Biosynthesis and Characterization of Silver Nanoparticles Produced by *Bacillus licheniformis*

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***Abstract*—Silver nanoparticles were synthesized using *Bacillus licheniformis* isolated from Al Thwara hot spring, Oman. Both supernatant and biomass of *B. licheniformis* were tested for their ability to reduce silver nitrate solution to silver nanoparticles by adding 1 and 3mM silver nitrate solutions. Silver nanoparticle production was observed both in supernatant and biomass *B. licheniformis*. Biosynthesis of silver nanoparticles occurred in presence of light. The particles maintained a good stability over time course. Small sized silver nanoparticles were produced when using 1 mM silver nitrate solution. The particle size ranges from 3 to 130 nm in supernatant with 1mM silver nitrate and 45-170 nm in supernatant with 3mM silver nitrate. On the other hand, 3 to 130nm and 45-168 nm particle size have been observed in biomass with 1mM and 3mM silver nitrate respectively. The average size of silver nanoparticles was 66 nm for 1 and 3mM silver nitrate solution with supernatant and 110 and 107 nm for 1 and 3mM silver nitrate solution with biomass respectively. However, AgNPs with 35 and 38nm size had a high percent intensity when 1mM $AgNO_3$ was used with supernatant and biomass.**

***Index Terms*—silver nanoparticles, *B. licheniformis*, stability of silver nanoparticles, DLS, supernatant, biomass**

I. Introduction

Silver nanoparticles (AgNPs) are considered to be an important molecule due to its extensive applications in biotechnology and biomedical fields [1]. In the past AgNPs were synthesized by chemical and physical methods. Synthesis of metal nanoparticles using physical and chemical methods have been used in nanotechnology due to the easy modulation in functional behavior of nanostructures and less expensive nature. Recent reports indicate that the toxic effects of various chemicals and organic solvents used in physical and chemical methods may possibly cause harmful effect to the living organisms. Hence, utilizing microbes as a biological machinery to synthesize metal nanoparticles would become more attractive [2]. Biological methods are mainly used as green chemistry approach to reduce the toxicity [1]. Bacteria, fungi and plants play an important role in the green synthesis of AgNPs. They play an active role in the remediation of toxic metals by reducing them to metal ions [3]. In biological synthesis of AgNPs using microbes, the extracellular method of biosynthesis of nanoparticles is mostly preferred because of its simplicity, large-scale production and easier downstream processing. Hence, the biological route to synthesize nanoparticles is developing into an important branch of nanotechnology [4]. Biologically synthesized AgNPs might be used as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling and as antimicrobials [5]. This study was conducted with an objective to biosynthesize the AgNPs using *Bacillus licheniformis* and characterization of the produced AgNPs.

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II. Materials and Methods

A. Source and Maintenance of Bacterium

B. licheniformis having optimum growth at 40 ℃ and pH 7 was isolated from Al Thwara hot spring, Nakhal, Oman. The bacterium was routinely maintained on nutrient agar slants and preserved in glycerol stock solutions at -20 ℃.

B. Synthesis of Silver Nanoparticles

Sterile nutrient broth medium was inoculated with *B. licheniformis* and incubated at 40 ℃ in an incubator shaker. The growth was measured periodically at 600 nm using spectrophotometer until the stationary phase. Once the stationary phase is reached, the culture was centrifuged at 5000 rpm. Tubes containing the supernatant were added with 1 and 3 mM $AgNO_3$ (Sigma, USA) solution separately and incubated in light for 2h. Tubes with only $AgNO_3$ without supernatant, only the supernatant and only with biomass were maintained as controls. A similar set of 2 tubes with supernatant and 1 and 3 mM $AgNO_3$ solution was incubated in dark for 2h. Same set of experiments were conducted with biomass also. After incubation, the extracellular synthesis of AgNPs was monitored visually by observing the change in color of the culture medium from a clear, light-yellow to brown and also by measuring the absorption spectrum of AgNPs in the samples using Shimadzu (model 9200) UV-visible spectrophotometer at a resolution of 1 nm. Unless otherwise stated, three independent runs were made for all experiments.

C. Characterization of Silver Nanoparticles

The stability of the produced AgNPs was studied by observing the UV–Vis absorption spectrum up to 11 days. The particle distribution size of AgNPs was measured

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using dynamic light scattering particle size analyzer (DLS- ciLas Nano DS). Further characterization involved Fourier Transform Infrared Spectroscopic (FTIR-PerkinElmer) analysis of the dried powder of AgNPs by scanning it in the range of 400–4000cm^{-1}.

