REVIEW

BIOTECHNOLOGY and BIOENGINEERING

Biologically Produced Nanosilver: Current State and Future Perspectives

Liesje Sintubin, Willy Verstraete, Nico Boon

Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium; telephone: +32-9-264-59-76; fax: +32-9-264-62-48; e-mail: nico.boon@ugent.be, www.labmet.Ugent.be

ABSTRACT: Silver nanoparticles are one of the most commercialized nanomaterials. They are widely applied as biocides for their strong antimicrobial activity, but also their conductive, optic and catalytic properties make them wanted in many applications. The chemical and physical processes which are used to synthesize silver nanoparticles generally have many disadvantages and are not eco-friendly. In this review, we will discuss biological alternatives that have been developed using microorganisms or plants to produce biogenic silver. Until now, only their antimicrobial activity has been studied more into detail. In contrast, a wide range of practical applications as biocide, biosensor, and catalyst are still unexplored. The shape, size, and functionalization of the nanoparticles is defined by the biological system used to produce the nanoparticles, hence for every application a specific biological production process needs to be chosen. On the other hand, biogenic silver needs to compete with chemically produced nanosilver on the market. Large scale production generating inexpensive nanoparticles is needed. This can only be achieved when the biological production system is chosen in function of the yield. Hence, the true challenge for biogenic silver is finding the balance between scalability, price, and applicability.

Biotechnol. Bioeng. 2012;xxx: xxx–xxx.

KEYWORDS: biogenic silver; antimicrobial; nanoparticles; catalyst

Introduction

Silver is used worldwide for its antimicrobial properties in innumerous applications such as personal care products, medical devices, wound dressings, washing machines, and computer keyboards (Edwards-Jones, 2009; Silver et al., 2006). Its popularity is due to its activity towards a broad range of microbial agents (Edwards-Jones, 2009; Elechiguerra et al., 2005; Morones et al., 2005; Yoon et al., 2007) and its relatively low toxicity towards humans (Lansdown, 2007). The use of bulk silver as an antimicrobial dates back to ancient times when water and wine were stored in silver vessels to prevent spoilage (Silver et al., 2006; Rai et al., 2009). In the 17th century, the silver ion (applied as the nitrate salt) was used to heal ulcers and chronic wounds, a therapy which probably originates from the Middle Ages and Paracelcus (Klasen, 2000a). In the 19th century, it was further used for the treatment of burns and the prevention of ophthalmic diseases in newborns. During the first decades of the 20th century, silver nitrate was still used to treat wounds and burns, but around the Second World War antibiotics treatments were developed and the interest in silver faded (Edwards-Jones, 2009; Klasen, 2000a,b; Silver et al., 2006). In 1965, the interest in silver revived thanks to a publication of Moyer et al. (1965) who suggested to use 0.5% of silver nitrate to treat burns. Silver sulphadiazine was introduced a couple of years later and today, it is still one of the most widely used burn therapies (Klasen, 2000b; Storm-Versloot et al., 2010).

In the beginning of the 20th century, Barns recognized that silver nitrate was caustic for the eyes of newborns and he invented argyrol, a protein-stabilized silver colloid (Silver et al., 2006). Around that same time, a similar colloidal silver solution was commercialized as collargol. These colloidal silver preparations were early nanosilver formulations (Nowack et al., 2011). In fact, they were not the earliest applications of silver nanoparticles. The Romans used silver nanoparticles to color the glass of the famous Lycurgus cup and also in later centuries nanosilver was used to stain glass (Evanoff and Chumanov, 2005). In those times, nanoparticles were not recognized as such and their production happened unintentionally. It was not until more recently that the term "nano" was introduced. The term "nano" indicates structures with a size between 1 and 100 nm.

Correspondence to: N. Boon
Contract grant sponsor: Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT)
Contract grant number: 71333
Contract grant sponsor: FWO
Received 19 September 2011; Revision revised 26 April 2012; Accepted 21 May 2012
Accepted manuscript online xx Month 2012;
Article first published online in Wiley Online Library (wileyonlinelibrary.wiley.com).
DOI 10.1002/bit.24570

Nanosilver is probably one of the most commercialized nanomaterials. It is used in over 200 consumer products. As an antimicrobial agent, it is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances (Maynard, 2007; Wijnhoven et al., 2009). Besides their antimicrobial features, silver nanoparticles also have other interesting properties. Nanosilver exhibits strong optical features making the nanoparticles suitable for biological sensing and imaging (Jain et al., 2008). Due to their high conductivity, silver nanoparticles are applied in conductive inks, adhesives and pastes for a range of electronic devices (Park et al., 2008). Silver nanoparticles are also used as catalysts in several chemical reactions such as the oxidation of styrene (Jiang et al., 2005; Xu et al., 2006).

Originally, silver nanoparticles were synthesized using chemical and physical methods such as liquid-phase synthesis, high-temperature reduction, vapor-phase condensation, laser ablation, photoreduction, and electrolysis (Abid et al., 2002; Mafune et al., 2000; Malynych and Chumanov, 2003; Wang et al., 2002; Zhu et al., 2000). Each method has advantages and disadvantages with common problems being costs, scalability, and wide size distribution (Evanoff and Chumanov, 2005). Also the instability of the nanoparticles upon application can be problematic since aggregation decreases the specific surface area lowering the antimicrobial and catalytic activity (Mafune et al., 2000). Moreover, customers and stakeholders demand eco-friendly production processes to avoid the use of solvents and toxic reagents. There are some green chemical methods reported using for example polysaccharides in water as the reduction medium (Huang and Yang, 2004; Vigneshwaran et al., 2006b). However, the current green nanotechnology focuses more on the use of biological systems to produce silver nanoparticles. Since Klaus et al. (1999) reported that a silver-resistant *Pseudomonas stutzeri* could produce silver nanoparticles, the number of publications using microorganisms and later on also plants has increased exponentially (Klaus et al., 1999). Although biological production shows a lot of potential, the possibilities, such as upscaling, have not been fully explored so far. Also about the application of biogenic silver—as the biologically produced silver nanoparticles will be called from now on—little is published. Therefore, in addition to a critical overview of the existing literature about biogenic silver, this review will focus on the perspectives for upscaling and applications.

