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# Biosynthetic potential assessment of four food pathogenic bacteria in hydrothermally silver nanoparticles fabrication

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**Abstract:** Silver nanoparticles (Ag NPs) were synthesized using four pathogenic bacterial extracts namely, *Bacillus cereus*, *E. coli*, *Staphylococcus aureus* and *Salmonella entericasubsp.enterica*. Synthesis process were hydrothermally accelerated using temperature, pressure and heating time of 121°C, 1.5 bar ad 15 min. Physico-chemical characteristics of the fabricated Ag NPs, including, particle size, polydispersity index (PDI), zeta potential, broad emission peak ($\lambda_{max}$) and concentration were evaluated using UV-Vis spectrophotometer and dynamic light scattering (DLS) particle size analyzer. Furthermore, main existed functional groups in the provided bacterial extracts were recognized using Fourier transform infrared spectroscopy. The obtained results revealed that two main peaks were detected around 3453 and 1636.5 $cm^{-1}$, for all bacterial extracts, were interrelated to the stretching vibrations of hydroxyl and amide groups which those had key roles in the reduction of ions and stabilizing of the formed Ag NPs. The results also indicated that, Ag NPs with much desirable characteristics, including minimum particle size (25.62 nm) and PDI (0.381), and maximum zeta potential (-29.5 mV) were synthesized using *S. e. subsp. enterica* extract. $\lambda_{max}$, absorbance and concentration values for the fabricated Ag NPs with this bacterial extract were 400 nm, 0.202% a.u. and 5.87 ppm.

**Keywords:** silver nanoparticles; biosynthesis; pathogens; bacteria strains; physico-chemical properties

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## 1 Introduction

Among metal and metal oxide nanoparticles, silver nanoparticles (Ag NPs) have attained more attention and interests due to their unique attributes, specially their enormous antimicrobial activity. As compared to bulk form of silver, Ag NPs have high surface to volume ratio which in turn, increases the surface energy of the Ag NPs to easily attach into the microorganisms cytoplasmic membrane and change their permeability and cause their death [1]. Furthermore, several studies indicated that there are another two mechanisms related to antimicrobial activity of Ag NPs, including inactivation of respiratory enzymes of mitochondria (near to the cell membrane) and DNA replication disruption [2,3]. Massive antimicrobial activity of Ag NPs against numerous microorganisms such as bacteria and fungi strains has developed their applications in various industries and fields including, food, biotechnology, waste water treatment, tissue engineering, paint, electronic devices, automobile and medicines [4,5].

Biogenic synthesis of metal NPs using microorganism is a novel branch of the nanotechnology which is known as nanobiotechnology and deals with great attention by the researchers, these days. In fact, nanobiotechnology is an emerging field of research at the crossroads of biotechnology and nanoscience which by intersection of inorganic and organic engineering solves critical problems in biology [1,6]. Presence of numerous biomolecules in the microorganisms, such as proteins, polysaccharides, lipids, nucleic acids and enzymes, make those attractive to reduce metal ions and convert them into the NPs, and stabilize the formed NPs. It has been revealed that, these biomolecules could be effectively used in the biosynthesis of inorganic NPs, as reducing and stabilizing agents [7,8]. Several studies have been done on biosynthesis of Ag NPs using different fungi (e.g. *Aspergillus fumigatus*) and bacteria (e.g. *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) strains [9,10].

There are two approaches in biological synthesis of metal NPs using microorganisms namely, intracellular and

extracellular [10]. In the intracellular method the metal salt, as ion source, is added into the broth culture media and after that provided microbe strain complemented to that. During incubation of the media, microorganisms growth and absorb the ions, and synthesis metal NPs in themselves. However, the extracellular synthesis can be implemented not only using some microorganisms whole cells, but also it is applicable employing cell filtrate, lysate, supernatant and cellular components of the microbe [1]. Due to antimicrobial activities of some metal ions and their inhibitory effects on the growth of microorganisms, extracellular approach has been preferred [9].

