Chemically synthesized silver nanoparticles as cell lysis agent for bacterial genomic DNA isolation

Gunajit Goswami, Himangshu Boruah, Trishnamoni Gautom, Dibya Jyoti Hazarika, Madhumita Barooah and Robin Chandra Boro

Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat-13, Assam, India

E-mail: gunajit@aau.ac.in and robinboro@gmail.com

Received 3 August 2017
Accepted for publication 6 October 2017
Published 31 October 2017

Abstract
Silver nanoparticles (AgNPs) have seen a recent spurt of use in varied fields of science. In this paper, we showed a novel application of AgNP as a promising microbial cell-lysis agent for genomic DNA isolation. We utilized chemically synthesized AgNPs for lysing bacterial cells to isolate their genomic DNA. The AgNPs efficiently lysed bacterial cells to yield good quality DNA that could be subsequently used for several molecular biology works.

Keywords: silver nanoparticles, DNA isolation, bacteria
Classification numbers: 2.03, 2.04, 5.08

1. Introduction

Silver metal has been long known to be toxic to bacteria and has been used for centuries as an antimicrobial agent in agriculture and healthcare industries. The advent of nanotechnology has witnessed the increased use of silver in the form of silver nanoparticles (AgNPs) [1–4]. The antibacterial property of AgNPs against gram-positive as well as gram-negative bacteria has been reported by different researchers [1–5]. The pronounced effect of AgNPs is on the metabolic activity of the cells as well as on the membrane leading to severe damage to the cells. The AgNPs anchor to the bacterial cell-wall and subsequently penetrate it, causing structural changes in the cell-membrane and ultimately leading to cell death [2–5]. Electron spin resonance spectroscopy results suggested that AgNPs trigger formation of free radicals in bacteria which damage the cell membrane culminating to cell death [6]. However, the release of silver ions by nanoparticles is also proposed as the main toxic mechanism as nanoparticles disperse faster in a given solution volume to release a higher amount of metal ions [2, 6]. Since most of the actions of AgNPs are related to cell-membrane rupturing, we hypothesized that AgNPs could be utilized as cell-lysis agent for bacterial DNA isolation. DNA isolation is an essential and a key step in important experimental studies pertaining to molecular biology such as polymerase chain reaction (PCR) amplification, sequencing, hybridization, cloning, biodetection etc. Earlier studies have reported the application of magnetic iron oxide nanoparticles for DNA isolation [7, 8]. However, synthesis of magnetic iron oxide nanoparticles requires relatively higher temperature. In addition, polymer coating and further surface modification is essential for effective DNA isolation [9]. Non-magnetic nanoparticles such as silver nanoparticles are considered more advantageous for their cost-effectiveness, lesser time consumption and ability to be used up without further surface modification. In this study, we used chemically synthesized AgNPs as cell-lysis agent, followed by phenol chloroform extraction and ethanol precipitation to extract DNA from both gram-positive and gram-negative bacteria. The protocol as described by Wilson [10] was adopted as reference protocol.
2. Experimental

2.1. Materials

All the chemicals used in this study were purchased from Merck (Germany) if not mentioned otherwise.

2.2. Bacterial strain and growth condition

Escherichia coli DH5α and Bacillus megaterium ATCC14581 were used for examining the cell membrane rupturing property of silver nanoparticles. Luria-Bertani (LB) medium was used to culture B. megaterium and E. coli and fresh cultures (optical density at wavelength of 600 nm = 1.0) were used to examine the efficacy of AgNPs as cell-lysis agent for DNA extraction.

2.3. Synthesis, characterization, and effect of AgNPs

Silver nanoparticles were synthesized and the concentration of the synthesized nanoparticles was evaluated according to Agnihotri et al (2014) [11].

Before beginning DNA isolation using AgNPs, we evaluated their effect on bacterial cell wall using LIVE/DEAD BacLight bacterial viability kit (Invitrogen, USA) after treating the bacteria with AgNPs for 10 min at...
2.4. Extraction of genomic DNA

Two milliliter of fresh cultures of each isolate was taken in 2 ml microfuge tube and harvested by centrifuging at 8000rpm for 5 min. The cell pellets of both the bacteria were re-suspended in equal volume (200 µl) of L-Lauryl Sarkosine and AgNP solution and mixed well followed by incubation at room temperature (treatment 1) and at 65 ºC (treatment 2) for 10 min. The treatments were carried out in triplicates.