Production of Biogenic Silver

When silver nanoparticles are chemically produced, three main components are needed: a silver salt (usually $AgNO_3$), a reducing agent (e.g., sodium borohydride) and a stabilizer or capping agent (e.g., polivinylpyrrolidone) to control the growth of the nanoparticles and prevent them from aggregating (Ledwith et al., 2007). In the case of the biological synthesis of silver nanoparticles, the reducing agent and the stabilizer are replaced by molecules produced by living organisms (Fig. 1). These reducing and/or stabilizing compounds can be present in bacteria, fungi, yeasts, algae, or plants (Gade et al., 2010; Narayanan and Sakthivel, 2010). The reduction can happen enzymatically or non-enzymatically and the stabilizer or capping agent is in many cases a protein. Depending on the biological system or biomass type used, a variety of nanoparticles with different sizes has been reported (Table I). Also diverse shapes have

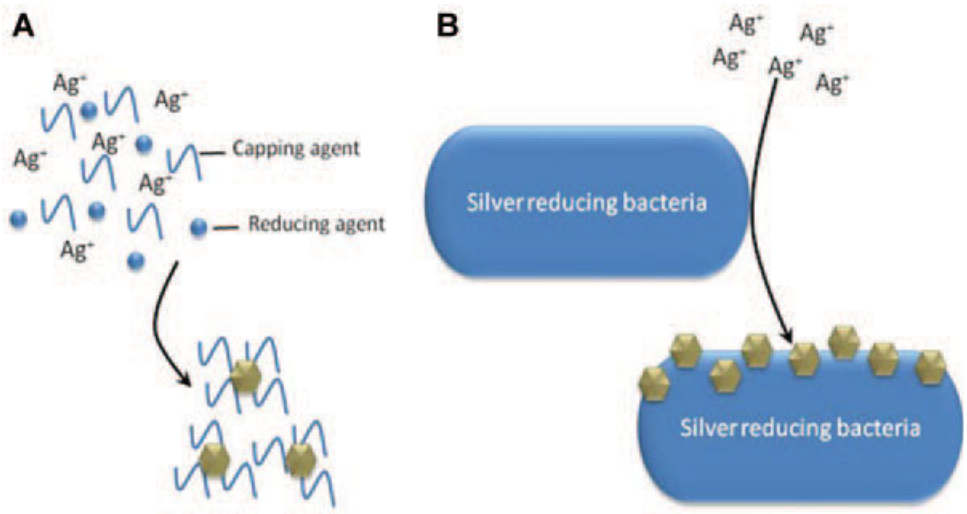

Figure 1. Chemical (**A**) versus biological synthesis using bacteria (**B**). In the case of chemical synthesis, silver ions, provided as a silver salt, are reduced by a chemical reducing agent forming a silver nucleus. The growth of the nucleus to a particle is controlled by a capping agent which also prevents aggregation by steric hindrance or electrostatic repulsion. In the case of biological synthesis the reducing and capping agent are both provided by microorganisms or plants. In this example (B) the ions are precipitated and stabilized by functional groups on the cell wall of bacteria.

Table I. Overview bacteria, fungi, yeasts, and plants which synthesize silver nanoparticles with their size and shape.

Species	Average nanoparticle size (distribution range; nm)	Nanoparticle shape	Reference
Bacteria			
Bacillus cereus	5	Spherical	Babu and Gunasekaran (2009)
Bacillus licheniformis	40–50	—	Kalishwaralal et al. (2008)
Bacillus thuringiensis	10–20	Various	Jain et al. (2010)
Brevibacterium casei	10–50	Spherical	Kalishwaralal et al. (2010)
Corynebacterium	5–15	—	Zhang et al. (2005)
Escherichia coli	50 [42.2–89.6]	Spherical	Gurunathan et al. (2009)
Escherichia coli	40–60	—	Natarajan et al. (2010)
Klebsiella pneumoniae	52.5 [28.2–122]	—	Shahverdi et al. (2007)
Lactobacillus farciminis	17.0 [14.3–19.7]	Spherical	Sintubin et al. (2009)
Lactobacillus fermentum	11.2 [10.3–12.1]	Spherical	Sintubin et al. (2009)
Lactobacillus plantarum	19.4 [16.8–21.9]	Spherical	Sintubin et al. (2009)
Lactobacillus rhamnosus	15.7 [13.6–17.8]	Spherical	Sintubin et al. (2009)
Pseudomonas stutzeri	<200	Triangular, hexagonal, spherical	Klaus et al. (1999)
Ureibacillus thermosphaericus	1–100	Spherical	Juibari et al. (2011)
Lactobacillus sp.	15–500	Polymorphus	Nair and Pradeep (2002)
Plectonema boryanum	1–15	Spherical	Lengke et al. (2007a)
	1–40	Spherical	
	5–200	Octahydral platelets	
Staphylococcus aureus	160–180	—	Nanda and Saravanan (2009)
Fungi			
Aspergillus niger	20	Spherical	Binupriya et al. (2009), Gade et al. (2008)
Aspergillus ochraceus	20	Spherical	Vijayakumar and Prasad (2009)
Aspergillus oryzae var. *viridis*	5–50	Spherical	Binupriya et al. (2010)
Cladosporium cladosproides	10–100	Spherical	Balaji et al. (2009)
Coriolus versicolor (proteins)	10	Spherical	Sanghi and Verma (2009a)
	25–75	Spherical	
Fusarium oxysporum	1.6	Spherical	Duran et al. (2007)
Fusarium oxysporum (enzyme)	25 ± 12	—	Kumar et al. (2007)
Fusarium solani	16.23 ± 0.78 [5–35]	Spherical	Ingle et al. (2009)
Neurospora crassa	11	Spherical, ellipsoidal	Castro-Longoria et al. (2011)
Penicillium brevicompactum	58.35 ± 17.88	—	Shaligram et al. (2009)
Penicillium fellutanum	5–25	Spherical	Kathiresan et al. (2009)
Phaenerochaete chrysosporium	50–200	Spherical	Vigneshwaran et al. (2006a)
Pleurotus sajor-caju (protein)	30.5 ± 4	Spherical	Vigneshwaran et al. (2007b)
Plerurotus sajor-caju	5–50	Spherical	Nithya and Ragunathan (2009)
Phoma glomerata	60–80	Spherical	Birla et al. (2009)
Phoma sp. 3.2883	71.06 ± 3.48	Spherical	Chen et al. (2003)
Phyllanthus amarus (protein)	30	Spherical	
Trichoderma viride	2–4	Spherical	Fayaz et al. (2010b)
Trichoderma viride	10–40	Spherical	Thakkar et al. (2010)
Verticillium sp.	25 ± 12	—	Sastry et al. (2003)
Volvariella volvacea	15	Spherical, ellipsoidal	Philip (2009)
Yeasts			
Silver-tolerant strain MKY3	2–5 [2–20]	Hexagonal, twinned cubic	Kowshik et al. (2003)
Algae			
Spirulina platensis	7–16	Spherical	Govindaraju et al. (2008)
Plants			
Agave americana	20 [5–50]	Spherical	Marciano et al. (2008)
Acalypha indica	20-30	—	Krishnaraj et al. (2010)
Azadirachta indica	5–35	—	Shankar et al., (2004)
Bryophyllum sp.	2–5	Spherical	Jha et al. (2009)
Calotropis gigantea	<20	Spherical	Rajasekharreddy et al. (2010)
Carica papaya	20–40	Irragular	Rajasekharreddy et al. (2010)
Chenopodium album	10–30	Spherical, triangular	Dwivedi and Gopal (2010)
Cinnamomum camphora	55–80	Triangles, anisotropic	Huang et al. (2007)
Cinnamomum camphora	5–80	Flat, rods	Huang et al. (2008)
	40–50	Nanowires	
Citrus aurantium	20–40	Irragular, ellipsoidal	Rajasekharreddy et al. (2010)
Coriandrum sativum	26 [8–75]	Spherical	Sathyavathi et al. (2010)

