed that *I. variabilis* IRSH1 produced AgNO3 more nanoparticles than *I. variabilis* IDAH9. Therefore, this strain was selected for further study. The FTIR results showed that the organic phytochemicals in the supernatant of the bacteria culture medium caused the bio-reduction of Ag+ ion to Ag^o (Fig 1).

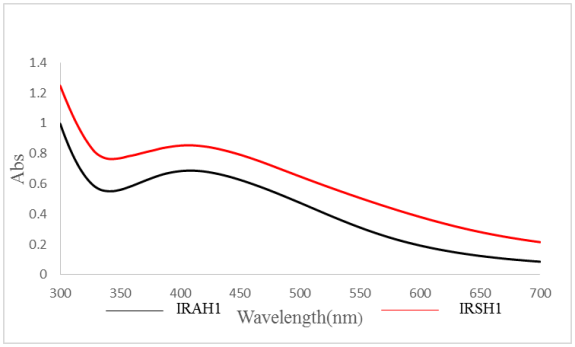
After the initial silver nanoparticles were biosynthesized and characterized, their production was optimized in two steps. Three significant factors, UV-Vis spectroscopy, average size, and polydispersity index, were determined in the first step (data not shown). Then, in the second step, the optimum conditions for the production of nanoparticles were determined. Finally, biosynthesis of silver nanoparticles was performed based on the optimum conditions. The results of UV-Vis spectroscopy showed that the silver nanoparticles optimized at a wavelength of 411 nm have the highest band absorption of surface plasmon resonance (SPR) of 1.55, while the initial

absorption was 0.85 (Figure 1). Furthermore, a comparison of UV-Visible absorption spectrums of colloidal silver nanoparticles from the reaction mixture at the initial and optimum conditions indicated that the band absorption for the optimized colloid nanoparticles is greater than the initial nanoparticles indicating the increase in the amount of nanoparticle production (Figure 1). (Hamedi et al., 2014).



A B

C



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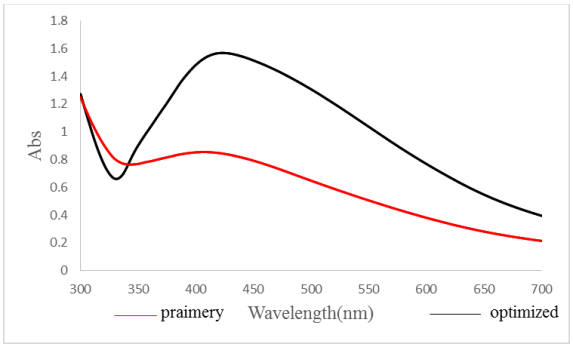

Figure 1. Evaluation of the silver nanoparticles synthesized by *Isoptericola variabilis* IRSH1 and IDAH9 using FT-IR spectroscopy of AgNPs and culture supernatants of bacteria (A, B) and the UV-Visible spectroscopy of colloidal silver nanoparticles resulting from the reaction mixture in the initial conditions (C) and optimum conditions (D).

After evaluating the number of silver nanoparticles made in the initial (basic) and optimum conditions, One Way ANOVA analysis ($p = 0.000$) was used to determine if there was a significant difference between the two. According to the values obtained in optimum conditions, optimizing silver nanoparticle production led to a 2.65-fold increase compared to initial conditions (Fig. 1).

Following production, the silver nanoparticles from both the basic and optimum conditions were characterized. Micrographs obtained by electron microscopy showed the optimized silver nanoparticles had dimensions less than 100 nm (average 65 nm) and were almost uniformly spherically, while in the initial state, particles were more heterogeneous and aggregated together (Figure 2).

Dynamic light scattering (DLS)_analysis was performed to study the polydispersity index and the average size of silver nanoparticles. Based on the results, the particle size distribution based on the size and number of silver nanoparticles synthesized in optimum conditions were determined for the 67 nm (47%) and 61 nm (97.7%), respectively.

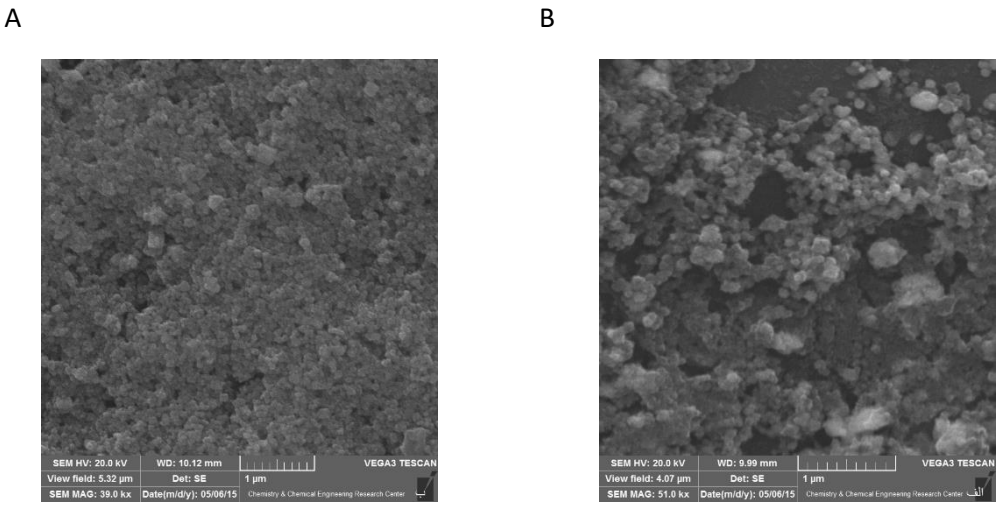

Figure 2. SEM images of silver nanoparticles synthesized by the strain *Isoptericola variabilis*, (A) IRSH1 Initial condition and (B) optimum condition.

