

Toward revealing the controversy of bacterial biosynthesis versus bactericidal properties of silver nanoparticles (AgNPs): bacteria and other microorganisms do not per se viably synthesize AgNPs

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Abstract In the last two decades, a large number of literature had focused on the biosynthesis of silver nanoparticles (AgNPs) from silver ions by bacteria and other microorganisms. This study infers that bacteria and other microorganisms do not per se synthesize AgNPs. All tested auto- and heterotrophic microorganisms in this study were killed by silver ions and could not as viable cells produce AgNPs. Microbial cell viability represented in colony-forming units and metabolic viability represented in aerobic respiration in all investigated microorganisms as well as photosynthesis in photoautotrophic microorganisms ceased by silver ions too early before AgNPs formation. The time required for AgNPs synthesis inversely related to the incubation temperature of the investigated microorganisms with silver ions where it requires only few minutes for nanoparticles formation at high temperature or autoclaving. The minimum inhibitory and minimum bactericidal and fungicidal concentrations of silver ions were significantly lower than AgNPs, indicating that silver ions are more efficient antimicrobial. The results presented in this study indicate that formation of AgNPs by eubacteria, cyanobacteria and fungi is not a vitally regulated cellular metabolic process and the mechanism occurs via bioreduction of silver ions to nanoparticles by organics released from the dead cells.

Keywords Antibacterial agents · Antimicrobial agents · Biosynthesis · Nanoparticles · Silver

Introduction

Over the past few decades, nanoparticles, with at least one dimension less than 100 nm, of metals attracted high attention for its new applications in various fields of industry. Among these nanomaterials, silver nanoparticles (AgNPs) seem to have attracted the most interest in terms of its potential applications. Chemical, physical and biological syntheses of AgNPs are hot topics of research due to the wide range of AgNPs applications and their antibacterial, antifungal and antiviral activities (Kim et al. 2007; Pal et al. 2007; Rai et al. 2009; et al. De Gusseme et al. 2010; Govindaraju et al. 2011; Lara et al. 2011; Brandt et al. 2012; Kim et al. 2012; Mohanty et al. 2012; Tamboli and Lee 2013; Eckhardt et al. 2013; Lok et al. 2014). Because of their antimicrobial properties, AgNPs are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics and plastics (Sharma et al. 2009; Li et al. 2010).

In spite of the antimicrobial properties of AgNPs, microbial synthesis of these nanoparticles (Klaus et al. 1999, 2001; Mandal et al. 2006; Gurunathan et al. 2009; Sintubin et al. 2009) has been proposed as one of the alternative ecofriendly green methods to chemical and physical procedures that are fraught with many problems including the use of toxic solvents, generation of hazardous by-products and high energy consumption (Thakkar et al. 2010). Many microorganisms have been used for intra- or extra-cellular biosynthesis of AgNPs. Silver-containing nanocrystals of different compositions were synthesized by *Pseudomonas stutzeri* AG259 bacterium (Klaus et al. 1999, 2001). The silver reduction by the

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bacterium *Proteus mirabilis* PTCC 1710 to produce intra- and extra-cellular nanoparticles was suggested to occur through energy-dependent processes (Samadi et al. 2009). Possible involvement of proteins in synthesizing AgNPs was observed in the filamentous cyanobacterium, *Plectononema boryanum* UTEX 485 (Lengke et al. 2006). The white rot fungus, *Phaenerochaete chrysosporium*, reduced silver ions to form AgNPs where a protein was suggested to cause the reduction (Vigneshwaran et al. 2006a). Jain et al. (2011) indicated that silver nanoparticle synthesis by *Aspergillus flavus* occurs initially by a 33-kDa protein followed by a 35-kDa protein electrostatic attraction which stabilizes the nanoparticles by forming a capping agent. *Geobacter sulfurreducens* reduced silver ions to nanoparticles via a mechanism involving *c*-type cytochromes (Law et al. 2008). Exposure of *Verticillium* fungal biomass to aqueous silver ions resulted in the intracellular formation of AgNPs below the cell wall surface, possibly due to the reduction of the metal ions by enzymes present in the cell membrane (Mukherjee et al. 2001). Intracellular formation of AgNPs was also reported in the filamentous fungus *Neurospora crassa* (Castro-Longoria et al. 2011). In *Fusarium oxysporum* fungus, the reduction of silver ions to nanoparticles was attributed to an enzymatic process involving NADH-dependent reductase (Durán et al. 2005). Synthesis of AgNPs using α -NADPH-dependent nitrate reductase and phytochelatin in vitro has been demonstrated (Anil-Kumar et al. 2007), and the nitrate reductase present in the bacteria was suggested to aid in the synthesis of AgNPs (Kalimuthu et al. 2008). Nitrate reductase was also suggested to initiate AgNPs formation from silver ions in a *Penicillium* species (Naveen et al. 2010). However, silver ions used for the biosynthesis of AgNPs can also be used as antimicrobial agent (Slawson et al. 1992; Feng et al. 2000; Yamanaka et al. 2005). This study was devoted to explore the controversy between biosynthesis of AgNPs by microorganisms and using AgNPs as antimicrobial agents.