Some of the microorganisms are pathogens and should be remove from the food and environment. Using these useless microorganisms to synthesis of metal NPs, such as Ag NPs, which those have antimicrobial activity and can be utilized in various areas, is valuable and more attractive subject during last years. Therefore, the main objectives of the present study were to i) evaluate the Ag NPs synthetic potential of four food pathogens bacteria namely, *Bacillus cereus, E. coli*, *Staphylococcus aureus* and *Salmonella entericasubsp. enterica* and ii) assess physico-chemical properties of the fabricated Ag NPs including, concentration, particle size, polydispersity index and zeta potential values.

# 2 Materials and methods

## 2.1 Materials

Silver nitrate (AgNO3) was purchased from Merck (Merck Co., Darmstadt, Germany). Standard Ag NPs colloid solution, with concentration of 1000 ppm and particle size of 10 nm, was provided from Tecnan-Nanomat Co. (Navarra, Spain). *Bacillus cereus* (PTCC 1015), *E. coli* (PTCC 1276), *Staphylococcus aureus* (PTCC 1431) and *Salmonella entericasubsp. enterica* (PTCC 1787) were obtained from microbial Persian type culture collection (PTCC, Tehran, Iran). Specifications of these four bacteria strains show in Table 1. Tryptic soy (CASO) broth, tryptic soy agar and nutrient agar were bought from Merck (Merck Co., Darmstadt, Germany).

## 2.2 Preparation of bacterial extract

In order to preparation of the bacterial biomass, after culturing the provided bacteria strains on the surface of the plates containing nutrient agar (for *E. coli*, *B. cereus* and *S. e. subsp. enterica*) and tryptic soy agar (for *S. aureus*) the plates were incubated at 37°C for 48 h excepted those containing *B. cereus*. The plates those were surfaced cultured with this bacteria strain were incubated at 30°C for 48 h. After that, all separated colonies of each bacterium strains were added into 40 mL of CASO broth and incubated in a laboratory incubator (M30, Memmert GmbH & Co.KG, Schwa Bach, Germany) adjusted at two different temperatures (30 and 37°C) for two days, as already has explained. After that, using a laboratory centrifuge (Microo 220 R, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) adjusted at 6000 rpm at 25°C for 10 min, the bacterial cells were separated and washed again using deionized distilled water (DDW) and centrifuged. Finally the provided biomass was added into 50 mL of DDW and kept in a refrigerated incubator (KB115, GmbH & Co.KG, Tuttlingen, Germany) for 48 h to cell disintegration. By centrifugation of the samples, bacterial cell free extract were separated from the cell debris and kept in the refrigerator (4°C) throughout the experiments.

**Table 1:** Specifications of four studied bacteria strains.

| Bacteria strain | Gram + / - | Need to $O_2$ | Shape | Optimum temperature to growth (°C) |
|---|---|---|---|---|
| *E. coli* | - | Facultative anaerobic | Rod | 37 |
| *S. aureus* | + | Facultative anaerobic | Spherical | 37 |
| *B. cereus* | + | Aerobic/ Facultative anaerobic | Rod | 30 |
| *S. e. subsp. enterica* | - | Facultative aerobic | Rod | 37 |

## 2.3 Synthesis of Ag NPs using bacterial extract

Based on the most literatures, a 1mM silver nitrate was prepared by dissolving of 0.017 g of $AgNO_3$ into the 100 mL DDW, as silver ions precursor [4,9,11]. After that, 1 mL of the silver salt solution was then added into the 3 mL of each bacterial extracts and the mixture solutions were placed in a laboratory autoclave (AM A240T, Astell Co., Sidcup, UK) adjusted at 121°C and 1.5 bar (pressure) for 15 min.