Changes in their antibacterial property were evaluated as an indicator of activity to assess the increased efficiency of the synthesized silver nanoparticles. For this purpose, the diameter of the inhibition zone for the bacteria *E. coli* in different concentrations of silver nanoparticles synthesized in the initial and optimum conditions was determined using the standard disk diffusion methodology (Kirby-Bauer). The results showed that the silver nanoparticles synthesized in optimum conditions have a strong antibacterial effect against *E. coli* compared to synthesized nanoparticles in initial conditions (Fig. 3).

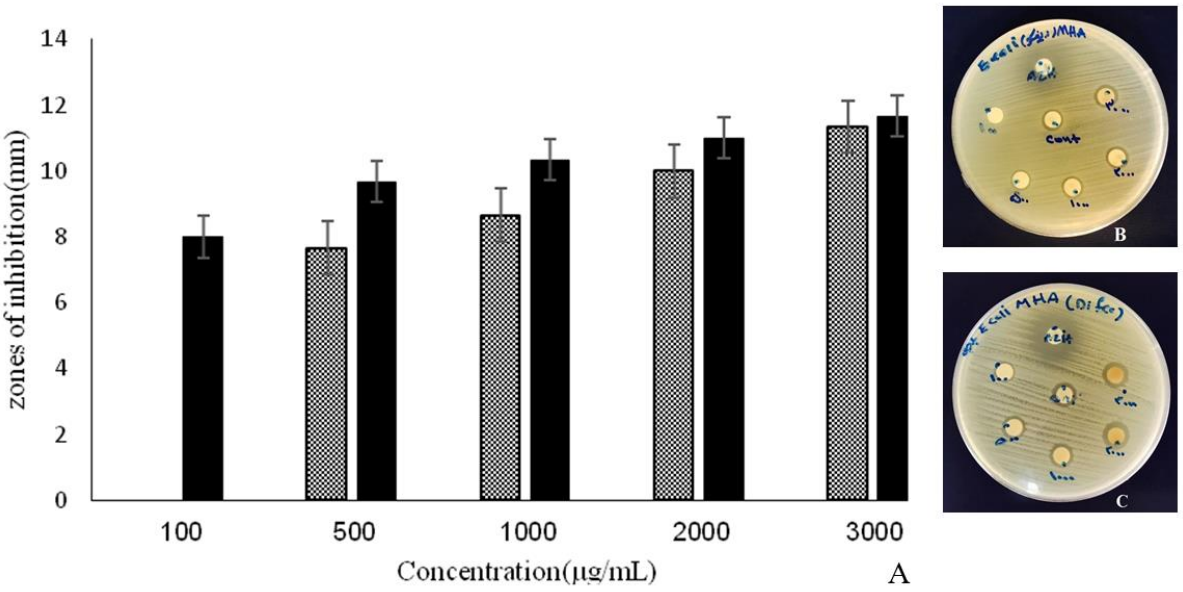

Figure 3. Comparison of antibacterial effect (A) shows the inhibition zone of the initial (gray columns) and optimized (black columns) silver nanoparticles synthesized in (B) initial and (C) optimum conditions against *E.Coli* at concentrations of 100, 500, 1000, 2000, and 3000 µg/mL.

Although several studies have been conducted on the antibacterial effect of silver nanoparticles, few studies have been done on optimizing silver nanoparticles and assessing their antibacterial

effect (Fathima & Balakrishnan, 2014; Nyakundi & Padmanabhan, 2015). Singh et al. produced the optimized silver nanoparticles from *Penicillium* fungus supernatant using the extracellular biologic synthesis method.

Their results showed that the nanoparticles produced had an average size of 25 to 30 nm, 80 µL (vs. 25 µL in this study) of a concentration of 0.05 mM, an inhibitory effect on the growth of the bacteria *E. coli* and *Staphylococcus aureus*, and the diameter of the growth inhibition zone for these bacteria was 17 and 16 mm, respectively (Singh et al., 2014). In the study conducted by Nayak et al. (2016), silver nanoparticles produced by a 'green' method from bark extracts of *Ficus benghalensis* and *Azadirachta indica* had an average size of 90.13 nm, 100 µL of nanoparticles with a concentration of 100 µg/mL had an inhibitory effect on the growth of *E coli* and *Pseudomonas aeruginosa*, and the inhibition zone diameter for these bacteria was 14 mm (Nayak et al., 2016).

Conclusion:

Based on the results of this study, the extracellular synthesis of silver nanoparticles using rice bran was contracted by *Isoptericola variabilis*, and the primary optimization of the AgNPs production significantly showed the increase in the amount of production of the silver nanoparticles with biological methods. Furthermore, in terms of increasing the efficiency of silver nanoparticles synthesized, nanoparticles produced in optimum conditions have a higher antibacterial effect than those synthesized in the initial conditions. In other words, the optimization process decreased the sensitivity threshold of silver nanoparticles required against *E. coli* to a fifth of the initial value (1/5).

Nowadays, the green synthesis of AgNPs is considered a safe and eco-friendly method (Ssekatawa et al., 2021). In conclusion, silver nanoparticles can be extracellularly produced by *Isoptericola variabilis*. Moreover, its optimization process leads to an increase in AgNPs productivity and improves its antibacterial efficiency against *E. coli*. Therefore, further studies are necessary on this potentially powerful tool in the fight against pathogens.

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgments

Iranian Research Organization for Science and Technology (IROST) is appreciated due to providing the facilities and opportunities for this research.

Ethical approval

This article does not contain any studies with human participants or animals.

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