Table I. (*Continued*)

Species	Average nanoparticle size (distribution range; nm)	Nanoparticle shape	Reference
Cyprus sp.	2–5	Spherical	Jha et al. (2009)
Datura metel	20–40	Irragular	Rajasekharreddy et al. (2010)
Emblica officinalis	16.8 [7.5–25]	Spherical	Ankamwar et al. (2005)
Garcinia mangostana	35 [6–57]	Spherical	Veerasamy et al. (2011)
Gliricidia sepium	27 [10–50]	Spherical	Rajesh et al. (2009)
Hibiscus rosa sinensis	13	Spherical, anisotropic	Philip (2010a)
Hydrilla sp.	2–5	Spherical	Jha et al. (2009)
Jatropha curcas	15–25	Spherical	Bar et al. (2009)
	30–50	Spherical	
Jatropha curcas	<20	Spherical	Rajasekharreddy et al. (2010)
Medicago sativa	5–51	Spherical	Lukman et al. (2011)
Parthenium hysterophorus	50 [30–80]	Irragular	Parashar et al. (2009)
Solanum melongena			Rajasekharreddy et al. (2010)
Syzygium aromaticum	—	—	Singh et al. (2010)
Tetrapanax papyriferus	<100	Cubic crystals	Zeng et al. (2007)
Tridax procumbens	<20	Spherical	Rajasekharreddy et al. (2010)

been constructed, with spherical particles being predominant. These nanoparticles are situated intracellularly or extracellularly on the cell wall when whole cells are used for the nanoparticle production or they are produced extracellularly in the cell supernatant (Narayanan and Sakthivel, 2010). The latter case allows the use of cell free extracts, containing the reducing and stabilizing agents, to produce biogenic silver minimizing the post-production extraction and purification of the nanoparticles.

Reduction Mechanism

The exact biological reduction mechanism of Ag^+ to Ag^0 is not fully understood, but generally enzymatic reduction can be distinguished from non-enzymatic reduction. In the current section, both reduction mechanisms will be generally discussed. However, for the detailed reduction reactions we refer to the specific research publications or the review written by Duran et al. (2011).

Mukherjee et al. (2001a) were among the first to suggest that enzymes might be responsible for the reduction of silver ions. They only observed the formation of silver nanoparticles on the mycelia of *Verticillium* sp. cells and not into solution. Therefore, they concluded that Ag^+ was trapped by negatively charged carboxylated groups of enzymes in the cell wall and subsequently was reduced there. Also Ahmad et al. (2003) believed that enzymes played a role in the production of silver nanoparticles by the fungus *Fusarium oxysporum*. With a protein assay they proved that the responsible enzyme was an NADPH-dependant reductase. This was confirmed by Kumar et al. (2007), who extracted α-NADPH-dependent nitrate reductase from *F. oxysporum* and used the pure enzyme to synthesize nanoparticles.

One of the disadvantages of the enzymatic reduction of silver is the slow rate of the reaction. The time required for reduction ranges has varied between 24 and 120 h, although Shahverdi et al. (2007) developed a rapid synthesis process using *Enterobacteria* supernatants. Within 5 min the supernatant of *Klebsiella pneumonieae*, *Escherichia coli*, and *Enterobacter* cloacae turned brown, indicating the formation of nanosilver. The addition of piperitone, an inhibitor of nitroreductases, prevented the production of nanoparticles, pointing out that the oxygen-insensitive nitroreductase NfsA is a keyplayer in the reduction of silver. Also *Bacillus licheformis* uses a nitrate reductase to reduce silver ions (Kalishwaralal et al., 2008).

The non-enzymatic reduction of silver is based on the chemical reduction, but in this case the reducing and stabilizing compounds are produced by microorganisms or plants (Kumar and Yadav, 2009; Sharma et al., 2009; Sintubin et al., 2009). Generally, the stabilizing compounds are proteins which cap the nanoparticles and prevent them from aggregation (Binupriya et al., 2010; Sathyavathi et al., 2010; Zhang et al., 2005). Reducing compounds, on the other hand, can be very diverse depending on the biomass type and/or species used. Lin et al. (2005) and Sintubin et al. (2009) reported the role of reducing sugars such as glucose being responsible for the reduction by *Lactobacillus* sp. bacteria. In the case of the fungus *Phaenerochaete chrysosporium* and honey, reducing sugars were mentioned to be the reducing agents as well (Philip, 2010b; Vigneshwaran et al., 2006a). The combination of reducing sugars and terpenoids were responsible for silver reduction in the case of neem leaf broth (Shankar et al., 2004). In addition to being capping agents, proteins also have been mentioned as reducing compounds in the case of the plants *Capsicum anuum*, *Hibiscus rosa sinensis* and *Coriandrum sativum* (Li et al., 2007; Philip, 2010a; Sathyavathi et al., 2010). For plants polyols, eugenol, quinines, and phyllantin have been also reported as reducing agents (Huang et al., 2008; Jha et al., 2009; Kasthuri et al., 2009; Singh et al., 2010).