## 2.4 Physico-chemical analysis

### 2.4.1 Bacterial extracts

Fourier transform infrared (FT-IR) spectra can easily detect and indicate the main functional groups presented in the bacterial extracts. These functional groups, based

on the literatures, can reveal the presence of some biomolecules such as proteins and polysaccharides in the extracts, which those act as reducing and stabilizing agents in the formation NPs [12,13]. For this reason, bacterial extracts were monitored in KBr pellets using a FT-IR spectrophotometer (Shimadzu 8400S, Shimadzu Co., Kyoto, Japan) adjusted at range of 4000-400 $cm^{-1}$.

#### 2.4.2 Synthesized Ag NPs

Ag NPs due to their surface Plasmon resonance (SPR) have a broad emission peak ($\lambda_{max}$) placed at wavelength of 380-450 nm, which this peak easily confirmed the formation of Ag NPs [2,9]. Therefore, using a UV-Vis spectrophotometer (UV-1800, Shimadzu Co., Tokyo, Japan) and scanning of the mixture solutions after hydrothermal process, fabrication of the Ag NPs using four different bacterial extracts were evaluated.

Furthermore, it is possible to calculate the concentration of the formed Ag NPs using UV-Vis spectroscopy. For this reason, a standard curve, using serial dilute solutions of the provided standard AgNPs (1-10 ppm) was prepared which in that, the absorbance (% a.u.) of the fabricated Ag NPs was correlated to their concentration.

Dynamic light scattering (DLS) particle size analyzer (Nanotrac Wave, Microtrac, USA) was also utilized to measure particle size, polydispersity index (PDI) and Zeta potential values and to monitor particle size distribution (PSD) of the formed Ag NPs.

### 2.5 Experimental design and statistical analysis

Experiments were design based on full factorial and all analysis related to the characteristics of the fabricated Ag NPs were completed in three replications. Analysis of variance (ANOVA) using Minitab v.16 statistical package (Minitab Inc., PA, USA) was used to statistical analysis. Tuky's comparison test was also used to compare the mean values, at 5% level of significance.

## 3 Results and discussions

### 3.1 FT-IR spectra of the bacterial extracts

Figure 1 shows FT-IR spectra of the four prepared bacterial extracts. As can be seen in this figure, there are

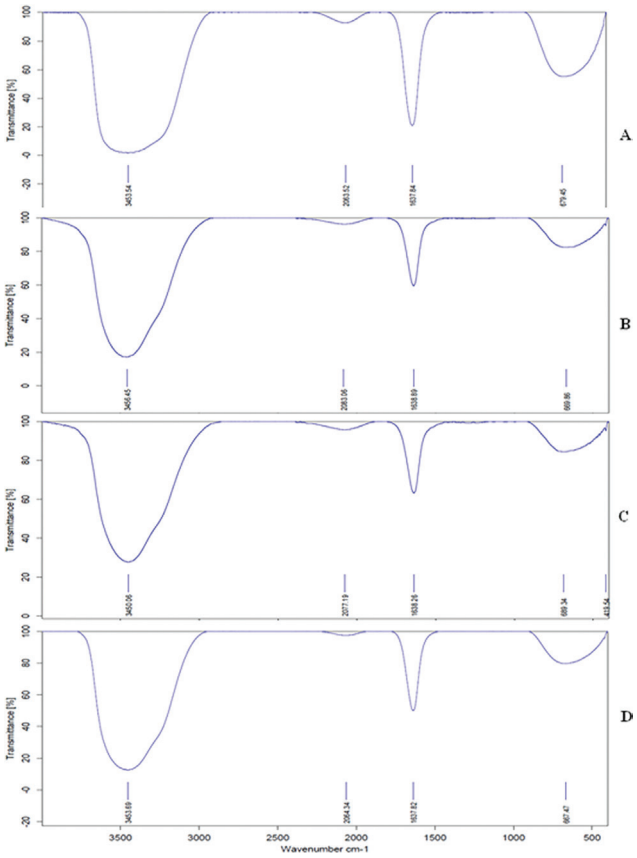


**Figure 1:** FT-IR spectrum of *E. coli* (a), *S. aureus* (b), *B. cereus* (c) and *S. e. subsp. enterica* (d) extracts.

