Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.2S,
July-December 2024: P. 1237-1242

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article
Content Available online at: www.bpasjournals.com

INTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES USING FOOD PROBIOTIC LACTOBACILLUS REUTERI WITH CHARECTERIZATIONS

Sara Ibrahim Othman[1]*, Fouad H. Kamil[2]†
1Departments of Biology, College of Science, University of Salahddin, Erbil, Kurdistan Region/ Iraq
2Department of Medical Laboratory Techniques, Erbil Medical Technical Institute, Erbil Polytechnic University, Erbil/ Iraq
†*Corresponding authors: sara.othman@epu.edu.iqfouad.kamel@epu.edu.iq;
S.I.O: https://orcid.org/0000-0003-1689-9068 F.H.K: https://orcid.org/0000-0001-5302-9200

Abstract
A green production of silver nanoparticles (AgNps) utilizing *Lactobacillus reuteri* is described in this work. Analysis using a scanning electron microscope, transmission electron microscope, and atomic force microscope revealed that the characterized AgNps had a nearly spherical shape. The nanoparticles exhibited a semi-textured surface, with certain particles showing surface protrusions and clearly defined edges.

Keywords: Silver nanoparticles, biosynthesis, *L.reuteri*, SEM, TEM, AFM analyses.

Introduction

By employing microorganisms [1, 2] or plant extracts [3-5], green biosynthesis techniques provide a straightforward and appealing alternative to conventional synthetic chemical and physical approaches for nanoparticle formation. Biological methods frequently include natural coating agents in the extracts to enhance nanoparticle stability and hence improve their antibacterial activity in comparison to uncoated counterparts [6, 7] Nevertheless, whereas microbes and plant extracts offer benefits, each method poses distinct difficulties. Strict control of microbial cultures can effectively reduce undesirable impurities that may impact the production or stability of nanoparticles. Several research groups have reported the effective production of AgNps using a biogenic method that involves microorganism isolates [1, 2, 8, 9]. Jalal et al. The distinctive physicochemical and biological characteristics of nanomaterials (with dimensions ranging from 1-100 nm) have made them a viable frontier for biomedical applications [10]These characteristics enable their application in many fields, including tissue engineering, medication administration, antibacterial treatments [11, 12], and medical imaging. Significantly, nanoparticles, whether administered alone or in conjunction with traditional antibiotics, present promising opportunities for the advancement of innovative nanomedicines. Metal nanoparticles, particularly silver nanoparticles (AgNps), have attracted considerable interest due to their strong antibacterial properties against a wide range of pathogens, such as fungus, viruses, and bacteria [3, 13]. Contemporary methodologies for the synthesis of AgNps include a

range of chemical and physical processes that allow for the formation of several shapes, including nanowires, nanorods, nanocubes, and nanowhiskers [5, 14]. Although traditional physical procedures have restricted yields of AgNPs, the chemical reduction approach is still the most commonly used technology. Nevertheless, this method sometimes includes the use of dangerous substances such as reducing agents, stabilizers, and organic solvents [15, 16]. Consequently, it is imperative to devise more ecologically sustainable, non-hazardous, and economically efficient methods for the synthesis of AgNps. These new techniques should ideally be environmentally friendly and straightforward.

Building on the existing information, the present study examines the application of an *L. reuteri* isolate as a stabilizing and reducing agent in the bioinspired formulation of silver nanoparticles. A comprehensive range of techniques, including scanning electron microscopy, transmission electron microscope, and atomic force microscope investigations, were used to precisely establish the physical and chemical characteristics of the biosynthesized AgNps.

Experimental

Materials and bacterial isolates

All chemicals and reagents used in the study were commercially available and were obtained from BDH (England). These included silver nitrate ($AgNO_3$), nutritional agar, and Muller-Hinton agar. The probiotic microorganism, Lactococcus. The JCM 1112 strain of reuteri was acquired at a municipal market in Erbil, Iraq. Upon the acquisition, a sequence of tests verified its identity.

Culturing and extraction of L. reuteri biocomponents

The biochemical constituents of the probiotic microorganism *L. reuteri* were collected using a previously outlined procedure [2] with minor adjustments. In brief, a solitary. colony of *L reuteri* was introduced into a specified volume of MRS broth medium. To ensure adequate bacterial growth, the culture was subsequently incubated at 37°C for 72 hours. Next, the culture was subjected to centrifugation at 10,000 revolutions per minute for 45 minutes in order to isolate the cell-free intracellular biomass from the bacterial cells. The collected supernatant, which contains the *L. reuteri* biocomponents were kept at 4°C until they were used as an as an additional in the synthesis of AgNps.

