Highly efficient *in vitro* biosynthesis of silver nanoparticles using *Lysinibacillus sphaericus* MR-1 and their characterization

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Abstract

Silver nanoparticles (AgNPs) have been widely used in diverse fields due to their superior properties. Currently the biosynthesis of AgNPs is in the limelight of modern nanotechnology because of its green properties. However, relatively low yield and inefficiency diminish the prospect of applying these biosynthesized AgNPs. In this work, a rapid mass AgNP biosynthesis method using the cell-free extract of a novel bacterial strain, *Lysinibacillus sphaericus* MR-1, which has been isolated from a chemical fertilizer plant, is reported. In addition, the optimum synthesis conditions of AgNPs were investigated. The optimum pH, temperature, dosage, and reaction time were 12, 70 °C, 20 mM AgNO₃, and 75 min, respectively. Finally, AgNPs were characterized by optical absorption spectroscopy, zeta potential and size distribution analysis, x-ray diffraction, electron microscopy, and energy-dispersive x-ray spectroscopy. The results revealed that these biosynthesized AgNPs were bimolecular covered, stable, well-dispersed face centered cubic (fcc) spherical crystalline particles with diameters in the range 5–20 nm. The advantages of this approach are its simplicity, high efficiency, and eco-friendly and cost-effective features.

Keywords: silver nanoparticles, green synthesis, *Lysinibacillus sphaericus*, biomaterials, characterizations

1. Introduction

Over the last few decades, silver nanoparticles (AgNPs) have received substantial attention due to their attractive electronic, chemical, and optical properties [1, 2], and they have been widely used in many fields, including catalysis, optical sensing, and electronics [3, 4]. Currently multi-drug resistance is a growing problem in the treatment of infectious diseases. AgNPs have been applied to disinfection and therapeutics, such as infected burn and wound reduction, medical device sterilization, tumor therapy, and cardiovascular implants [5, 6]. AgNPs are also incorporated into daily-life products, such as apparel, cosmetics, and plastics because of their antimicrobial properties [7, 8]. The antimicrobial activity of AgNPs makes them an excellent choice for multiple uses in the medical field.

AgNPs are generally synthesized by physical and chemical methods [9]. Although these two methods have been able to efficiently produce large quantities of AgNPs with a defined size and shape, they are expensive and often involve the use of toxic and hazardous chemicals, which pose an environmental risk [10]. Furthermore, other problems have generally been associated with these two conventional
synthetic routes, such as the aggregation of AgNPs [11, 12]. Therefore, there is growing awareness of the need to develop environmentally friendly and sustainable methods. Microbial synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and microbial biotechnology. Following the initial report on the formation of AgNPs in Pseudomonas stutzeri [13], many reports on the synthesis of AgNPs using fungi or bacteria have appeared in the literature [14]. Using microorganisms, especially their cell-free extracts, for the synthesis of AgNPs can be advantageous compared with other biological processes because microbial resources are abundant in nature, are easy to culture, and have the potential to be scaled up for large-scale synthesis. However, the biosynthesis of AgNPs by microorganisms has usually involved the use of a low concentration of Ag⁺ (i.e., 1 mM) and a long reaction time (on the order of hours or days), which have been two major obstacles to rapid production [15]. Syed and coworkers have reported the reduction of 1 mM of AgNO₃ into AgNPs within 96 h via the mycelia of the thermophilic fungus Humicola sp. [16]. Qian et al have investigated the assisted synthesis of AgNPs in 24 h via the cell filtrate of the endophytic fungus Epichocym nigrum by using 1 mM of AgNO₃ [17]. Also, Malhotra and coauthors have revealed the biosynthesis of AgNPs in 16 h using 1 mM of AgNO₃ via the cell-free supernatant of a novel marine strain of Stenotrophomonas [18]. Consequently, for the purpose of commercial use, the inefficiency and low yield of AgNP biosynthesis need to be overcome urgently.