The non-enzymatic reduction is often fast, taking only a couple of minutes (Shankar et al., 2004; Sintubin et al., 2009). Moreover, the non-enzymatic production of silver nanoparticles can handle more extreme parameters such as a

high pH or temperature which possibly can accelerate the synthesis rate. For example, at a pH of 11.5, Sintubin et al. (2009) succeeded in producing nanoparticles with *Lactobacillus* sp. within one minute. Also Sanghi and Verma (2009a), Fu et al. (2006) and Govindaraju et al. (2008) noticed a faster reaction rate when working in alkaline conditions. Not only do alkaline conditions increase the reaction rate, they can enhance recovery as well. The recovery with *Lactobacillus fermentum* at pH 11.5 was 1.6 times higher compared to the recovery at pH 7, and 6.4 times higher compared to pH 2 (Sintubin et al., 2009). Lengke et al. (2007a) also noticed a higher silver recovery when increasing the temperature: at 100°C all silver was taken up by the biomass. Also Zhang et al. (2005) and Huang et al. (2008) reported that higher temperatures were favorable for reduction, increasing the reduction rate (Kaviya et al., 2010; Zhang et al., 2005). Finally, the non-enzymatic reduction of silver allows to work with $AgNO_3$ concentrations of 1–10 g L^{-1} which is 10–100 times higher than for enzymatic reduction (0.01–0.1 g L^{-1}) (Sintubin et al., 2009; Zhang et al., 2005, 2007).

The Use of Whole Cells or Cell Extracts

For the production of biogenic silver whole cells or cell extracts can be used. When whole cells are used, silver reduction can happen intracellularly, as in the case of *P. stutzeri* and *Verticillium* sp. (Klaus et al., 1999; Mukherjee et al., 2001a), or extracellularly with the formation of silver nanoparticles on the cell wall in the case of *Lactobacillus* sp. (Nair and Pradeep, 2002; Sintubin et al., 2009). Another case of extracellular synthesis is the formation of nanoparticles in the medium or supernatant by reducing and capping agents excreted by microorganisms or plants (Duran et al., 2007; Kowshik et al., 2003). Since these nanoparticles do not need whole cells for their synthesis, they can be produced with a cell-free extract. In the case of microorganisms, such cell extracts can be made in two ways: (1) by washing the biomass and dissolving the cells in water or a buffer for a certain amount of time to extract the reducing compounds (Binupriya et al., 2010; Duran et al., 2007), or (2) by using the medium in which the biomass has grown (Nanda and Saravanan, 2009; Nithya and Ragunathan, 2009). The latter might contain in addition to excreted microbial components, such as enzymes or carbohydrates, also reducing compounds originating from the medium. Hence, one must take into account that the production of silver nanoparticles might not entirely be biological. In the case of plants, extracts are the most common formulation to produce silver nanoparticles. They are usually made by soaking fresh or dried plant material in cold or boiling water (Philip, 2010a; Sathyavathi et al., 2010; Veerasamy et al., 2011).

Cell extracts and whole cells are constituted out of a large variety of biological molecules, such as carbohydrates, proteins and nucleic acids, or structures, such as cell wall, S-layers or cuticula, thus usually contain more than one reducing and/or capping agent. Weak reducing agents usually produce larger nanoparticles and strong reducing agents smaller nanoparticles (Tolaymat et al., 2010); hence, a combination of them with various capping agents will lead to the production of nanoparticles with a wide range of sizes and shapes or a high polydispersity. This is what generally happens when silver nanoparticles are produced biologically, which is in contrast to chemical production processes which use only one reducing agent and create more monodisperse nanoparticles (Binupriya et al., 2010). To avoid this heterogeneity, a possibility in the biological production process is the use of biological molecules or cellular structures extracted from eukaryotic or prokaryotic cells, which will give rise to more monodisperse silver nanoparticles. Chen et al. (2008), for example, used the separated squama inner coat of an onion as a template for silver reduction and created monodisperse spherical nanoparticles with a size between 4.6 and 7.9 nm (Chen et al., 2008). The extraction and purification of these biomolecules to produce monodisperse nanoparticles, will require one or more extra steps in the production process and, therefore, will be more time-consuming and less economic. An economic exception would be the use of simple molecules such as reducing sugars and polysaccharides. Since microorganisms and plants exist of highly organized structures, the cells or biomass parts can be used as a template for nanoparticle precipitation with an external reducing agent. Zeng et al. (2007) precipitated silver nanoparticles in the pores a rice-paper plant with $NaBH_4$ as a reducing agent. Saifuddin et al. (2009), on the other hand, treated a *Bacillus subtilis* extract with microwave irradiation to create nanoparticles. Also Marciano et al. (2008) applied microwave irradiation to reduce the silver ions adsorbed to the plant cuticula of *Agave Americana*.

Biogenic Versus Chemically Produced Nanoparticles

One of the main advantages of biogenic silver compared to chemically produced nanosilver, is its green synthesis avoiding organic solvents and toxic reagents. Moreover, the biological production of nanoparticles allows [one] to recycle silver containing waste streams, for example, photo processing wastewater (Kononova et al., 2007; Nakiboglu et al., 2003; Nowack, 2010; Veglio and Beolchini, 1997; Wang et al., 2009). The recycling, recovery, and regenerating of this silver is important due to limited resources and the fluctuating market prices. Moreover, the biological production of silver nanoparticles converts a waste stream into a product with an added value as biocide, catalyst or biosensor (Hennebel et al., 2009). In addition, the reducing microbial or plant material used for recycling could also be a waste stream recovered from food or agricultural processes (Rajani et al., 2010). When the biomass would not be able to precipitate silver, it could still be used as a template for nanoparticle formation in combination with microwave

irradiation or the addition of an external reducing agent. The biological production of biogenic silver has an additional advantage when whole microbial cells are used: when non-toxic $AgNO_3$ concentrations are used, the cells can synthesize silver nanoparticles without dying, creating the possibility for a continuous production process (Mukherjee et al., 2001a).