Bioinspired synthesis of silver nanoparticles using L. reuteri isolate

Bioinspired methodology was used to synthesize AgNps utilizing *L. reuteri* isolate. Briefly, 5 mL of cell-free *L. reuteri* isolate was added to a 95 mL solution containing 1 mM $AgNO_3$. The mixture underwent sonication to facilitate the interaction between the bacterial components and silver ions. The sonication parameters were set at a flow rate of 0.2 mL/min, ultrasonic power of 100 W, and frequency of 42 kHz for 20 minutes. Following sonication, the mixture was stirred at 800 rpm and maintained at 25°C for 30 minutes under dark conditions to promote the reduction process. The solution was then stored in dark bottles for 24 hours. To isolate the synthesized AgNps, the solution was centrifuged at 10,000 rpm for 10 minutes at 4°C. This step separated the AgNps from the remaining

biological components. The pellet containing AgNps was subsequently washed with deionized distilled water to remove any residual bacterial components. The final product, a colloidal suspension of AgNps, was stored in opaque containers to prevent light exposure. A color change from a slightly yellowish solution (*L. reuteri* suspension) to a translucent dark brownish color indicated the successful formation of AgNps.

SEM, TEM, and Atomic Force Microscopy (AFM)

The morphology and size of the biosynthesized AgNPs were characterized using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). TEM analysis was performed with a ZEISS instrument (LEO 906 E, Oberkochen, Germany) operating at an accelerating voltage of 80 kV. This technique provided high-resolution micrographs for detailed observation of the AgNP morphology and size distribution. The FE-SEM analysis was conducted using a ZEISS Gemini (Oberkochen, Germany) to acquire complementary information on the AgNps surface features. ImageJ software was then employed for quantitative analysis of particle size and morphology based on the TEM and SEM micrographs. The size, shape, and surface topography of the AgNps were further characterized using atomic force microscopy (AFM) from Oxford Instruments (UK). This technique provided high-resolution imaging data, complementing the information obtained from other characterization methods.

Results and discussion

Cell-free extract from *L. reuteri* containing diverse metabolites (enzymes, organic acids) mediatedAgNps formation, as confirmed by color change and characterization techniques (AFM, SEM, and TEM). These metabolites likely functioned as reducing and capping agents.

Characterization of biosynthesized AgNps

AFM analysis of AgNps

Fig.1 presents the results obtained from AFM analysis of the biosynthesized AgNps. The picture displays great heterogeneity in the morphology of the AgNps. Notably, the particles have a slightly rough surface texture with visible protrusions, implying some degree of roughness.

Additionally, some AgNps appear to have uneven spherical forms rather than ideal spheres, presumably reflecting inconsistent development patterns. The average height of the surface protrusions is around 48.07 nm, with a total particle height of 105.4 nm. The breadth, however, ranges from 1.56 nm to 2.98 nm, possibly because of the uneven forms. The measured particle size distribution varies from 71.93 nm to 107.1 nm, with an average size of 89.65 nm. These data addressing the morphological variety and morphologies of the AgNps are comparable with studies reported by Rasheed et al. [17]. In their investigation, AgNps produced using Artemisia vulgaris also demonstrated morphological variation and polydispersity. This shows that the biosynthetic process might naturally alter the morphology and size distribution of the resultant nanoparticles, the AgNps.

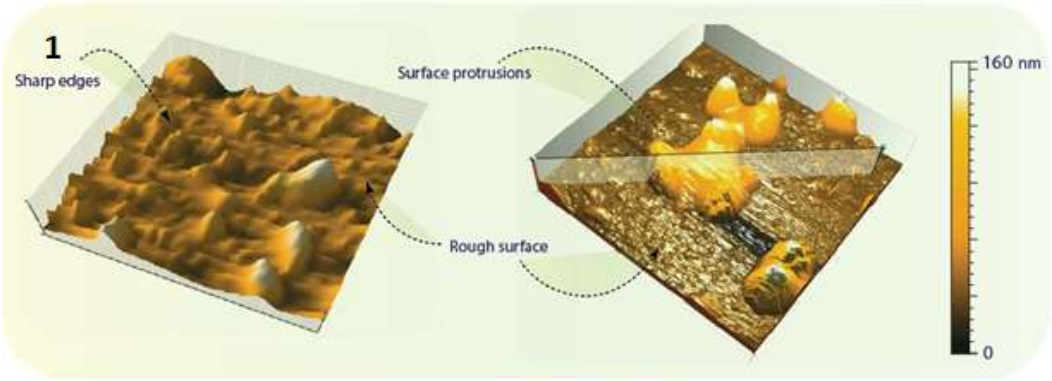

Fig.1. AFM (b)