4 dominated peaks for all the extracts which those are centered at 3450-3456, 2063-2083, 1637 and 667-689 $cm^{-1}$. The two main peaks were detected around 3453 and 1636.5 $cm^{-1}$, for all bacterial extracts, were interrelated to the stretching vibrations of hydroxyl and amide groups, respectively. Hydroxyl group which is the main functional group of the polysaccharides, nucleic acids and other main components existed in the bacterial cell has main role in reduction of silver ions and finally synthesis of Ag NPs [5]. Amide group is also the main functional group which is presented in proteins and enzymes and has a key role in stabilizing of the fabricated Ag NPs [9]. The obtained results revealed that all four selected bacteria strains had synthetic and stabilizing potentials to fabricated stable Ag NPs.

### 3.2 SPR and concentration of the fabricated Ag NPs

Due to SPR character of the metal NPs which is related to the combined vibration in resonance between free electrons and the light wave, fabricated Ag NPs had broad

emission peaks ($\lambda_{max}$) were located at wavelength ranging 380-450 nm [1,3,11]. There is a direct relation between peak height and concentration of the synthesized Ag NPs [5]. According to the provided standard curve, using serial dilute solutions of the provided standard Ag NPs (1-10 ppm), the following equation (Eq. 1) was generated:

$$C = 32.775\ X - 0.7474 \quad (1)$$

where, c is the concentration of the formed Ag NPs in the colloidal solution and X is the absorbance of the solution at $\lambda_{max}$. Table 2 shows the values of $\lambda_{max}$, absorbance and concentration of the fabricated Ag NPs using different four bacterial extracts. As clearly observed in Table 2, the Ag NPs with highest concentration were synthesized using *E. coli* extracts. Figure 2 indicates UV-Vis spectra of the formed Ag NPs using *E. coli* extract.

## 3.3 Particle size, PDI and zeta potential of the synthesized Ag NPs

Physico-chemical characteristics of the synthesized Ag NPs using four different bacterial extracts show in Table 3.

**Table 2:** $\lambda_{max}$, absorbance and concentration of the fabricated Ag NPs using bacterial extracts.

| Bacteria strain | $\lambda_{max}$ (nm) | Absorbance (% a.u.) | Concentration (ppm) |
|---|---|---|---|
| *E. coli* | 428 | 0.253 | 7.55 |
| *S. aureus* | 400 | 0.086 | 2.07 |
| *B. cereus* | 450 | 0.103 | 2.63 |
| *S. e. subsp. enterica* | 400 | 0.202 | 5.87 |

Data are mean values of three replications.

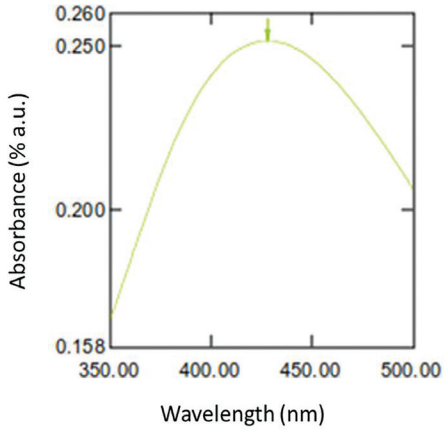


**Figure 2:** Surface Plasmon resonance spectrum of the synthesized Ag NPs using *E. coli*.

The obtained results indicated that Ag NPs with much desirable characteristics including minimum particle size (25.62 nm) and PDI (0.381), and maximum zeta potential (-29.5 mV) were synthesized using *S. e. subsp. enterica* extract. Silambarasan and Abraham synthesized exteracellulary Ag NPs using B. cereus with particle size