Upon application in liquid environments, chemically produced nanoparticles are known to aggregate to larger clusters (Mafune et al., 2000). This decreases their high specific surface area and therefore also their catalytic and antimicrobial activity. Biogenic silver, however, can be stable over large periods of time. Bfilainsa and D'Souza (2006) noticed that the extracellularly produced nanoparticles of *Aspergillus fumigates* were stable for 4 months and Saifuddin et al. (2008) even found the particles synthesized with *B. subtilis* to be stable for 6 months (Bfilainsa and D'Souza, 2006; Saifuddin et al., 2009). Sintubin et al. (2011b) found biogenic silver produced with *L. fermentum* to be stable upon application. These nanoparticles were still associated with the matrix of the bacteria; hence the bacterial cell wall structures prevented aggregation by covering the nanoparticles which lead to an antibacterial activity which was 20 times higher compared to chemically produced nanoparticles. The latter formed large clusters during application (Sintubin et al., 2011b).

Moreover, the biological molecules, mostly proteins, and even whole cells which stabilize biogenic silver, easily allow functionalization of the nanoparticles with other biomolecules (Hennebel et al., 2009). Functionalization can increase the antimicrobial activity by improving the interactions with microorganisms (Botes and Cloete, 2010), or can make the nanoparticles useful for biomedical sensing or imaging (Khlebtsov and Dykman, 2010).

Finally, the formation of nanoparticles on biomass also allows easy separation of the nanoparticles from the reaction medium or up-concentration by centrifugation (Sintubin et al., 2009).

Current Applications

Although in the beginning research was mainly focused on the production of biogenic nanosilver and the finding of new species being able to reduce silver, more recently, their potential applications are more explored.

Antimicrobial Properties

Because of their strong antimicrobial properties, silver nanoparticles are applied in a large variety of applications to control microbial growth. The antimicrobial activity, however, depends on the size of the nanoparticles. The smaller the nanoparticles, the higher the specific surface area and therefore also their activity (Lok et al., 2007; Morones et al., 2005; Pal et al., 2007). Pal et al. (2007) also discovered that the shape of the nanoparticles was of importance, with triangular nanoparticles displaying a higher antibacterial activity. Furthermore, the type of molecule which stabilizes the nanoparticles can influence the activity by altering the interaction with the target microorganisms (Cho et al., 2005; Goodman et al., 2004). Because each type of biogenic silver is unique due to their size, shape and the proteins or other bio-molecules which functionalize their surfaces, it is important to thoroughly characterize their antimicrobial activity in order to formulate the most effective concentration for every application.

Commonly used techniques to determine the antimicrobial activity of biogenic silver, are the minimal inhibitory concentration (MIC), the minimal bactericidal concentration (MBC), time-kill, the half effective concentration (EC_{50}), well-diffusion method and disk-diffusion method (Table II). Depending on the technique used, different information can be gathered which is useful for certain applications. For example, in the case of applications which prevent microbial growth it is useful to determine the MIC. On the other hand, the MBC is a more valuable parameter for applications which do not only prevent microbial growth but also treat microbial contaminations. However, since each researcher uses the method which suites him most against the species and strain which interests him most, it is very hard to compare the antimicrobial activity between the different types of biogenic silver. This is shown in Table II where the concentrations of MIC and MBC range from 10 to 200 mg L^{-1} and the disk- and well-diffusion tests are characterized by 0–15 mm of zone of inhibition (ZOI). Additional factors which might influence the antimicrobial activity of biogenic silver, are the composition of the medium in which the test occurs, and the starting inoculums. Taking all these factors into consideration, it is clear standardized protocols are needed to test the antimicrobial activity of biogenic silver. The European Committee for Standardization, the International Organization of Standardization and the Clinical and Laboratory Standards Institute already provide protocols which define the criteria which have to be fulfilled during antimicrobial susceptibility testing. A couple of parameters which are outlined, are the cell concentration of the inoculum, the number of replicates which need to be made for a representative test, the use of a neutralizer and the necessary controls. These protocols also specify how the result of an antimicrobial susceptibility test should be interpreted (CALS, 2008; International Organization of Standardization, 2007; NEN-EN, 1997).

The antimicrobial activity of silver nanoparticles is still not fully understood. It is generally believed that three different mechanisms determine the antimicrobial activity of silver nanoparticles: (1) direct physical contact between the nanoparticles and the microbes causing structural damage (Kim et al., 2007; Sondi and Salopek-Sondi, 2004); (2) the generation of reactive oxygen species (ROS) damaging the membrane (Kim et al., 2007; Kumar et al., 2003); (3) the slow release of silver ions which interfere with

Table II. Overview of the antimicrobial activity of biogenic silver produced by different biological systems (biomass type and whole cell/cell extract).