In this study, a novel approach to the highly efficient and rapid biosynthesis of metallic AgNPs using the cell-free extract of a novel bacterial strain, Lysinibacillus sphaericus MR-1, was investigated. The cell-free extract of L. sphaericus MR-1 reduced the high concentration of Ag⁺ into AgNPs within several tens of minutes through the use of 20 mM of AgNO₃. The biosynthesized AgNPs were characterized by various techniques such as ultraviolet-visible absorption spectroscopy (UV–vis), Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), field-emission scanning electron microscopy (FESEM) combined with energy-dispersive x-ray spectroscopy (EDX), and high-resolution transmission electron microscopy (HRTEM).

2. Material and methods

2.1. Strain, medium, and chemicals

The strain was isolated from soil samples collected from a chemical fertilizer plant in Huaian by the dilution plate technique on a nutrient agar plate (1% peptone, 0.3% beef extract, 0.5% NaCl, 2% agar, pH 7.5) at 37 °C. It was identified by 16 S rRNA gene sequencing performed by Sangon Biotech (Shanghai) Co., Ltd. A yeast extract, peptone, and KNO₃ (YPK) medium (pH 7.5), which consisted of 0.15% yeast extract, 0.25% peptone, and 0.1% KNO₃, was employed to culture the isolate. AgNO₃ was purchased from Sinopharm Chemical Reagent Co., Ltd. All the reagents were of analytical grade and were used as purchased without any further purification.

2.2. Cell-free extract preparation

For inoculum preparation, a single clone of the isolate was transferred from the nutrient agar plate into 100 ml of sterile nutrient broth in a 250 ml Erlenmeyer flask. The flask was incubated at 37 °C for 12 h on a rotary shaker at 200 rpm. Then 10 ml of mid-log phase culture (optical density at 600 nm OD600 = 1, monitored by UV–vis spectrophotometer) were inoculated into 1000 ml Erlenmeyer flasks containing 500 ml of YPK medium. The inoculated flasks were incubated at 37 °C and shaken at 200 rpm again for 36 h, and then the cell-free extract was obtained by centrifugation (10 000 rpm, 10 min at room temperature) and decantation.

2.3. Biosynthesis of AgNPs

For the biosynthesis of the AgNPs, 0.085 g of solid AgNO₃ was added into 100 ml of cell-free extract in 250 ml Erlenmeyer flasks. The reaction was carried out at 45 °C overnight in the dark on the rotary shakers. The YPK medium with the solid AgNO₃ and the cell-free extract without the addition of the AgNO₃ were treated under the same conditions as the controls. The visual color change in the reaction mixture from light yellow to dark brown was observed overnight with reference to the controls. The formation of silver nanoparticles was confirmed by UV–vis spectrophotscopy.

2.4. Optimization of the AgNP synthesis conditions

The effect of three variables (substrate concentration, pH, and temperature) on the production of AgNPs was optimized by varying one parameter at a time, such as the substrate concentration (1–20 mM solid AgNO₃), pH (6, 7, 8, 8.5, 9, 10, 11, 12, and 13), and temperature (20, 30, 35, 40, 45, 50, 60, 70, 80, and 90 °C). After biosynthesis, 1 ml of the AgNP mixture was taken out and centrifuged, and the pellets were washed three times. Then the pellets were re-suspended in 1 ml of deionized water with pipettes, and the solution was diluted with 10 folders and characterized with UV–vis. Under optimal conditions, the efficiency of the AgNP synthesis was evaluated by characterizing the samples made at different time intervals (0, 15, 30, 45, 60, 75, 90, 120, 180, and 240 min).