**Table 3:** Physico-chemical characteristics of the fabricated Ag NPs using bacterial extracts.

| Bacteria strain | Particle size (nm) | PDI | Zeta potential (mV) |
|---|---|---|---|
| *E. coli* | 84.05 | 0.564 | -15.1 |
| *S. aureus* | 57.28 | 0.510 | -17.2 |
| *B. cereus* | 94.64 | 0.658 | -18.7 |
| *S. e. subsp. enterica* | 25.62 | 0.381 | -29.5 |

Data are mean values of three replications.

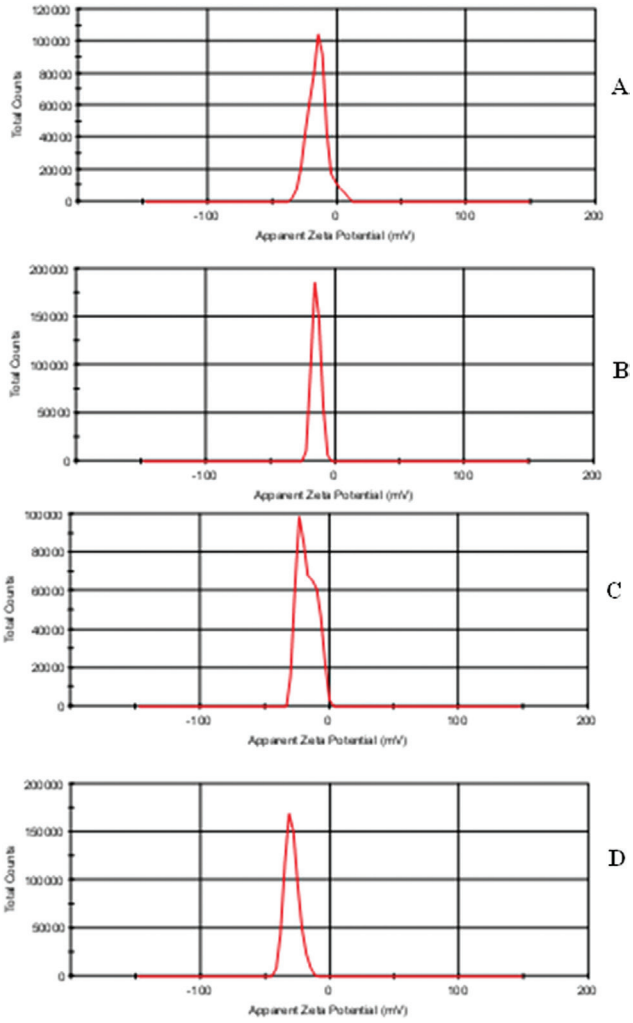


**Figure 3:** Zeta potential distribution of the synthesized Ag NPs using *E. coli* (a), *S. aureus* (b), *B. cereus* (c) and *S. e. subsp. enterica* (d) extracts.

of 62.8 nm and $\lambda_{max}$ of 440 nm [14]. Shah et al. fabricated Ag NPs using *E. coli* extract wiyh particle size and zeta potential values of 297.7 nm and -12.4 mV, respectively [15]. Nanda and Saravanan exteracellulary synthesized Ag NPs using S. aureus with mean particle size of 170 nm and $\lambda_{max}$ of 420 nm [16].

Obtained results also indicated small value for the PDI of the synthesized Ag NPs using *S. e. subsp. enterica* extract which in turn revealed that the monodispersed Ag NPs were formed. The obtained result was in line with finding of Mohammadlou et al. [4]. They fabricated monodispersed Ag NPs using *Pelargonium* leaf extract while the PDI of the fabricated NPs was 0.413. Furthermore, higher value of zeta potential for the fabricated Ag NPs with this bacteria strain illustrated their highest stability in the colloidal solution. This result was in agreement with achievement of Torabfam and Jafarizadeh-Malmiri [3]. They fabricated much more stable Ag NPs using chitosan with zeta potential of +50 mV. Figure 3 shows the zeta potential distribution of the synthesized Ag NPs using four different bacterial extracts.