Materials and methods

Organisms and growth conditions

The cyanobacterium *Spirulina platensis* strain IAM M-135 was grown photoautotrophically in *Spirulina*–Ogawa–Terui (SOT) medium (Ogawa and Terui, 1970) under continuous illumination at 32 ± 1 °C with aeration (Morsy et al. 2008a). Growth was assessed by chlorophyll *a* (chl *a*) content. Chl *a* was dark-extracted under cooling in 90 % methanol overnight, clarified by centrifugation under cooling (4 °C) at $7000 \times g$ for 15 min and quantified spectrophotometrically according to the equations of Holden (1976).

The mutant *Escherichia coli* HD701 constructed previously (Sauter et al. 1992) from wild-type *E. coli* MC4100 was maintained aerobically on nutrient agar and grown on nutrient broth with shaking (100 rpm) (Penfold et al. 2003; Morsy 2011a) at 35 °C. Growth of *E. coli* was followed spectrophotometrically at 600 nm. *Aspergillus terreus* was maintained on Sabouraud dextrose agar (Moss and McQuown 1969) slants and grown on Sabouraud sucrose broth (Koutb and Morsy 2011) at 30 °C. The composition of Sabouraud sucrose broth (SSB) is (g/L): sucrose 40, peptone 10, Actidione 0.5 and chloramphenicol 0.25. Growth of *A. terreus* was assessed by dry mass per 100 ml culture.

Bioreduction of silver ions and formation of AgNPs

Three culture replicates were incubated with shaking in dark in case of *E. coli* and *A. terreus* and in light in case of *S. platensis*. Silver nitrates were added to the 100-ml culture of each organism up to 1 mM AgNO₃ at the exponential phase of the growth. The formation of the AgNPs was monitored by UV–visible spectroscopy at 430 nm. The effect of temperature on AgNPs synthesis was investigated.

Microbial cell viability during biosynthesis of AgNPs

Microbial cell viability was followed by monitoring the colony-forming units (CFU) in time intervals after addition of silver nitrates to each culture. Synthesis of AgNPs and the revival of *E. coli* and *A. terreus* cells as well as metabolic cell viability represented in its oxygenic respiration activity at aerobic conditions were followed in nutrient broth and Sabouraud dextrose broth, respectively. Formation of AgNPs by the cyanobacterium *S. platensis* as well as the cyanobacterial cell viability represented in its photosynthesis and respiration activities was followed up for 7 days in SOT medium.

Photosynthesis and respiration

Photosynthetic oxygen evolution in case of *S. platensis* and aerobic respiration for all tested microorganisms were followed in time intervals after addition of silver ions to each culture. Photosynthetic oxygen evolution of *S. platensis* was measured using a Clark-type oxygen electrode computerized to an oxygen monitoring system (OMS; Hansatech Instruments, Inc.). Oxygen evolution was measured in actinic light ($44.5 \mu\text{E m}^{-1} \text{s}^{-1}$) passed through a red optical filter (Morsy 2011b). All measurements were taken at 32 ± 1 °C stabilized by a circulating water bath. Respiratory O₂ uptake by each investigated microorganism was measured using the same above-mentioned Clark electrode and OMS at respective optimum temperature of each microorganism but in the dark.

Reduction of silver ions to nanoparticles by cyanobacterial polysaccharides of *S. platensis*

Exopolysaccharides (EPS) of *S. platensis* were prepared as described previously (Filali-Mouhim et al. 1993), lyophilized and stored at 4 °C for further experiments. The reduction of silver ions to nanoparticles by cyanobacterial polysaccharides of *S. platensis* was conducted at 15 psi and 121 °C for 5 min. The effect of silver ions and EPS concentrations on AgNPs formation was investigated.

Characterization of AgNPs

After formation of AgNPs, the cultures were centrifuged at 4000×g for 5 min at 20 °C. The pellet was discarded, and the supernatant containing AgNPs was centrifuged at 15,000×g for 20 min at 20 °C. After washing the precipitate (AgNPs) three times with distilled water, it was suspended in distilled water and stored in the dark at room temperature for further characterizations.

UV–visible spectroscopy

The wavelength absorption spectrum of AgNPs was monitored by UV–visible spectroscopy by recording the spectra between 300 and 900 nm and monitoring the appearance of the characteristic peak at 400–440 nm using UV–vis spectrophotometer (Thomson Scientific, model: Evolution 3000).

Transmission electron microscopy (TEM)

The morphology of AgNPs was characterized by TEM. An aliquot (10 µL) of aqueous suspension of AgNPs was transferred onto a carbon-film-coated copper grid and allowed to dry for 30 min. The grid was scanned, and the images of AgNPs were obtained by a JEOL-JEM-2010XII microscope operated at 200 kV with a magnification of up to 5×10^5 times. The TEM data of AgNPs shape and size were analyzed.

Minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) and fungicidal (MFC) concentrations of silver ions versus nanoparticles

The concentration of silver nanoparticle solutions was calculated using the Beer–Lambert law, which correlates the optical density (OD) with concentration. Due to the linear relationship between OD and concentration, silver nanoparticle concentration in diluted solutions was calculated from a standard curve of the optical density at 430 nm (maximum absorbance wavelength) versus mass concentration (ppm). The minimum inhibitory concentration (MIC)

of silver ions and nanoparticles was determined as the lowest concentration that inhibits the growth of a microorganism. A series of proper medium broth of each subject microorganism were mixed with the serially diluted silver ions or nanoparticles solutions, and a standard inoculum of the microorganism was added. The minimum inhibitory concentration (MIC) was determined as that in the first broth in which growth of the microorganism has been inhibited by the antimicrobial effect of silver ions or nanoparticles. The minimum bactericidal (MBC) and fungicidal (MFC) concentrations were determined from broth dilution MIC tests by subculturing to appropriate medium agar plates of each subject microorganism without silver ions or nanoparticles. MBC and MFC were determined as the lowest concentration of silver ions or nanoparticles required to kill the microorganism where no colony appeared upon subculturing.