2.5. Characterization of AgNPs

The AgNP mixture was centrifuged at 10 000 rpm for 30 min to isolate the AgNPs from free proteins or other compounds present in the solution, following which the pellets were re-dispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The process of centrifugation and re-dispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the mixture. The localized surface plasmon resonance (SPR) of the AgNPs was characterized with a UV–vis spectrophotometer (UV-2401PC, Shimadzu, Japan) at a resolution of...
1 nm in a wavelength range between 300 and 600 nm. The hydrodynamic diameter and the zeta potential of the AgNPs were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS 90 (Worcestershire, UK). HRTEM images were recorded using a Tecnai G2 F30 S-TWIN microscope operated at an accelerating voltage of 200 kV. Samples for HRTEM imaging were prepared by placing a drop of the solution sample in deionized water onto a carbon-coated Cu grid, drying it in air, and loading it into the electron microscope chamber. Then the purified pellets were freeze-dried, and the powders were subjected to FTIR and XRD measurement. The Fourier transform infrared spectra were recorded by a Nicolet 5700, Thermo Electron Co., USA, in the range 400–4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). X-ray powder diffraction measurements were carried out on a Bruker D8 Advance (Germany) instrument in Bragg–Brentano mode with Cu K\(\alpha\) radiation from 35 to 90° 2\(\theta\). These purified solid AgNPs were further ultrasonically dispersed with ethanol, dropped on glass slides, air dried, and examined by FESEM combined with EDX (Quanta 250, USA). The numerical data of these characterizations were processed by the software package Origin Pro 8.5.

3. Results

3.1. Morphology and molecular analysis of the isolate

The pure pale-white bacterial X11 (figure 1(a)) obtained on the nutrient agar plate was identified as \emph{L. sphaericus} based on molecular analysis through 16 S rRNA sequencing studies (figure 1(b)) and was named \emph{L. sphaericus} MR-1.

3.2. Biosynthesis of AgNPs

In the experiment, the formation of AgNPs was visually confirmed by the color change of the mixture from pale yellow to dark brown. This change did not occur in the negative controls (figure 2(a)). The preliminary investigation of the biosynthesized AgNPs was carried out by UV–vis spectroscopic analysis. It can be readily observed that the characteristic surface plasmon resonance band peak of the mixture was at 416 nm (figure 2(b)), indicating the presence of AgNPs [19].

3.3. Optimization of the AgNP synthesis conditions

While the effect of temperature on the synthesis of AgNPs was being investigated, it was found that the maximum absorbance of the reaction mixture had increased from 20 to 70 °C, whereas it had decreased from 70 to 90 °C. As shown in the inset of figure 3(a), a nearly linear relationship between the maximum absorbance and the temperature in the range 20–70 °C was presented. The result suggested that moderately elevated temperature accelerated the reduction process.

In general, the reduction reaction of metallic ions is sensitive to the pH of a solution [20, 21]. The current study involved a systematic analysis of pH-dependent changes in the reaction mixture for the synthesis of AgNPs. It was observed that the maximum absorbance had increased when pH increased from 6 to 12 (figure 3(b)). The result indicated that an alkaline pH favored the formation of AgNPs.

In addition, we evaluated the effect of different concentrations of AgNO\(_3\) on the synthesis of AgNPs. The maximum synthesis of AgNPs occurred with respect to Ag\(^+\) concentration in the range 18–20 mM (figure 4(a)), and a few earlier researchers also showed that optimum AgNP accumulation occurred under this condition [22].

The UV–vis spectrum of AgNP synthesis under optimal conditions as a function of time is shown in figure 4(b). After 45, 60, and 75 min, the reduction was 71.5%, 96%, and 100% completed, respectively. It can be seen that the reduction was quite rapid and was much faster than previously reported bioreduction processes, which were on the order of hours or days, and was comparable to or faster than many chemical or physical methods [15, 23].

3.4. Characterization of AgNPs

The typical XRD pattern of the biosynthesized AgNPs is shown in figure 5(a). Five diffraction peaks at 20 values of 38.116°, 44.277°, 64.426°, 77.472°, and 81.536°, corresponding to the d-spacing values 2.359, 2.044, 1.445, 1.231, and 1.180 Å of the AgNPs, were observed. They were assigned to the (1 1 1), (2 0 0), (2 2 0), (3 1 1), and (2 2 2) crystalline planes of the face centered cubic (fcc) crystalline matter.
structure of metallic silver, respectively (JCPDS file no. 00-004-0783). The broad nature of the XRD peaks could be attributed to the nanocrystalline nature of the AgNPs. The small peaks appeared to have possibly originated from the AgCl or Ag₂O crystals in the sample. The surface chemistry of the biosynthesized AgNPs was investigated using FTIR spectroscopy (figure 5(b)). The peaks seen at 3739.3, 3410.9, 2947.9, 2436.0, 1602.6, and 1383.1 cm⁻¹ were assigned to the O–H, N–H, –O–CN, –CN, C≡C, and C–O groups, respectively [24–26]. These functional groups may have an effective role in the green synthesis of AgNPs. The overall result confirmed the presence of bimolecular in the sample. It is reported that proteins can bind to nanoparticles through either free amine groups or cysteine residues in the proteins [29]. Therefore, reduction and stabilization of the AgNPs by proteins in the cell-free extract may have occurred in this procedure.