Particle size distribution (PSD) of the synthesized Ag NPs using *E. coli*, *S. aureus*, *B. cereus* and *S. e. subsp. enterica* extracts show in Figures 4a, 4b, 4c, and 4d, respectively. The presence of sharp and narrow peaks for PSD of all four groups of the fabricated Ag NPs using different bacteria revealed that the formed Ag NPs were monodisperesed. The results were reconfirmed by the small values of the PDI for the synthesized Ag NPs with all selected bacteria strain extracts, as can be seen in Table 3.

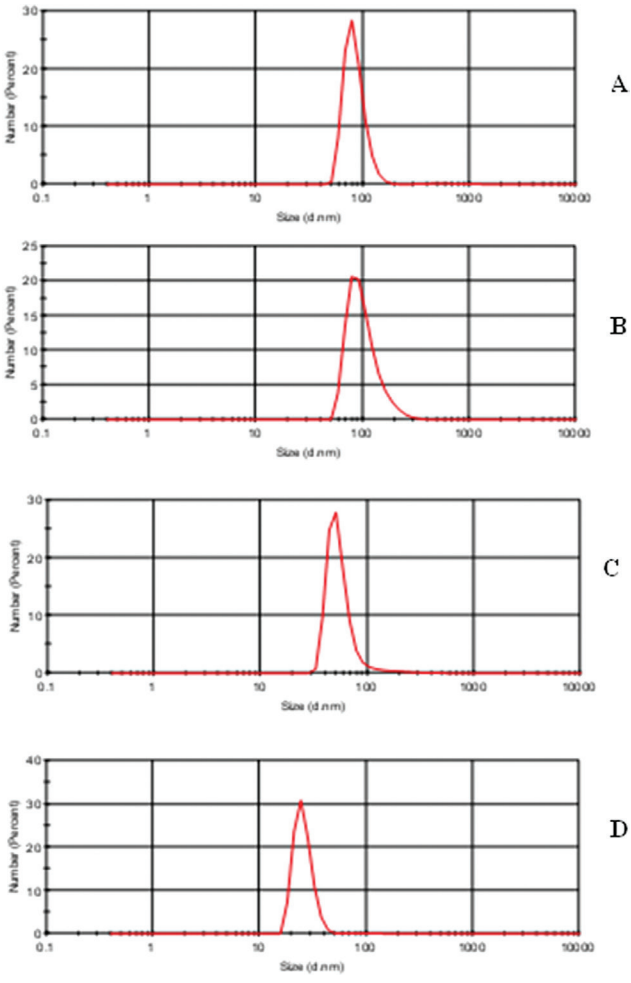


**Figure 4:** Particle size distribution of the synthesized Ag NPs using *E. coli* (a), *S. aureus* (b), *B. cereus* (c) and *S. e. subsp. enterica* (d) extracts.

## 4 Conclusions

Biological synthesis of Ag NPs using bacteria strains, especially pathogens, is a boon for advance research in nanobiotechnology. However, pathogens with toxin production must be removed from the food products and killed, but, those have great potential in metal NPs synthesis, such as Ag NPs which those have strong antibacterial activity against vast microorganisms, especially the pathogens. The obtained results revealed that four selected pathogens in the present study namely, *E. coli*, *S. aureus*, *B. cereus* and *S. e. subsp. enterica* had appropriate synthetic potential for extracellular fabrication of monodispersed and stable Ag NPs with small particle size. Using pathogens in Ag NPs synthesis, according to the developed manner in the present study can be used widely in the synthesis of other noble metal and metal oxide NPs.

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**Conflicts of interest:** All authors declare no conflict of interest.

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