III. Results and Discussion

Once the growth of *B. licheniformis* reached the stationary phase, biomass was separated and both the supernatant and biomass were used for the synthesis of AgNPs. After 2h, the tubes incubated in light with supernatant turned into brown which indicates the reduction of $AgNO_3$ into AgNPs. Similar result was obtained for $AgNO_3$ and biomass incubated in light. But no such colour change was observed in tubes incubated under dark. This specifies the significant role of light in $AgNO_3$ reduction. Silver nanoparticle synthesis was also dependent on the growth phase of the culture. It was reported that among the supernatants harvested from various growth phases, the culture supernatant obtained from the stationary phase resulted in the rapid synthesis of AgNPs when compared with the supernatants from other growth phases [6]. Maximum synthesis of AgNPs by *B. licheniformis* was observed during the stationary phase. This is apparent from the increased absorbance values in the 420 nm region of the spectrum.

A. Surface Plasmon Resonance

The primary characterization of synthesized nanoparticles by UV-visible spectroscopy is an important technique for the analysis of nanoparticles [7]. In the UV-visible absorption spectrum, a strong, broad peak, between 400 and 500 nm, was observed for nanoparticles synthesized using the culture supernatant and biomass of *B. licheniformis* after 2 h incubation (Fig. 1). The absorbance of supernatant with 3mM $AgNO_3$ was higher than that of supernatant with 1mM $AgNO_3$. But the absorbance of biomass with 1mM and 3mM $AgNO_3$ was almost same level. Observation of this peak is due to surface plasmon which is well documented for various metal nanoparticles ranging in size from 2 to 100 nm [8]. Surface plasmon resonance is a collective excitation of the electrons in the conduction band around the nanoparticle surface. Electrons conform to a definite vibration mode by size and shape of the particle. Hence, the metallic nanoparticles exhibit characteristic optical absorption spectra in the UV-visible region [9]. Biosynthesis of silver nanoparticles can't be carried out by all the organisms. Organisms having the silver resistance machinery can synthesize silver nanoparticles if supplied with optimum concentration of silver ions. The supernatant from *B. licheniformis* may act both as reducing and capping agents in AgNPs synthesis. Enzymes, proteins, amino acids, polysaccharides and vitamins present in the supernatant play an active role in the reduction of Ag^+ ions. The widely accepted mechanism for the synthesis of silver nanoparticles is the reduction mediated by the nitrate reductase enzyme [10], [6]. NADH and NADH-dependent nitrate reductase enzyme are important factors in the biosynthesis of metal nanoparticles [9]. *B. licheniformis* is known for the secretion of cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, that might be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of AgNPs [6].

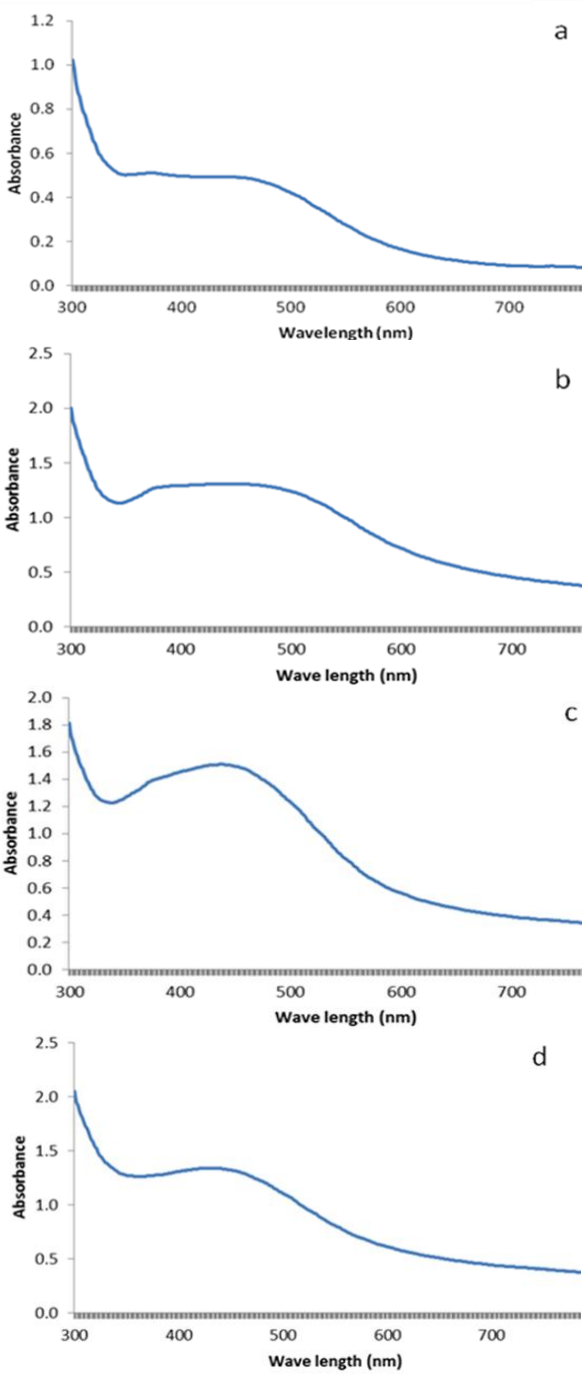

Figure 1. AgNps synthesis by *B. licheniformis* a)1mM b) 3mM silver nitrate solution with supernatant, c)1mM b) 3mM silver nitrate solution with biomass.