Statistical analysis

The results of the MIC and MBC of silver ions versus nanoparticles were subjected to analysis of variance (one-way ANOVA) using PCSTAT program (version 1A). The treatment means were compared using the least significant difference (LSD) values at a significance level of $P < 0.05$.

Results

This study was devoted to explore the controversy between the biosynthesis of AgNPs by microorganisms and the antimicrobial activities of these nanoparticles. For this purpose, experiments were conducted to follow up simultaneously the microbial cell viability and the formation of AgNPs in time intervals after addition of silver ions to each microbial culture. Autotrophic microorganisms represented by the cyanobacterium *S. platensis* and heterotrophic microorganisms represented by the eubacterium *E. coli* HD701 and the fungus *A. terreus* were used in this investigation.

Upon addition of silver ions to the cultures, microbial cell viability was followed by monitoring the colony-forming units (CFU) in time intervals (Fig. 1). All tested auto- and heterotrophic microorganisms in this study were killed within 2 to 4 h of exposure to silver ions (Fig. 1). Metabolic viability represented in aerobic respiration in all investigated microorganisms as well as photosynthesis in autotrophic microorganisms was followed in time intervals after addition of silver ions to the culture (Fig. 2) of each microorganism at the exponential growth phase. For *S. platensis*, silver ions were added at the third day of culture where photosynthesis and respiration ceased within one hour of silver ions addition (Fig. 2a). The growth represented by chlorophyll *a* concentration in the culture ceased

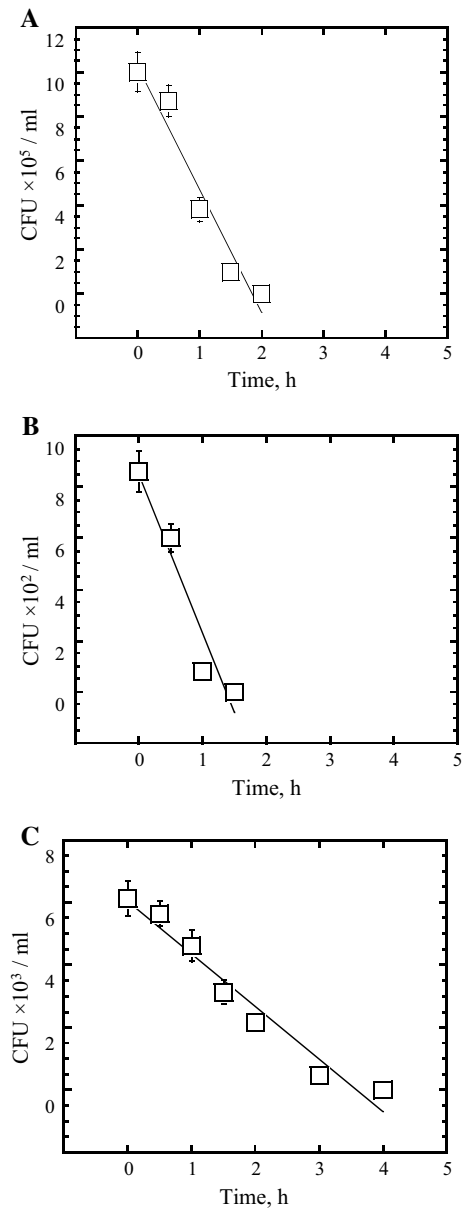


Fig. 1 Microbial cell viability represented as colony-forming units (CFU) of *E. coli* (a), *S. platensis* (b) and *A. terreus* (c) in response to 1 mM silver ions. Viable cells were enumerated on an agar plates of the appropriate medium and growth conditions for each microorganism as described in Materials and methods. The bars show the standard errors of three replicates

after addition of silver ions, and a decline phase appeared (Fig. 2a). For *E. coli*, silver ions were added at the fourth hour of culture. Aerobic respiration of *E. coli* (Fig. 2b) ceased upon addition of silver ions to the culture. Growth curve represented by bacterial optical density declined after silver ion addition (Fig. 2b). Similar results were obtained for the wild-type strain *E. coli* EGY previously isolated and identified by 16 sRNA (Abd-Alla et al. 2011). For the fungus *A. terreus*, silver ions were added at the second day of