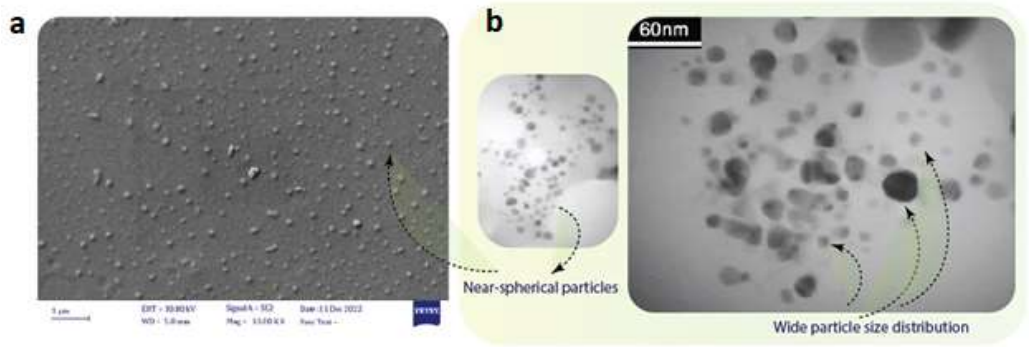

Fig.2. (a) SEM (b) TEM micrographs of AgNps

SEM and TEM analysis of AgNps

Fig.2a depicts the SEM micrograph of the biosynthesized AgNps. The picture mostly exhibits near-spherical nanoparticles with minor signs of agglomeration or aggregation. This data correlates well with the zeta potential measurements, demonstrating mild electrostatic repulsion between AgNps particles, preventing aggregation and sedimentation, thereby leading to a stable AgNps suspension. Upon careful analysis using ImageJ software (see inset, Fig. 2a), the AgNps show a certain level of roughness, with certain particles exhibiting sharp edges and surface protrusions.
The transmission electron microscopy (TEM) study (Fig.2b) showed a broader range of sizes for the AgNps compared to scanning electron microscopy (SEM), spanning from 25.29 nm to 109.8 nm assessed using ImageJ software. Both scanning electron microscopy (SEM) and atomic force microscopy (AFM) investigations support this wider size distribution. Crucially, a significant number of the AgNps exhibited reduced dimensions, resulting in an increased ratio of surface area to volume.

Conclusion

The present work effectively showcased the environmentally friendly production of silver nanoparticles (AgNps) by the utilization of *L. reuteri*. Characterization identified AgNps as spherical. The scanning electron microscopy (SEM) image mostly shows very small nanoparticles with little signs of clustering or aggregation. Transmission electron microscopy (TEM) examination showed a broader range of sizes for the silver nanoparticles (AgNps) compared to the SEM measurementsReferences

1. Jalal, M., et al., *Biosynthesis of silver nanoparticles from oropharyngeal Candida glabrata isolates and their antimicrobial activity against clinical strains of bacteria and fungi.* 2018. 8(8): p. 586.

2. Tharani, S., et al., *Extracellular green synthesis of chitosan-silver nanoparticles using Lactobacillus reuteri for antibacterial applications.* 2020. 30: p. 101838.

3. Ohiduzzaman, M., M. Khan, and K.J.J.o.M.S. Khan, *Green synthesis of Carica papaya mediated silver nanoparticles: Characterization, antibacterial activity, and bioelectricity generation for sustainable applications in nanotechnology.* 2024. 1317: p. 139141.

4. Nassar, M.Y., et al., *Synthesis and Characterization of Lemon Leaf Extract-Mediated Silver Nanoparticles: An Environmentally Friendly Approach with Enhanced Antibacterial Efficacy.* 2024: p. 138753.

5. Khalaf, Y.H., Y. Dawood, and A.A.J.R.i.C. Khashan, *Green biosynthesis of berberine-mediated silver nanorods: their protective and antidiabetic effects in streptozotocin-induced diabetic rats.* 2023. 5: p. 100722.

6. Nguyen, K.C., et al. *Comparison of toxicity of uncoated and coated silver nanoparticles*. in *Journal of Physics: Conference Series*. 2013. IOP Publishing.

7. Ferreira, A.M., et al., *How similar is the antibacterial activity of silver nanoparticles coated with different capping agents?* 2023. 13(16): p. 10542-10555.

8. Mustapha, T., et al., *A review on plants and microorganisms mediated synthesis of silver nanoparticles, role of plants metabolites and applications.* 2022. 19(2): p. 674.

9. Anandaradje, A., et al., *Microbial synthesis of silver nanoparticles and their biological potential.* 2020: p. 99-133.

10. Mabrouk, M., et al., *Nanomaterials for biomedical applications: production, characterisations, recent trends and difficulties.* 2021. 26(4): p. 1077.

11. Díez-Pascual, A.M.J.N., *Recent progress in antimicrobial nanomaterials.* 2020. 10(11): p. 2315.

12. Owida, H.A., et al., *Carbon nanomaterials advancements for biomedical applications.* 2023. 12(2): p. 891-901.

13. Sabzevar, A.H., F. Aflakian, and G.J.J.o.M.S. Hashemitabar, *Characterization and evaluation of antibacterial, antioxidant and cytotoxic activities of green synthesized silver nanoparticles using Haloxylon persicum.* 2024. 1304: p. 137615.

14. Ha, H., et al., *Mini review of synthesis strategies of silver nanowires and their applications.* 2022. 50: p. 100663.

5. Rekha, C., et al., *Synthesis of highly stable silver nanorods and their application as SERS substrates.* 2018. 3(2): p. 196-205.

16. Zhu, Y.-p., et al., *Sonochemical synthesis of silver nanorods by reduction of sliver nitrate in aqueous solution.* 2010. 17(4): p. 675-679.

17. Rasheed, T., et al., *Green biosynthesis of silver nanoparticles using leaves extract of Artemisia vulgaris and their potential biomedical applications.* 2017. 158: p. 408-415.