After incubation, the tubes were centrifuged at 12 000rpm for 5 min and the supernatant was collected in a new 2 ml tube. To the supernatant equal volume of phenol (Tris-equilibrated) was added and again centrifuged at 12 000rpm for 10 min at room temperature. The upper aqueous phase was collected and equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added and again centrifuged at 12 000rpm for 10 min at room temperature. The upper aqueous phase was again collected and equal volume of chloroform: isoamyl alcohol (24:1) was added followed by centrifugation at 12 000rpm for 10 min at room temperature. To the upper aqueous phase, 1/10th volume of 3 M sodium acetate (pH 5.2) was added and mixed gently followed by addition of double volume chilled absolute ethanol. It was mixed gently and centrifuged at 12 000rpm for 10 min at 4 ºC. The pellet obtained was washed twice with 70% ethanol by centrifugation at 5000rpm for 5 min at 4 ºC. The pellet was air dried and dissolved in 50 µl of 1 × TE buffer (10 mM Tris, 1 mM ethylenediaminetetraacetic acid) and stored at 4 ºC until use. The purity and yield of the DNA were determined by electrophoresis as well as using spectrophotometer. We also evaluated the effect of different DNA were determined by electrophoresis as well as using spectrophotometer. We also evaluated the effect of different DNA isolation. Result from these treatments were determined by electrophoresis as well as using spectrophotometer. We also evaluated the effect of different DNA isolation. Result from these treatments

2.5. Quality evaluation of extracted DNA

Amplification of 16S rRNA was carried out from both the bacteria using the primer set U16SF-5’-AGAGTTTGATCMTGGCTCAG-3’. The U16SR-5’-TACGYYTACCTTGATCGACTT-3’ and primer set ProF-5’-ATCAAAAAACAAAGTTGC-3’ and ProR-5’-AATTAGTACTTGCCACT-3’ was used to amplify the proC gene from both the bacteria. The polymerase chain reaction (PCR) reaction mixture contained PCR 5 × buffer (20 mM Tris-HCl, 50 mM KCl), 20 mM of each deoxynucleotide, 1.5 mM MgCl2, 20 pmol of each primer, 2U Taq DNA polymerase and 50ng genomic DNA, in a final volume of 50 µl. PCR amplification was performed using a thermal cycler (Perkin-Elmer, Norwalk, USA). The PCR program was as follows: initial denaturing step of 94 ºC for 3 min, followed by 35 cycles of denaturation at 94 ºC for 30s, annealing at 55 ºC (for 16s) and 60 ºC (for proC) for 1 min and extension at 72 ºC for 1.5 min (for 16s rRNA) and 1 min (for proC); a final extension step at 72 ºC for 7 min. The amplified products were analyzed in 1% agarose gel. In addition, to see the stability of the DNA upon storage, we stored the DNA for 3 months at 4 ºC and performed agarose gel electrophoresis.

3. Results

3.1. Synthesis and characterization of AgNPs

The co-reduction approach was employed for the synthesis of silver nanoparticles using sodium borohydride and tri-sodium citrate as reducing agents. Appearance of light yellow color in the reaction mixture indicated the synthesis of AgNPs. The surface plasmon resonance (SPR) peak recorded at 403 nm in the UV–vis spectrum and transmission electron microscopy (TEM) micrograph revealed the synthesis of spherical silver nanoparticles mostly of 5–10 nm in size with a very narrow size distribution (figure 1). The concentration achieved was nearly 6 × 1012 particles per ml [14]. The x-ray diffraction (XRD) spectrum of the synthesized AgNPs produced four sharp peaks at 37.96, 44.00, 64.36, and 77.32 in the 2 theta region, corresponding to (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes face centered cubic (fcc) silver [11]. X-ray diffractogram indicates the predominant orientation of (1 1 1) plane of the synthesized AgNPs (figure 2).

3.2. Effect of AgNPs on cell lysis

Fluorescent microscopy revealed the formation of pores in the bacterial cell wall after treating the cells with AgNP and thus the cells appeared red after both treatments (figure 3). Cells with unaffected cell walls were observed with green fluorescence and no red fluorescence, while AgNP treated cells were observed with red fluorescence.

3.3. Extraction of genomic DNA

We applied two treatments to assess the effect of each condition on DNA isolation. Result from these treatments
revealed that AgNP had cell-lysis capability and thus DNA bands were observed after agarose gel electrophoresis (figure 4). However, yield is slightly lower at room temperature. The concentration of DNA was in the range of 200–400 ng µl⁻¹ with ratio of 260:280 ≥ 1.8. We also evaluated the effect of different reagents on the yield and quality of the DNA (see supplementary information) (stacks.iop.org/ANSN/8/045015/mmedia).