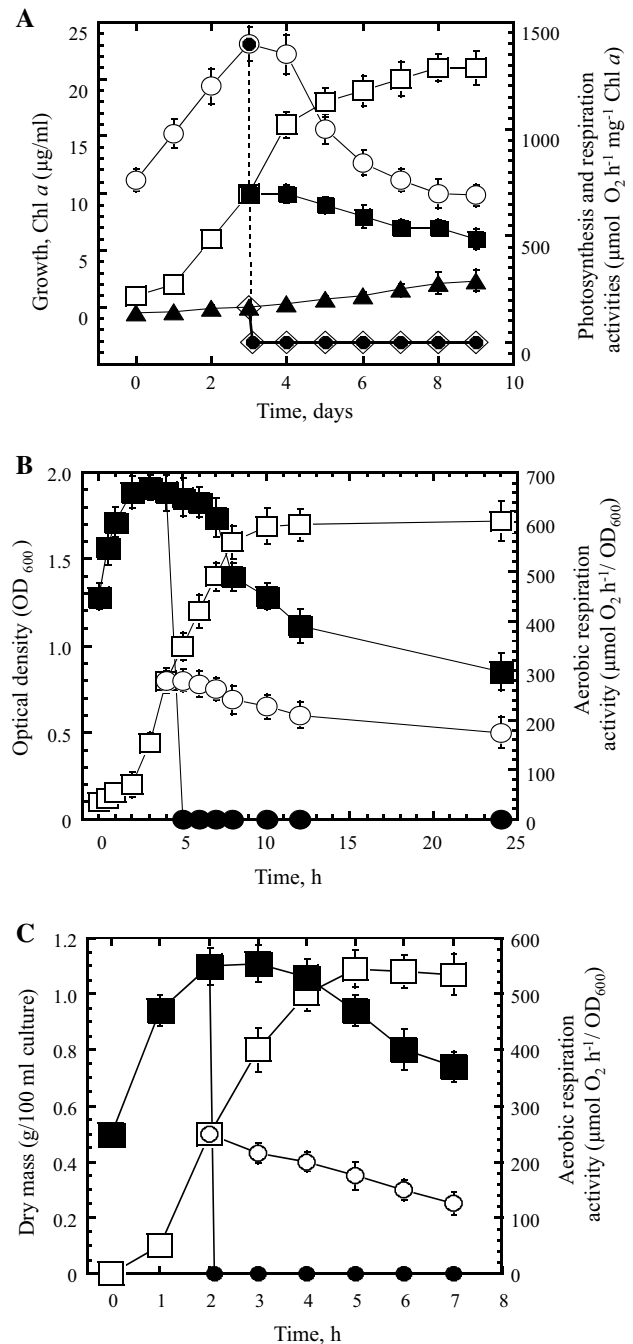


Fig. 2 Cease of growth, photosynthesis and aerobic respiration by silver ions. a Photosynthesis, respiration and growth of *S. platensis* in response to 1 mM silver ions. Growth (open squares), photosynthesis (open circles) and respiration (closed triangles) were monitored at normal growth condition as described in Materials and methods. At the third day of culture, silver nitrate was added to the culture up to 1 mM and the growth (closed squares), photosynthesis (closed circles) and respiration (open cubes) were followed. b, c Cease of growth and aerobic respiration of *E. coli* and *A. terreus*, respectively, by silver ions. Growth (open squares) and aerobic respiration (closed squares) were monitored as described in Materials and methods. Upon addition of silver nitrate to the culture up to 1 mM, the growth (open circles) and aerobic respiration (closed circles) were also followed. The bars show the standard errors of three replicates

culture where aerobic respiration ceased within 2 h of silver ions addition and the growth curve declined (Fig. 2c).

In contrast to the fast loss of microbial cell viability upon addition of silver ions to the cultures (Figs. 1, 2), the formation of AgNPs (Fig. 3) required several days at normal growth temperature (Fig. 4) of each microorganism where cells of all investigated microorganisms were killed by silver ions too early before formation of AgNPs. The bioreduction of silver ions to nanoparticles was strongly enhanced at high temperature (Fig. 4) which enhance the cell lyses as well as the bioreduction rate of silver ions to nanoparticles by the released cellular organics. The prolonged exposure to silver ions of all investigated microorganisms leads to the formation of silver particles in the nanorange (Fig. 3), indicating that the formation of AgNPs by the released cellular organics is not a specific metabolic process to a certain microorganism. The formed nanoparticles by *E. coli*, *S. platensis* and *A. terreus* (Fig. 3) showed a brown to reddish color in aqueous solution and showed a typical wavelength spectrum of AgNPs with a maximum wavelength of around 430 nm. The synthesized AgNPs by *E. coli*, *S. platensis* and *A. terreus* (Fig. 3) showed a size range of 5–30, 10–40 and 4–21 nm, respectively, as analyzed using transmission electron microscope (Fig. 3).

The cyanobacterium *Anabaena variabilis* ATCC 29413 grown photoautotrophically in liquid cultures in an eight-fold dilution of the medium of Allen and Arnon (1955) also non-viably formed AgNPs (Fig. 5a) after several days of exposure to silver ions and showed similar results to *S. platensis*. Mixed phytoplankton collected from the Nile River as previously described (Morsy 2011c) and maintained in Bold's basal medium (Bold 1949, Bischoff and Bold 1963) formed AgNPs (Fig. 5b) also after full death of cells and could not as viable cells produce these nanoparticles similar to *S. platensis* and *A. variabilis*. Average size of various AgNPs synthesized in this study as analyzed using TEM is shown in Table 1.