The average size distribution of silver nanoparticles in colloidal solution was found to be 14.8 ± 1.2 nm (figure 6(a)).
observed in the current study that represents the ideal surface charge (figure 6(b)). A high absolute value of zeta potential denotes a high electrical charge on the surface of AgNPs, which can cause a strong repulsive force among the particles to prevent agglomeration and which thus might be responsible for the stable nature of the AgNPs.

An FESEM image of the AgNPs synthesized under optimum conditions using 20 mM of silver nitrate solution revealed that the particles were spherical and well dispersed and had diameters of 5–20 nm (figure 7(a)). The EDX spectrum (figure 7(b)) confirmed the formation of AgNPs. The presence of Cl may have come from the glass slides used for the EDX sample preparation. The HRTEM images (figure 8) further demonstrated the nature of AgNPs. The particle diameter was around 5–10 nm. The size seemed a little smaller than that revealed by the FESEM image and size distribution analysis, which might be explained as a result of the bimolecular coating on the AgNPs.

4. Discussion

Biogenic silver nanoparticles are an interesting alternative to chemically and physically produced AgNPs due to their green properties. But to be commercially available and able to compete with chemically and physically synthesized AgNPs, the production of biogenic AgNPs needs to be improved. This can be achieved by carefully choosing biological resources such as plants and microorganisms. Some plants have been used to produce AgNPs with high yield. For example, Maria.
et al. used the extract of the stem bark of Z. xylopyrus obtained by the reflux extraction method to synthesize AgNPs. Under the condition of pH 11, 28 ± 2 °C, and 10 mM AgNO₃, the extract reduced about 60% of the AgNO₃ into 60–70 nm AgNPs after 24 h [30]. However, large-scale production of biogenic AgNPs by the microbial method has not been explored so far. In this paper, the cell-free extract of Lysinibacillus sphaericus MR-1 was used to synthesize AgNPs. Under the condition of pH 12, 70 °C, and 20 mM of AgNO₃, the extract reduced AgNO₃ into 5–20 nm AgNPs in 75 min. Since the reducing agent (cell-free extract of L. sphaericus MR-1) can be easily obtained through fermentation and this microorganism resource is a renewable green material, this method for biosynthesis of AgNPs can be suitably scaled up for large-scale commercial synthesis.

In his review Berry concluded that L. sphaericus bacteria were usually used as an insect pathogen because of their parasporal crystal (BT) endotoxins [31]. Also, Fayaz et al. investigated the synergistic effect of biosynthesized AgNPs and antibiotics [32]. Since AgNPs have a significant antimicrobial effect, the discovery of the considerable capability of this strain to synthesize AgNPs may open the door to developing multifunctional agriculture products. For example, a product functions as an insecticide and at the same time an anti-pathogen. Liong et al. and Otsuka et al. used modified silver nanoparticles synthesized by a chemical method as drug carriers [33, 34]. Since the AgNPs synthesized by the cell-free extract of L. sphaericus MR-1 were covered by bimolecular, such AgNPs can be used as drug carriers in the field of biomedicine because of the functional groups on their surface.

5. Conclusions

In this study, we report rapid mass biosynthesis of AgNPs with the features of simplicity, high reduction rate, and high yield. A novel bacterial strain, L. sphaericus MR-1, was isolated from the soil of a chemical fertilizer plant. The cell-free extract of the L. sphaericus MR-1 reduced a high concentration of Ag⁺ to AgNPs rapidly with high yield. Under the condition pH 12, 70 °C, and 20 mM of AgNO₃, the reduction was completed within 75 min. These AgNPs were

Figure 6. Dynamic light scattering measurements for (a) particle size distribution analysis and (b) zeta potential measurements of AgNPs.