Biomass type	Whole cell/ Cell extract	Antimicrobial method	Pathogen	Inhibition	Reference
Aspergillus oryzae (fungus)	Cell extract	MIC[a] in liquid broth	*Staphylococcus aureus*	50 mg L^{-1}	Binupriya et al. (2010)
Tetrapanax papyriferus (plant)	Whole cell	MIC on agar	*Escherichia coli*	14.1 mg L^{-1}	Zeng et al. (2007)
			Candida albicans	28.1 mg L^{-1}	
Staphylococcus aureus (bacterium)	Cell extract	Well-diffusion method	MRSA	ZOI (2 μg)	Nanda and Saravanan (2009)
			MRSE	ZOI (2 μg)	
			Streptococcus pyogenes	ZOI (2 μg)	
			Salmonella Typhi	ZOI (2 μg)	
			Klebsiella pneumoniae	ZOI (2 μg)	
			Vibrio cholerae	No ZOI (2 μg)	
Pleurotus sajor caju (fungus)	Cell extract	Well-diffusion method	*Pseudomonas aeruginosa*	ZOI	Nithya and Ragunathan (2009)
			Escherichia coli	ZOI	
			Staphylococcus aureus	ZOI	
Acalypha indica (plant)	Cell extract	MIC in liquid broth	*Escherichia coli*	10 mg L^{-1}	Krishnaraj et al. (2010)
			Virbrio cholerae	10 mg L^{-1}	
Camellia sinensis (plant)	Cell extract	MIC on agar	*Vibrio harveyi*	35 mg L^{-1}	Vaseeharan et al. (2010)
Cinnamomum zeylanicum (plant)	Cell extract	EC_{50}[b]	*Escherichia coli*	11 mg L^{-1}	Sathishkumar et al. (2009)
Aspergillus clavatus (fungus)	Cell extract	Well-diffusion method	MRSA	ZOI (10 μL)	Saravanana and Nanda (2010)
		Disk-diffusion method	MRSE	ZOI (10 μL)	
			MRSA	ZOI (10 μL)	
			MRSE	ZOI (10 μL)	
Phoma glomerata (fungus)	Cell extract	Disk-diffusion method	*Escherichia coli*	ZOI (15 μL)	Birla et al. (2009)
			Staphylococcus aureus	ZOI (15 μL)	
			Pseudomonas aeruginosa	ZOI (15 μL)	
Aspergillus ochraceus (fungus)	Whole cell	Time kill	*Escherichia coli*	2 h (7 mg L^{-1})	Vijayakumar and Prasad (2009)
			Bacillus subtilis	2 h (7 mg L^{-1})	
Gliricidia sepium (plant)	Cell extract	Disk-diffusion method	*Staphylococcus aureus*	ZOI (50 μL)	Rajesh et al. (2009)
			Escherichia coli	ZOI (50 μL)	
			Pseudomonas aeruginosa	ZOI (50 μL)	
			Klebsiella pneumoniae	ZOI (50 μL)	
Pleurotus sajor-caju (fungus)	Cell extract	Well-diffusion method	*Staphylococcus aureus*	ZOI	Vigneshwaran et al. (2007b)
			Klebsiella pneumoniae	ZOI	
Lactobacillus fermentum (bacterium)	Whole cell	MIC	*Escherichia coli*	12.5 mg L^{-1}	Sintubin et al. (2011b)
			Pseudomonas aeruginosa	12.5 mg L^{-1}	
		MBC[c]	*Staphylococcus aureus*	50 mg L^{-1}	
			Escherichia coli	25 mg L^{-1}	
			Pseudomonas aeruginosa	100 mg L^{-1}	
			Staphylococcus aureus	200 mg L^{-1}	
Bacillus thuringiensis (bacterium)		Disk-diffucion method	*Escherichia coli*	13 mm ZOI (50 μg mL^{-1})	Jain et al. (2010)
			Pseudomonas aeruginosa	15 mm ZOI (50 μg.mL^{-1})	
			Staphylococcus aureus	9 mm ZOI (50 μg mL^{-1})	

For each type of biogenic silver, the used antimicrobial method and pathogens are given. The antimicrobial activity is described as the concentration needed to inhibit pathogens (MIC), the necessary time (log kill) or the presence of a zone of inhibition (ZOI) (well-diffusion and disc-diffusion method). In the latter case, the applied biogenic silver concentration or volume is given where possible.

[a]MIC = minimal inhibitory concentration.

[b]EC50 = half effective concentration.

[c]MBC = minimal bactericidal concentration.

the DNA replication and inhibit enzymes by reacting with thiol groups (Feng et al., 2000; Liau et al., 1997; Morones et al., 2005). In the case of biogenic silver, however, the nanoparticles are coated with functional groups or cell parts of microorganisms or plants which can influence the antimicrobial activity. Sintubin et al. (2011b), for example, examined the mechanism of biogenic silver produced with *L. fermentum* and discovered the release of silver ions being the dominant factor, while the influence of ROS formation and direct contact seemed to be negligible. In this study, the silver nanoparticles were fixed on the cells of *L. fermentum*, which might have prevented true physical contact with the target bacteria, thus it cannot be excluded that direct contact might be an important antimicrobial mechanism for other types of biogenic silver (Sintubin et al., 2011b).

Water Treatment

Silver is a popular disinfectant for the treatment of drinking water. Aquapure and QSI-Nano, for example, are commercially available home water purification systems (Maynard, 2007). These systems contain chemically produced nanoparticles. De Gusseme et al. (2010) was the first who investigated the use of biogenic silver, produced with *L. fermentum*, for the removal of viruses out of drinking water (De Gusseme et al., 2010). A concentration of $5.4\,mg\,L^{-1}$ biogenic silver eliminated the two model viruses—bacteriophage UZ1 and murine norovirus 1 (MNV-1)—within 1–3 h. To disinfect drinking water in a continuous way, biogenic silver was coated on a cartridge filter which greatly enhanced the antiviral activity compared to an uncoated filter. In a second paper, they used biogenic silver powder immobilized in PVDF membranes for the removal of viruses from drinking water (De Gusseme et al., 2011). A virus decline of more than 3 log was observed at a flux rate of $72\,L\,m^{-2}\,h^{-1}$. The antiviral activity was the result of the slow release of Ag^{+}. This means that in time the antiviral activity will decrease making the membrane technology not applicable for long-term and large-scale continuous water treatment. However, for small scale water treatment, these membranes show a lot of potential.

The same research group also investigated the influence of biogenic silver produced with *L. fermentum* on solar disinfection (SODIS). They found that low concentrations of biogenic silver enhanced SODIS during the first 3 h of disinfection. Moreover, the presence of biogenic silver prevented regrowth of bacteria during storage. This was in contrast with SODIS alone or when TiO_2 was used in combination with SODIS (Sintubin et al., 2011a).

At their turn, Vaseeharan et al. (2010) used biogenic silver synthesized with tealeaf extract to combat *Vibrio* infections in shrimps. Concentrations above $35\,\mu g\,cm^{-3}$ removed 95% of *Vibrio harveyi*. At the same time, the mortality of the shrimp *Feneropenaeus indicus* was decreased with 71% over a period of 14 days compared to the control. This indicates that biogenic silver can be an alternative for antibiotic treatment in aquaculture. However, more research is needed on the toxicology and bioaccumulation of biogenic silver.