To confirm that the biosynthesis of AgNPs by microorganisms is just a bioreduction process of silver ions by the organics released from the dead cultures, the synthesis of AgNPs (Fig. 6a) by cyanobacterial exopolysaccharides (EPS) of *S. platensis* cells was investigated. Various initial concentrations of silver nitrate (0.0 to 10 mM) were used to study the effect of silver ion concentration on AgNPs formation in the presence of 10 mg/ml polysaccharides (Fig. 6b), and the reaction was autoclaved at 15 psi and 121 °C for 5 min. The formation of AgNPs improved by increasing the initial concentration of silver salt (Fig. 6b). The effect of EPS concentration (0.0–14 mg/ml) on AgNPs formation was followed in the presence of 8 mM silver ions. Increasing the initial concentration of EPS increased the formation of AgNPs (Fig. 6c). The synthesized nanoparticles had

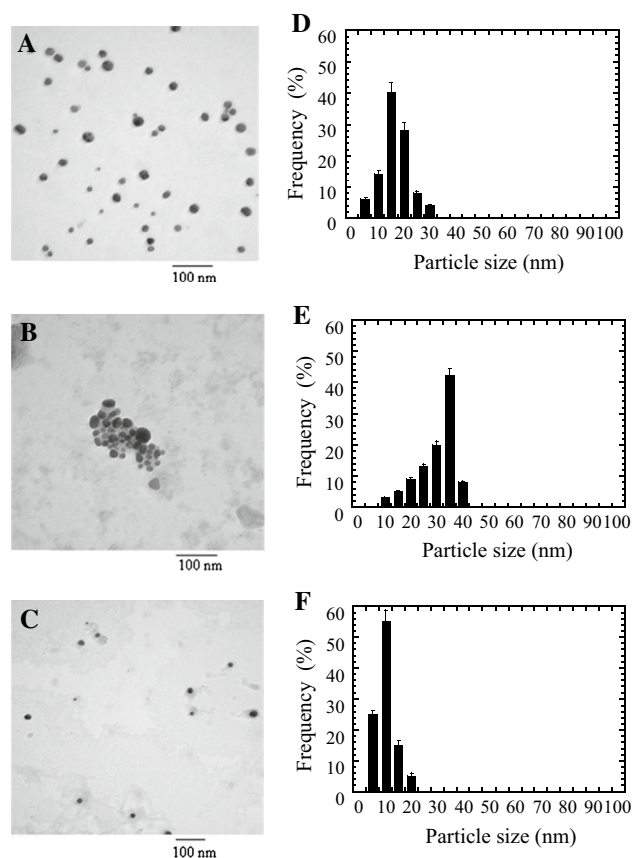


Fig. 3 Transmission electron microscope (TEM) images of silver nanoparticles prepared by *E. coli* (a), *S. platensis* (b) and *A. terreus* (c). Histograms d, e and f are for the particle size frequency derived by counting over multiple TEM images of AgNPs prepared by *E. coli*, *S. platensis* and *A. terreus*, respectively

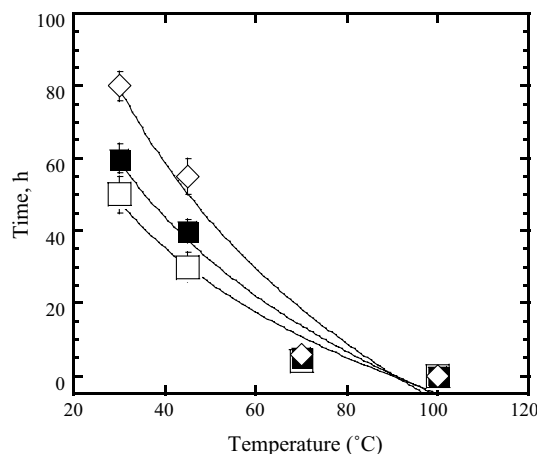


Fig. 4 Temperature versus time during the synthesis of silver nanoparticles. The temperature and time required for the synthesis of silver nanoparticles ($OD_{430} = 1$) were recorded for *E. coli* (closed squares), *S. platensis* (open squares) and *A. terreus* (open cubes). The bars show the standard errors of three replicates

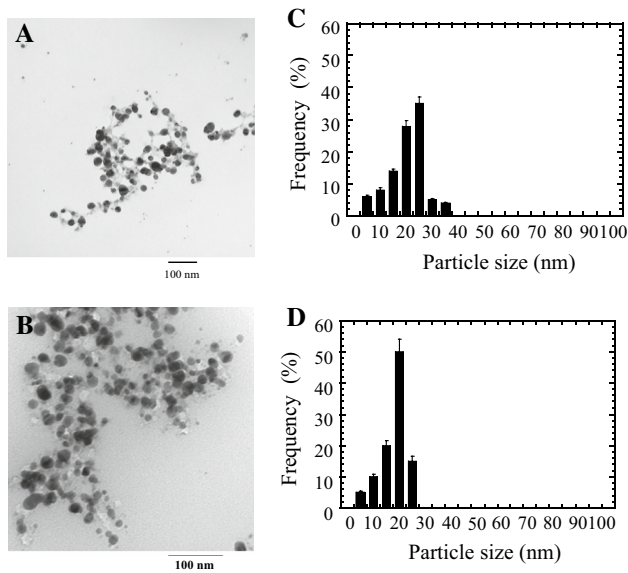


Fig. 5 Transmission electron microscope (TEM) images of silver nanoparticles obtained by the bioreduction of silver ions to nanoparticles by *Anabaena variables* ATCC 29413 (a) or mixed phytoplankton collected from the Nile River (b). Histograms c and d are for the particle size frequency derived by counting over multiple TEM images of AgNPs prepared by *Anabaena variables* and mixed phytoplankton, respectively

a brown to reddish color in aqueous solution and showed a typical wavelength spectrum of AgNPs (Fig. 7) with a maximum wavelength of 430 nm. The synthesized AgNPs showed a size range of 8–39 nm as analyzed using transmission electron microscope (Fig. 6D; Table 1).

Minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) and fungicidal (MFC) concentrations of silver ions versus nanoparticles were determined. The formed AgNPs by *E. coli* culture was used for investigating its bactericidal effect against the same microorganism where these nanoparticles showed antibacterial properties against that microorganism supporting that *E. coli* did not viably synthesize AgNPs (Fig. 8a). In addition, silver ions showed a more efficient antibacterial activity against *E. coli* (Fig. 8b) where the MIC and MBC of silver ions were significantly lower than AgNPs (Fig. 9). Similarly, AgNPs

synthesized by other microorganisms were investigated as antimicrobial agent against all investigated microorganisms in this study. All AgNPs prepared in this study showed antimicrobial properties against all tested microorganisms even the same microorganism used for its synthesis (Table 2). Silver ions showed also antimicrobial properties against all tested microorganisms (Table 2). Both silver ions and nanoparticles showed a slight difference between the MIC and MBC or MFC against each microorganism (Table 2), suggesting that both act as a potent microbicidal agent against all subject microorganisms (*E. coli*, *S. platensis* and *A. terreus*). Although both silver ions and nanoparticles showed antimicrobial activities (Table 2), the minimum inhibitory concentration and minimum bactericidal and fungicidal concentrations of silver ions were significantly lower than AgNPs (Fig. 9; Table 2) indicating that silver ions are more efficient antimicrobial.

Discussion

Due to its wide range of applications and in spite of its antimicrobial properties, the biosynthesis of silver nanoparticles (AgNPs) by bacteria and other microorganisms became a hot topic of research in the recent two decades seeking a cheap mass scale industrial production of these nanoparticles. The results shown in this study reveal the controversy among literature on the biosynthesis of AgNPs by microorganisms and those describing the use of AgNPs as antimicrobial agent against similar organisms in other literature. Upon addition of silver ions to the cultures, all tested auto- and heterotrophic microorganisms in this study were killed and could not as viable cells produce AgNPs. Photosynthesis and respiration of the cyanobacterium *S. platensis* ceased by silver ions. Similar results were obtained for *Anabaena variabilis* ATCC 29413. Aerobic respiration of *E. coli* and *A. terreus* also ceased upon the addition of silver ions to the culture medium. Similar results were obtained for *Epicoccum purpurascens* and *Aspergillus fumigatus*. The formation of AgNPs by all investigated microorganisms at normal growth conditions was very slow process. Such formation of AgNPs occurs

Table 1 Average size of various AgNPs synthesized in this study as analyzed using transmission electron microscope (TEM)

Microorganisms used for synthesis of AgNPs	Size range of AgNPs (nm)	Average size of AgNPs (nm)
<i>E. coli</i>	5–30	15.5 ± 0.9
<i>S. platensis</i>	10–40	35.6 ± 1.8
<i>A. terreus</i>	4–21	8.8 ± 0.6
EPS of <i>S. platensis</i>	8–39	35 ± 1.9
<i>Anabaena variabilis</i>	4–35	23 ± 1.3
Mixed phytoplankton	3–25	19 ± 1.1

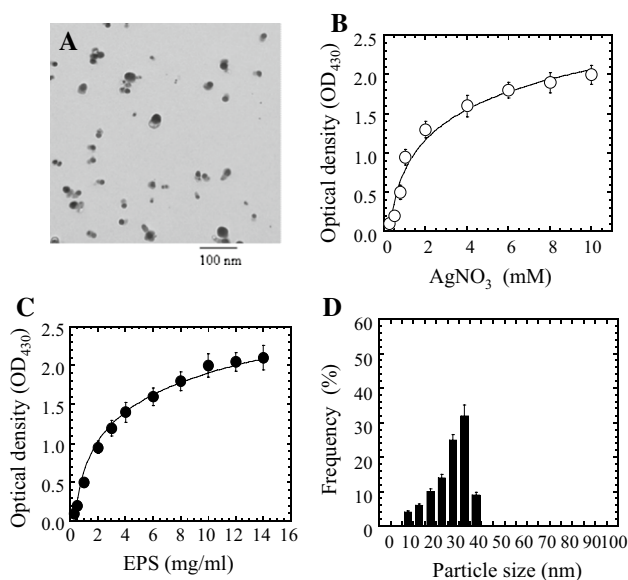


Fig. 6 Bioreduction of silver ions to nanoparticles by exopolysaccharides (EPS) of *S. platensis*. TEM image (a). **b** The effect of silver ions concentration in the presence of 10 mg/ml EPS on AgNPs formation. The effect of EPS concentration on AgNPs formation was followed in the presence of 8 mM silver ions (c). The bars show the standard errors of three replicates. The histogram (d) is for the particle size frequency derived by counting over multiple TEM images of AgNPs prepared by EPS of *S. platensis*