To evaluate the broad efficacy of the AgNP, we further isolated DNA from other bacteria such as L. plantarum, P. validus, S. aureus, P. putida, and observed satisfactory results (figure 5). The yield of DNA was found in the range of 200–500 ng with no appearance of DNA shearing.

3.4. Quality evaluation of extracted DNA

In order to examine the applicability of the DNA samples for downstream molecular application, we amplified 16S rRNA and proC gene using the extracted DNA. Agarose gel electrophoresis revealed the presence of expected product size of ~1500 bp and ~950 bp corresponding to 16S rRNA and proC, respectively (figure 6). Further, we evaluated the effect of storage on quality of the isolated DNA and found that the isolated DNA remained intact after 1 and 3 months of storage at 4 °C (figure 7).

4. Discussion

Several genomic DNA isolation protocols and kits are available which employ different mechanisms to break the cells and purify the DNA. Some protocols use enzymatic lysis followed by treatment with chaotropic reagents. Other protocols use mechanical lysis of cells using glass beads or sand particles. In some protocols, lysis is carried out with the help of detergents in presence of buffer. Earlier studies suggested that AgNPs cause damage to bacterial cell wall [12, 13] and our microscopic (live versus dead staining) study also...
confirmed pore formation on bacterial cell wall. Pore formation resulted in the entry of fluorescent dye propidium iodide into the bacterial cells that gave red fluorescence. Therefore we used AgNPs to lyse bacterial cells. The size of the nanoparticles is also an important factor for it to be an effective cell lysis agent. Previous reports indicated that smaller-sized nanoparticles have better penetration power and are more effective in damaging the cell wall [12]. We therefore, synthesized AgNPs in size ranging from 5–10 nm as per the protocol described earlier [11]. The characteristics of the synthesized AgNPs in terms of size, shape and concentration was found to be similar to the report of Agnihotri et al (2014) [11]. Although AgNPs form pore in the bacterial cell wall, it is not sufficient to lyse the cells.
effectively. Addition of detergent like N-Lauroylsarcosine increases the lysis efficiency. N-Lauroylsarcosine is used as lysis agent in DNA isolation protocol [14] as well as in protein purification as it helps in solubilizing the bacterial cell membrane [15]. The heat treatment at 65 °C also helped in lysis of the cells and improves the yield. The reference protocol [10] also had a step of heat treatment at 65 °C and another protocol also used the same temperature to lyse the cells effectively [16]. The probable mechanism involved in this protocol may be that AgNPs make the bacterial cell wall porous and thus helps N-Lauroylsarcosine enter into the pores. N-Lauroylsarcosine degrades the cell membrane. Heat treatment destabilizes the membrane resulting in lysis of the cells. Thus, after centrifugation, DNA remains in the supernatant and cell debris along with AgNPs settles at the bottom. The DNA can be then purified from the supernatant by phenol chloroform extraction. The schematic representation of mechanism of action of AgNPs is depicted in figure 8.

Having standardized the protocol for DNA isolation, we went on to apply this protocol for isolation of genomic from other bacteria. Our protocol worked well for bacteria at both room temperature and 65 °C. We also examined the quality of the extracted DNA so that it can be used in downstream processes. Amplification of 16S rDNA and proC gene indicated that the extracted DNA is suitable for PCR analysis. In our study, for gram-positive bacteria we achieved good results with treatment 2 and for gram negative ones treatment 1 showed good results.

Our DNA extraction protocol has several advantages. First, bacterial lysis solution containing silver nanoparticles is effective even after storage up to 60 days at room temperature (kept in dark) indicating that it can be stored at room temperature thus saving on the time and cost of preparing fresh (figure 1). Second, our protocol used silver nanoparticles that can be synthesized by a simple, less time consuming process and used directly for DNA isolation without any surface modifications (figure 1). Second, our protocol used silver nanoparticles that can be synthesized by a simple, less time consuming process and used directly for DNA isolation without any surface modifications. Third, this protocol can be effectively used to isolate DNA from both gram-positive and gram-negative bacteria unlike some protocol which use different reagents for gram positive and gram negative bacteria. This protocol does not require the use of enzymes such as lysozyme and proteinase K which saves on use of additional chemicals and cost.

5. Conclusion
Silver nanoparticles have been used in many biological applications, but this is for the first time we report its use in DNA isolation from bacteria. Our study proves that this protocol can be virtually applied to both gram positive and negative bacteria. However, further application of this protocol on other recalcitrant bacteria will provide a clearer picture about the wide applicability of this protocol.

Acknowledgments
The authors offer sincere gratitude to Department of Agricultural Biotechnology and DBT-AAU Centre, Assam Agricultural University, Jorhat (India) for providing the necessary facilities to carry out this work.

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