The UV-visible spectra recorded from the $AgNO_3$ and cell free extract and $AgNO_3$ and biomass as a function of time of reaction is shown in Fig. 2. It can be observed that the silver surface plasmon occurs around 420nm and steadily increased in intensity as a function of time of reaction. The absorption values for silver nanoparticles

synthesized using 3mM silver nitrate solution was higher than the nanoparticles produced with 1mM silver nitrate solution using both supernatant and biomass. The silver nanoparticles synthesized by 3mM $AgNO_3$ with supernatant and biomass remained stable up to 11 days. But the nanoparticles synthesized using 1mM $AgNO_3$ were stable up to six days only. The stability studies indicate that the nanoparticles dispersed into the aqueous solution might be without any aggregation. The stability of AgNPs varies with different bacterial biosynthesis. AgNP solution of gold and silver remained stable for 60 days with no sign of aggregation [9] to few minutes [11]. The stability in the surface plasmon band in the silver nanoparticle solution indicates that the particles are dispersed into the aqueous solution without aggregation. The stability of silver nanoparticles could be due to a capping agent released by *B. licheniformis* [12].

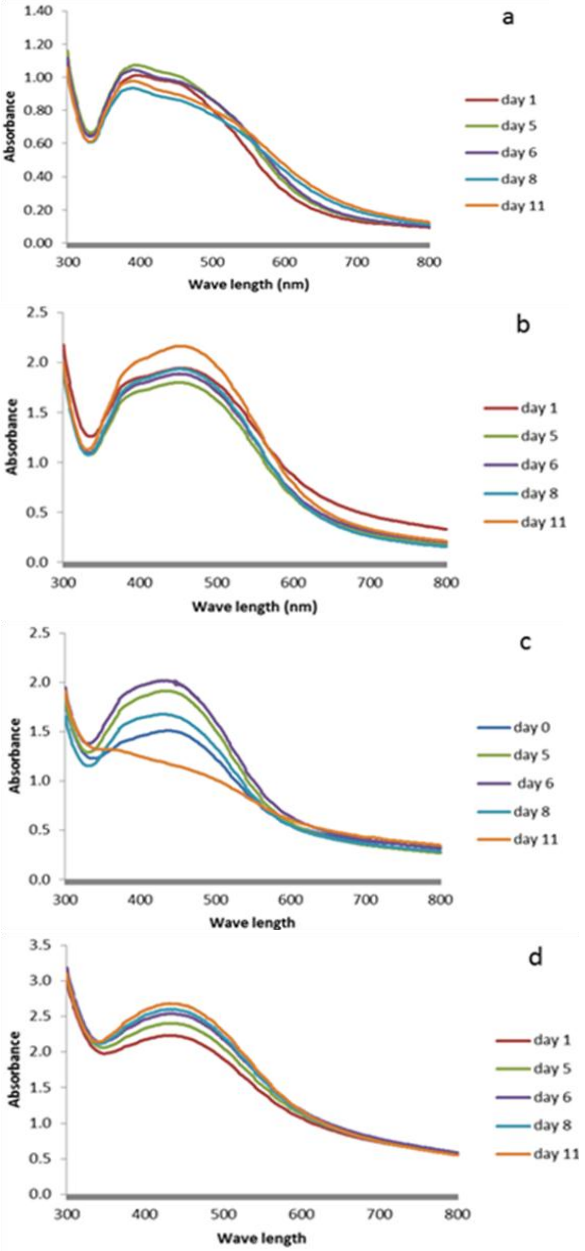

Figure 2. Stability of AgNPs synthesized by *B. licheniformis* a)1mM b) 3mM silver nitrate solution with supernatant, c)1mM b) 3mM silver nitrate solution with biomass.

The FTIR spectra reveal the presence of different functional groups like alkanes, amines and nitro compounds. The bonds or functional groups such as –C–O–C–, –C–O– and –C C– are derived from heterocyclic compounds like proteins, which are present in the supernatant would be the capping ligands of the nanoparticles produced by *B. licheniformis* [13].