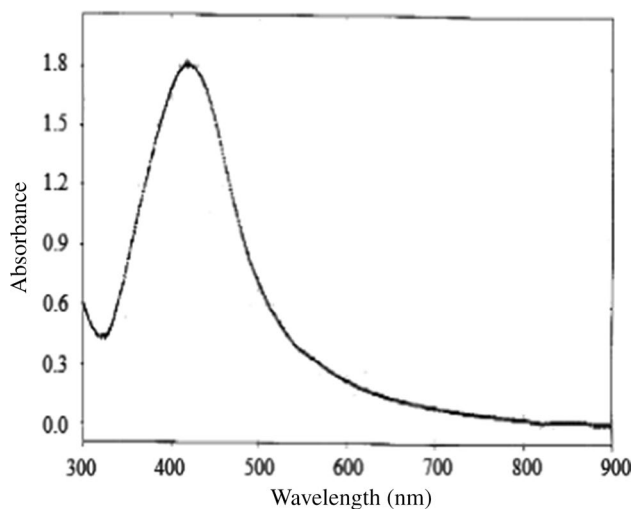


Fig. 7 UV-visible spectrum of AgNPs prepared by the bioreduction of silver ions to nanoparticles by exopolysaccharides (EPS) of *S. platensis*

after full culture death and requires several days of incubation at normal growth temperature of all tested microorganisms. Mixed phytoplankton could not also as viable cells produce AgNPs and showed similar results to *S. platensis* and *Anabaena variabilis*. Such slow bioreduction was

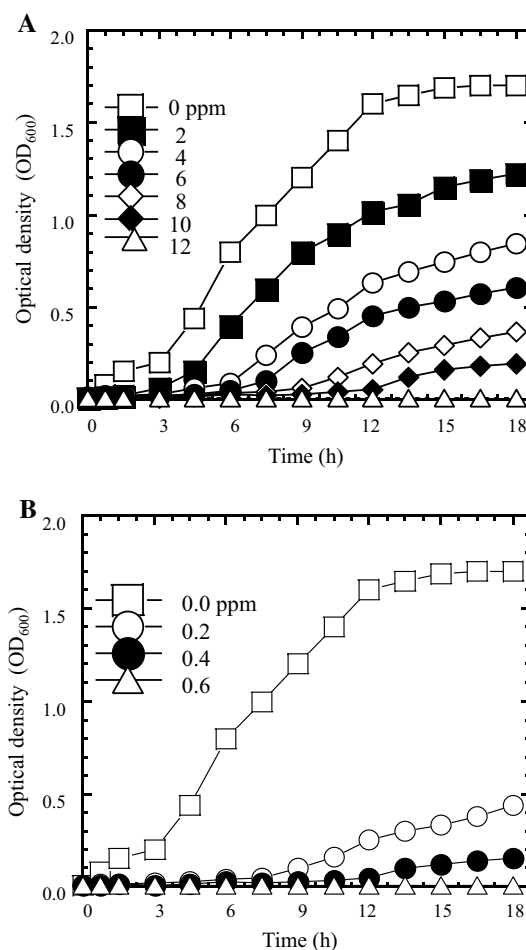


Fig. 8 Determination of the minimum inhibitory concentration. The MIC of AgNPs (a) and silver ions (b) was determined by following the inhibitory effect on growth of *E. coli* as described in “Materials and methods”

concomitant with the slow microbial cell lyses and release of organics to the culture medium. The bioreduction was strongly enhanced and occurs within few minutes at high temperature or autoclaving which enhance the cell lyses and the bioreduction rate of silver ions to nanoparticles by the released cellular organics.

The results presented in this study showed two similar antimicrobial characteristics of silver ions and nanoparticles. First, both silver ions and nanoparticles showed a broad-spectrum antimicrobial activities against all subjected microorganisms where all synthesized AgNPs in this study showed bactericidal and fungicidal properties even against microorganisms used for its synthesis supporting that the biosynthesis is not a vital metabolic process. Second, both silver ions and nanoparticles act as microbicides. As antimicrobials are usually regarded as bactericidal if the MBC is no more than four times the MIC (French 2006), the slight difference between the MIC and MBC or MFC

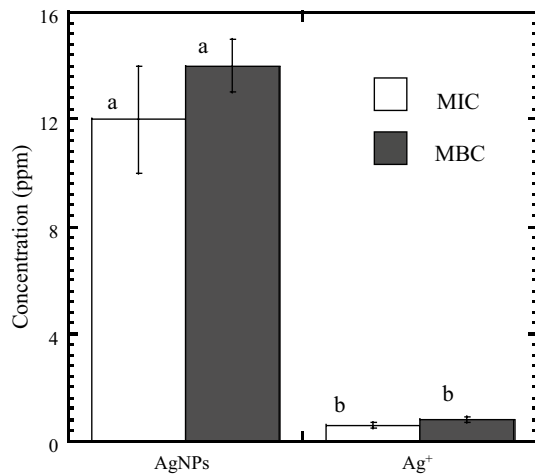


Fig. 9 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver ions versus nanoparticles against *E. coli*. The bars on columns show the standard errors of three replicates. Columns followed by the same letter are not significantly different at LSD ($P < 0.05$)

of either silver ions or nanoparticles suggests that both of them act as potent microbicidal agents against all tested microorganisms. However, silver ions are more efficient as antimicrobial than its nanoparticles. Thus, the microbial cell death occurs by silver ions too early before formation of AgNPs.

Cell extracts of all investigated microorganisms could also reduce silver ions to form AgNPs at high temperature in a fast rate and at low rate at normal growth temperature of each microorganism. Even the cyanobacterial polysaccharides fraction extracted from *S. platensis* cells could efficiently reduce silver ions to nanoparticles in a fast rate

at high temperature or autoclaving and at low rate at normal growth temperature of that microorganism. These results suggest that the biosynthesis of AgNPs by microorganisms is just a non-specific bioreduction process of silver ions into nanoparticles by the organics released in the microbial cell lysate from the dead cultures. Such bioreduction of silver ions into nanoparticles is similar to that bioreduction occurs by using many reported organic materials including pure and crude organics such as starch and plant extracts which have been successfully used for AgNPs formation (Raveendran et al. 2003; Vigneshwaran et al. 2006b; Mittal et al. 2013).