Figure 7. (a) FESEM image of silver nanoparticles, which were 5–20 nm in size, spherical, and well dispersed. (b) EDX spectrum of samples recorded in the area-profile mode, which clearly shows the Ag signals.
characterized by various techniques, including UV–vis, XRD, FTIR, FESEM-EDX, HRTEM, zeta potential, and size distribution analysis. Results revealed that these AgNPs were monodispersed, stable, spherical in the range of 5–20 nm, and coated with biological molecules. The molecules covering the surface provide ample room for functionalized modifications, which indicates that AgNPs possess significant potential for application in the field of biomedicine.

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References

[1] Austin L A, Mackey M A, Dreaden E C and El-Sayed M A 2014 The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery Arch. Toxicol. 88 1391–417
[2] McConnell W P, Novak J P, Brousseau L C, Fuierer R R, Tenent R C and Feldheim D L 2000 Electronic and optical properties of chemically modified metal nanoparticles and molecularly bridged nanoparticle arrays J. Phys. Chem. B 104 8925–30
[3] Majdalawieh A, Kanan M C, El-Kadri O and Kanan S M 2014 Recent advances in gold and silver nanoparticles: synthesis and applications J. Nanosci. Nanotechnol. 14 4757–80
[4] Prathna T, Raichur A M, Chandrasekaran N and Mukherjee A 2014 Recent developments on biosynthesis of noble metal nanoparticles: synthesis, characterization and potential applications Rev. Adv. Sci. Eng. 3 239–49
[5] Chaloupka K, Malam Y and Seifalian A M 2010 Nanosilver as a new generation of nanoparticle in biomedical applications Trends Biotechnol. 28 580–8
[6] Siripattanakul-Ratpukdi S and Fürhacker M 2014 Review: issues of silver nanoparticles in engineered environmental treatment systems Water Air Soil Poll. 225 1–18
[7] Kokura S, Handa O, Takagi T, Ishikawa T, Naito Y and Yoshikawa T 2010 Silver nanoparticles as a safe preservative for use in cosmetics Nanomedicine: Nanotechnol., Biomed. 6 570–4

[8] Marambio-Jones C and Hock E M 2010 A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment J. Nanopart. Res. 12 1531–51

[9] Abou El-Nour K M M, Eftaiha A A, Al-Warthan A and Ammar R A A 2010 Synthesis and applications of silver nanoparticles Arab J. Chem. 3 135–40

[10] Poulouse S, Panda T, Nair P P and Theodore T 2014 Biosynthesis of silver nanoparticles J. Nanosci. Nanotechnol. 14 2038–49

[11] Abbasi E, Milani M, Fekri Aval S, Kouhi M, Akbarzadeh A, Tayefi Nasrabadhi H, Nikasa P, Joo S W, Hanifepour Y and Nejati-Koshki K 2014 Silver nanoparticles: synthesis methods, bio-applications and properties Crit. Rev. Microbiol. 1–8

[12] Li X, Xu H, Chen Z-S and Chen G 2011 Biosynthesis of nanoparticles by microorganisms and their applications J. Nanomater. 2011 270974

[13] Klaus T, Joeger R, Olsson E and Granqvist C-G 1999 Silver-based crystalline nanoparticles, microbially fabricated Proc. Natl Acad. Sci. USA 96 13611–4

[14] Narayanan K B and Sakthivel N 2010 Biological synthesis of metal nanoparticles by microbes Adv. Colloid. Interface. 156 1–13

[15] Vivekanandhan S, Schreiber M, Mason C, Mohanty A K and Misra M 2014 Maple leaf Acer sp. extract mediated green process for the functionalization of ZnO powders with silver nanoparticles Colloid. Surface. B 113 169–75