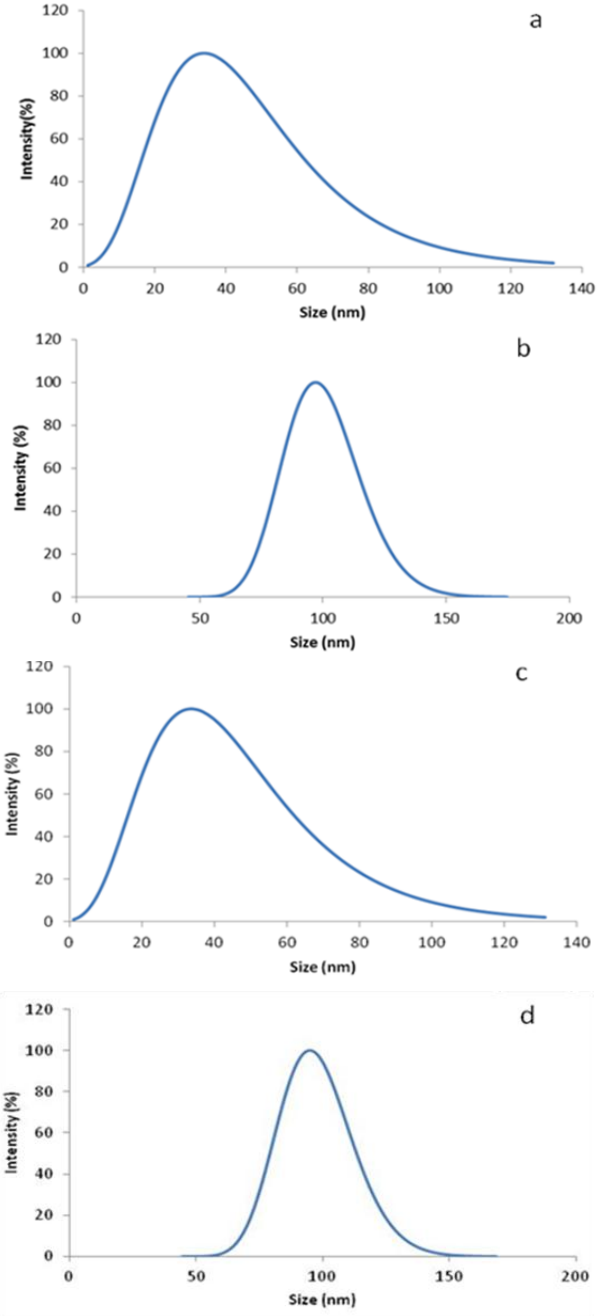

Figure 3. Size of AgNPs synthesized by *B. licheniformis* a)1mM b) 3mM silver nitrate solution with supernatant, c)1mM b) 3mM silver nitrate solution with biomass

B. Size Distribution Analysis by DLS

The size of the synthesized AgNPs from *B. licheniformis* was examined using DLS (Fig. 3). The particle size ranges from 3 to 130 nm in 1mM $AgNO_3$ with supernatant and 45-170 nm in 3mM $AgNO_3$ with supernatant. On the other hand, 3 to 130nm particle size

in 1mM $AgNO_3$ with biomass and 45-168 nm in 3mM $AgNO_3$ with biomass was observed. More than fifty percent of AgNPs synthesized by supernatant with 1mM $AgNO_3$ solution and supernatant with 3mM $AgNO_3$ solution were less than 35nm. But in biomass with 1mM $AgNO_3$ fifty percent of particles are between 50-95nm and 48-90nm in biomass with 3mM $AgNO_3$.

These results clearly depict that 1 and 3mM $AgNO_3$ solution does make difference in the size intensity of biosynthesized nanoparticles. It was reported that the particles range in size from 10 to 120 nm and possess an average size of 40 nm synthesized by *B. marisflavi* [14]. It has also been reported that AgNPs produced by *Klebsiella pneumoniae* range in size from 28.2 to 122 nm and possess an average size of 52.5 nm [15]. The average size of AgNPs synthesized using biomass of *B. licheniformis* [6] and supernatant of *E. coli* were about 50 nm [16]. AgNPs synthesized by spore crystal mixture of *B. thuringiensis* had an average size of 15 nm and the structure was cubic and hexagonal [17]. Though the average size of AgNps is little high, a good intensity of minimum size is achieved in this study. The smaller-size of AgNPs has many positive attributes, such as good conductivity, chemical stability, catalytic and antibacterial activity, which would make them suitable for many applications. The particle size distribution and its aggregation properties affect the toxicity of AgNPs [18]. Small particles easily bind to bacterial cell membrane and pass through the membranes to interact with cellular enzymes [19], [20]. Aggregation may reduce the effective surface area of nanoparticles, reducing stability in solution and surface reactivity.

Hence, conditions which would influence the synthesis of minimum size AgNps should be standardized. The clear curve in DLS studies depict that the synthesized AgNps might have a spherical shape. Therefore, additional characterization with TEM is needed. Both supernatant and biomass of the *B. licheniformis* used in this study have the ability to synthesize AgNPs.

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