Although photoreduction of silver ions to nanoparticles was previously reported (Tan et al. 2007; Sato-Berrú et al. 2009), in this study, photoreduction of silver ions to nanoparticles did not occur in control experiments in light using SOT medium without *S. platensis* cells or pure EPS. This might be because SOT medium is inorganic, while photoreduction of silver ions to nanoparticles requires the presence of organic materials such as sodium citrate (Sato-Berrú et al. 2009) or a mixture of branched polyethyleneimine (BPEI) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) where the mechanism for the reduction of silver ions in the BPEI/HEPES mixtures entails oxidative cleavage of BPEI chains that results in the formation of positively charged BPEI fragments enriched with amide groups as well as in the production of formaldehyde, which serves as a reducing agent for silver ions (Tan et al. 2007). Control experiments were also conducted in dark to explore whether there is a role of photoreduction in AgNPs formation or not in the presence of *S. platensis* cells or EPS. In these control experiments conducted in dark using *S. platensis* at normal growth temperature of 32 °C, formation of

Table 2 Minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) and fungicidal (MFC) concentration of silver ions versus nanoparticles

	Microorganisms used for synthesis of AgNPs	Susceptible microorganisms to AgNPs	MIC ($\mu\text{g ml}^{-1}$)	MBC or MFC ($\mu\text{g ml}^{-1}$)	
AgNPs	<i>E. coli</i>	<i>E. coli</i>	12 ± 2	14 ± 1	
		<i>S. platensis</i>	18 ± 2	24 ± 3	
		<i>A. terreus</i>	16 ± 2	20 ± 2	
	<i>S. platensis</i>	<i>E. coli</i>	14 ± 1	20 ± 2	
		<i>S. platensis</i>	20 ± 2	28 ± 3	
		<i>A. terreus</i>	18 ± 2	26 ± 3	
	<i>A. terreus</i>	<i>E. coli</i>	8 ± 1	14 ± 1	
		<i>S. platensis</i>	16 ± 2	20 ± 2	
		<i>A. terreus</i>	12 ± 1	16 ± 1	
	EPS of <i>S. platensis</i>	<i>E. coli</i>	<i>E. coli</i>	12 ± 1	20 ± 2
			<i>S. platensis</i>	18 ± 2	26 ± 3
			<i>A. terreus</i>	16 ± 1	22 ± 2
Ag ⁺		<i>E. coli</i>	0.6 ± 0.1	0.8 ± 0.1	
		<i>S. platensis</i>	0.9 ± 0.1	1 ± 0.2	
		<i>A. terreus</i>	0.8 ± 0.2	0.9 ± 0.1	

AgNPs was also observed similar to light conditions indicating that neither light nor vitality of *S. platensis* cells was required for AgNPs formation. In addition, purified EPS also reduced silver ions to nanoparticles in dark at 32 °C confirming that organics are the bioreductant of silver ions to nanoparticles with no role of photoreduction or cell vitality. These results confirm that organics released from dead *S. platensis* cells reduce silver ions to nanoparticles.

It is well known that a microorganism that synthesizes an antibiotic or a toxin has a mechanism for protecting itself from the microbicidal action of such substances. In contrast to biologically synthesized antibiotics, AgNPs act as antimicrobial and a powerful microbicide for even the microbes that have been hypothesized to biosynthesize these nanoparticles. Unlike iron magnetic nanoparticles in magnetotactic bacteria, it is not reported that intracellular biosynthesis of AgNPs is a natural phenomenon in any microorganism. Besides, a natural intracellular biosynthesis of AgNPs would require a mechanism for active influx and accumulation of silver ions inside the cell. The magnetotactic bacteria that in vivo synthesize magnetite nanoparticles in magnetosomes are believed to synthesize siderophores (Bazylinski and Frankel 2004) which have a high affinity for Fe^{3+} to be transferred to inside the cell and then to be reduced to Fe^{2+} and accumulated in magnetosomes by active iron transport proteins (Nakamura et al. 1995; Schübbe et al. 2003) where magnetite (or greigite) nanocrystals are formed. In contrast, no gene in currently sequenced microbial genomes is functionally confirmed by knockout as encoding for silver ion influx transporter in microorganisms. A survey in NCBI (National Center for Biotechnology Information) Web site and all sequenced genomes revealed nothing for a gene expressing a biochemically or physiologically characterized silver ion influx transporter in any of the current genome-sequenced microorganisms. Unlike iron, silver is not considered as a mineral macro-nutrient or even micro-nutrient for microorganisms and is not actively accumulated for a natural phenomenon. One better alternative to microorganisms for the biosynthesis of AgNPs is the plant extracts which are easily available, safe and have a broad variety of metabolites that can aid in the reduction of silver ions faster than microorganisms in the synthesis. The use of microbial cell extracts (Morsy 2014) or fractions such as cyanobacterial polysaccharides which can be massively prepared (Morsy et al. 2008b) for the bioreduction of silver ions to nanoparticles (Morsy et al. 2014) at high temperature would also be economically feasible for industrial-scale preparations of AgNPs.

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