[16] Syed A, Saraswati S, Kundu G C and Ahmad A 2013 Biological synthesis of silver nanoparticles using the fungus Humicola sp. and evaluation of their cytotoxicity using normal and cancer cell lines Spectrochim. Acta. A. 114 144–7

[17] Qian Y, Yu H, He D, Yang H, Wang W, Wan X and Wang L 2013 Biosynthesis of silver nanoparticles by the endophytic fungus Epicoccum nigrum and their activity against pathogenic fungi Bioproc. Biosyst. Eng. 36 1613–9

[18] Malhotra A, Dolma K, Kaur N, Rathore Y S, Ashish S, Mayilrajr and Choudhury A R 2013 Biosynthesis of gold and silver nanoparticles using a novel marine strain of Stenotrophomonas Bioresour. Technol. 142 727–31

[19] Amendola V, Bakr O M and Stellacci F 2010 A study of the surface plasmon resonance of silver nanoparticles by the discrete dipole approximation method; effect of shape, size, structure, and assembly Plasmonics 5 85–97

[20] Yin T, Walker H W, Chen D and Yang Q 2014 Influence of pH and ionic strength on the deposition of silver nanoparticles on microfiltration membranes J. Membrane. Sci. 449 9–14

[21] Badawy A M E, Luxton T P, Silva R G, Scheckel K G, Suidan M T and Tolaymat M T 2010 Impact of environmental conditions (pH, ionic strength, and electrolyte type) on the surface charge and aggregation of silver nanoparticles suspensions Environ. Sci. Technol. 44 1260–6

[22] Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian S R, Muniyandi J, Hariharan N and Eom S H 2009 Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli Colloid. Surface. B 74 328–35

[23] Song K C, Lee S M, Park T S and Lee B S 2009 Preparation of colloidal silver nanoparticles by chemical reduction method Korean. J. Chem. Eng. 26 153–5

[24] Tamuly C, Hazarika M, Borah S, Das M R and Borah M P 2013 In situ biosynthesis of Ag, Au and bimetallic nanoparticles using Piper pedicellatum C.DC: green chemistry approach Colloid. Surface. B 102 627–34

[25] Cruz D, Fale P L, Mourato A, Vaz P D, Serralheiro M L and Lino A R 2010 Preparation and physicochemical characterization of Ag nanoparticles biosynthesized by Lippia citriodora (Lemon Verbena) Colloid. Surface. B 81 67–73

[26] Vigneshwaran N, Ashtaputre N M, Varadarajan P V, Nachane R P, Paralikar K M and Balasubramanya R H 2007 Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus Mater. Lett. 61 1413–8

[27] Sathiyarayanan G, Kiran G S and Selvin J 2013 Synthesis of silver nanoparticles by polysaccharide bi flocculant produced from marine Bacillus subtilis MSBN17 Colloid. Surface. B 102 13–20

[28] Suman T Y, Radhika Rajasree S R, Kanchana A and Elizabeth S B 2013 Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using Morinda citrifolia root extract Colloid. Surface. B 106 74–8

[29] Mandal S, Phadare S and Sastry M 2005 Interfacing biology with nanoparticles Curr. Appl. Phys. 5 118–27

[30] Maria B S et al 2014 Synthesis of silver nanoparticles using medicinal Zizyphus xylopyrus bark extract Appl. Nanosci. doi:10.1007/s13204-014-0372-8

[31] Berry C 2012 The bacterium, Lysinibacillus sphaericus, as an insect pathogen J. Invertebr. Pathol. 109 1–10

[32] Fayaz A M, Balaji K, Girilal M, Yadav R, Kalaichelvan P T and Venkatesan R 2010 Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria Nanomedicine: Nanotechnol., Biol. Med. 6 103–9

[33] Liang M, Angelos S, Choi E, Patel K, Stoddart J F and Zink J I 2009 Mesosctructured multifunctional nanoparticles for imaging and drug delivery J. Mater. Chem. 19 6251–7

[34] Otsuka H, Nagasaki Y and Kataoka K 2003 PE Gylated nanoparticles for biological and pharmaceutical applications Adv. Drug. Deliver. Rev. 55 403–19