Medical Applications

Because of the emergence of antibiotic-resistant bacteria and the increasing number of hospital-acquired infections, the interest to use silver in the medical field is rising (Chaloupka et al., 2010). Nanosilver not only possesses a broad antimicrobial activity but also has a anti-inflammatory effect (Nadworny et al., 2010) and accelerates wound-healing (Tian et al., 2007). This makes silver nanoparticles suitable for several biomedical applications such as implantable devices, catheters and wound dressings (Chaloupka et al., 2010).

To fight multi-resistant bacteria, Fayaz et al. (2010a) tested the synergetic of biogenic silver, produced with the fungus *Trichoderma viride*, with several antibiotics such as kanamycin, erythromycin, chloramphenicol, and ampicillin against a range of bacteria. Biogenic silver increased the size of ZOI for every combination, but the effect was the highest for the combination with ampicillin. Gajbhiye et al. (2009) performed a similar experiment with biogenic silver, produced with the fungus *Alternaria alternate*, in combination with fluconazole against a series of fungi. They saw a significant enhancement of the antifungal activity of fluconazole in the presence of biogenic silver.

Nanda and Saravanan (2009) used only biogenic silver, produced with *Staphylococcus aureus*, against a range of resistant bacteria. The highest activity was seen against methicillin-resistant *S. aureus*, methicillin-resistant *Streptococcus epidermidis* and *Streptococcus pyogenes*. The other bacteria were only moderate susceptible (Nanda and Saravanan, 2009).

Although these three examples indicate that biogenic silver is active against antibiotic-resistant bacteria and fungi, it is not clear if they can be applied in medical devices. As Chaloupka et al. (2010) previously mentioned, biogenic silver synthesized with microorganisms could possibly cause a contamination with pathogens when used in biomedical applications. In concrete terms, biogenic silver synthesized with a conditional pathogen such as *S. aureus* might still contain living cells causing infection upon application in wound dressings. Also the biocompatibility of biogenic silver could be an issue (Chaloupka et al., 2010). Hence, the toxicity and biocompatibility need to be thoroughly tested before biogenic silver can be implied in medical applications.

Challenges and Future Perspectives

Production and Upscaling

To economically compete with chemically produced nanoparticles, biogenic silver needs to be produced in large scale to suppress costs. Nevertheless, until now reports of the large-scale biosynthesis of silver nanoparticles are rare. Huang et al. (2008) reported the continuous-flow

production (0.5–1 mL min^{-1}) of nanosilver with *Cinnamonum camphora*. Although the continuous synthesis of silver nanoparticles is important for future industrial production, Huang et al. (2008) performed their research in a tubular micro-reactor using volumes of 50 mL. Therefore, it is still not proven that large-scale production of biogenic silver is possible.

Many times, the choice of microorganisms and especially plants to produce silver nanoparticles is based on the added value of the biological material itself. For example, the algal cells of *Spirulina platensis* was chosen because it also possesses pharmaceutical and neutraceutical properties (Govindaraju et al., 2008), whereas plants such as *Acalypha indica*, *Aloe vera*, and Indian propolis are known for their medicinal properties (Chandran et al., 2006; Krishnaraj et al., 2010; Roy et al., 2010). Although this is very interesting for future applications, one must also take into account the production efficiency of the chosen biological system and the coupled costs. More specifically, high productivity is needed combining high titer and short production times. Rapid growing microorganisms, for example, have been genetically engineered to overexpress metal binding phytochelatins (Park et al., 2010).

There are two main factors which largely determine the costs of the large scale production of biogenic silver nanoparticles: the price of the biomass or biological material and the price of $AgNO_3$. The price of the biomass is influenced by its cultivability: the more biomass can be achieved in a short amount of time, the cheaper it is. Also the type of biomass will define the price. Plant material is often cheaper than microbial biomass (Raskin, 1996; Raskin et al., 1997). Moreover, the use of plant extracts eliminates the maintenance of a microbial culture, which can be an elaborate process (Sathyavathi et al., 2010). However, the use of extracted and purified bio-molecules, such as enzymes, even if they originate from plants is less economic (Kumar et al., 2007). A cheaper alternative for using pure cultures or plants, could be the waste products of large scale industrial fermentation processes like beer breweries, or agricultural crop waste.

The price of $AgNO_3$ is determined by the stock market and/or the supplier. The last years, the silver price has increased substantially. In contrast to the biomass, the price of $AgNO_3$ cannot be influenced by the production process. Therefore, it is the recovery of the added $AgNO_3$ which, together with the price of the biomass, defines the actual cost of the production of biogenic silver. In other words, the production yield should be high enough avoiding the loss of unreduced silver ions and the coupled wastewater treatment costs. This means that the reduction capacity of the biomass needs to be high enough. Hence, the choice of the microorganism or plant to produce silver nanoparticles at industrial scale should be rather focused on its reducing capacity, instead of on its additional medicinal or pharmaceutical value. The silver recovery can also be increased by varying the $AgNO_3$:biomass ratio towards more biomass and less silver, increase the reaction time or change physical parameters such as pH and temperature (Govindaraju et al., 2008; Lengke et al., 2007a; Sintubin et al., 2009; Vigneshwaran et al., 2007a; Zhang et al., 2005, 2007). It must be taken into account, however, that changing the reaction parameters might also influence the physico-chemical characteristics of the nanoparticles such as shape and size.

Bimetallic and Trimetallic Nanoparticles

When silver is merged with another metal forming bimetallic nanoparticles, the properties of both metals can be combined creating nanoparticles with enhanced activity. The metals can be present in an alloy or a core/shell structure (Toshima, 2008). The combination of silver with other metals can form nanoparticles with enhanced antimicrobial or catalytic features (Nino-Martinez et al., 2008; Toshima and Yonezawa, 1998). One step further than bimetallic nanoparticles, are trimetallic nanoparticles which form even more powerful catalysts according to Toshima (2008).

The reports on biogenic bimetallic nanoparticles containing silver are rather few and on biogenic trimetallic nanoparticles they are non-existent. The first reported biogenic bimetallic nanoparticles contained a gold core surrounded with a silver shell, and were produced using a plant-extract of Neem leaves (Shankar et al., 2004). More recently, also the algae *S. platensis* and the fungi *Fusarium semisectum*, *Volvariella volvacea* and *Neursospora crassa* were able to synthesize silver–gold bimetallic nanoparticles (Basavaraja et al., 2008; Castro-Longoria et al., 2011; Philip, 2009). This particular metal combination might not be surprising since the mentioned organisms were proven previously to produce monometallic gold and silver nanoparticles. Thus, most probably other organisms which are able to produce silver nanoparticles and other monometallic nanoparticles, could combine these metals creating bimetallic or even trimetallic nanoparticles. Table III gives an overview of organisms of which has been reported that they produce in addition to silver also other metal nanoparticles and which might be interesting candidates for the biogenic production of bimetallic and trimetallic nanoparticles.

Functionalization

Functionalization is the modification of the surface of nanoparticles with organic or inorganic ligands, adding more attractive features to the nanoparticles. Functionalization can improve the stability of the nanoparticles and their compatibility, but also their self-organization in more complex structures or meta-structures (Neouze and Schubert, 2008). Functionalization can also improve the interaction of nanoparticles with other molecules. For example, it can enhance the adsorption of substrates on silver catalysts, or the added ligand can improve the detection of molecules by silver biosensors (Kennedy et al., 2011; Zhang et al., 2011).

Table III. Species which can produce, in addition to nanosilver, other nanoparticles.

Species	Nanoparticles	Reference
Aspergillus niger (fungus)	Au	Bhambure et al. (2009)
Aspergillus oryzae (fungus)	Au	Binupriya et al. (2009)
Azadirachta indica (plant)	Au	Shankar et al. (2004)
Bacillus licheniformis (bacterium)	Au	Kalishwaralal et al. (2009)
Brevibacterium casei (bacterium)	Au	Kalishwaralal et al. (2010)
Chenopodium album (plant)	Au	Dwivedi and Gopal (2010)
Cinnamomum camphora (plant)	Au, Pd	Huang et al. (2007), Yang et al. (2009)
Citrus aurantium (plant)	Au	Rajasekharreddy et al. (2010)
Coriolus versicolor (fungus)	CdS	Sanghi and Verma (2009b)
Emblica officinalis (plant)	Au	Ankamwar et al. (2005)
Escherichia coli (bacterium)	Au	Du et al. (2007)
Fusarium oxysporum (fungus)	Au, CdS, Pt, Zr	Ahmad et al. (2002), Bansal et al. (2004), Mukherjee et al. (2002), Riddin et al. (2006)
Hibiscus rosa sinensis (plant)	Au	Philip (2010a)
Klebsiella pneumoniae (bacterium)	Se	Fesharaki et al. (2010)
Lactobacillus sp. (bacterium)	Au, Ti	Nair and Pradeep (2002), Prasad et al. (2007)
Medicago sativum (plant)	Au, Pt	Bali et al. (2010), Gardea-Torresdey et al. (2002)
Neurospora crassa (fungus)	Au	Castro-Longoria et al. (2011)
Penicillium sp. (fungus)	Au	Du et al. (2010)
Phyllanthus amarus (fungus)	Au	Kasthuri et al. (2009)
Plectonema boryanum (bacterium)	Au, Pd, Pt	Lengke et al. (2006a), Lengke et al. (2006b), Lengke et al. (2007b), Lengke et al. (2006c)
Pseudomonas stutzeri (bacterium)	Cu	Varshney et al. (2010)
Solanum melongena (plant)	Au	Rajasekharreddy et al. (2010)
Spirulina platensis (algae)	Au	Govindaraju et al. (2008)
Syzygium aromaticum (plant)	Au	Deshpande et al. (2010)
Verticillium sp. (fungus)	Au	Mukherjee et al. (2001b)
Volvariella volvacea (fungus)	Au	Philip (2009)

Biogenic silver is already functionalized with organic ligands, such as proteins, polysaccharides, or polyols, originating from the biomass used to produce the nanoparticles. As a result, biogenic nanoparticles are often more stable compared to chemical nanoparticles. In the case of biogenic silver produced with *L. fermentum*, for example, the nanoparticles were stabilized on a whole cell resulting in an antibacterial activity which was up to 20 times higher compared to chemically produced nanoparticles (Sintubin et al., 2011b). However, the exact outcome of this functionalization in terms of activity and behaviour of the nanoparticles, is not known for other types of biogenic silver and needs to be studied into detail for each type of biogenic silver. On the other hand, the nanoparticles can be functionalized further with new molecules. The organic groups originating from the stabilizing biogenic molecules, allow easy conjunction with new ligands such as antibodies and DNA altering the nanoparticles in such a way that they could be implemented in very specific areas of application. In the case of biosensors, for example, the originating silver probe can bind with a target (e.g., cancer proteins) resulting in a change in the resonance frequency which can be detected with surface enhanced RAMAN spectroscopy (Kennedy et al., 2011; Zhang et al., 2011).

Future Applications

In theory, biogenic nanoparticles can be implemented in every possible application which already uses chemically produced silver nanoparticles, for example, as catalysts and biosensors, in antimicrobial surfaces, biomedical applications, etc. At the moment, a lot of studies have been performed to analyze the general antimicrobial activity of biogenic silver. However, except for water disinfection treatments, the implementation of biogenic silver into practical applications has not been studied intensively, creating a major field of unexplored possibilities. The antimicrobial activity of silver nanoparticles in combination with their anti-inflammatory and wound healing features, makes them interesting for biomedical applications such as bandages for the treatment of burns (Rodriguez-Carmona and Villaverde, 2010). As stated earlier, one must take into account that the microbial fabrication of biogenic silver could possibly cause a contamination with pathogens and subsequent infection (Chaloupka et al., 2010). Hence, biogenic silver produced with pathogenic bacteria or fungi such as *S. aureus* and *Aspergillus niger* is not implementable in this case. Instead, biogenic silver produced with probiotic microorganisms or plants forms a better alternative. Moreover, certain plants have medical properties which could give an added value to the nanoparticles. Nevertheless, the biocompatibility and toxicity must be tested carefully for every type of biogenic silver. The number of antimicrobial applications for biogenic silver does not limit itself to water treatment and the biomedical field but can also be extended to food packages, textiles, and self-cleaning coatings of computer keyboards and mobile phones. Just as for biomedical applications, pathogens should not be the first choice for the production of biogenic silver for these cases. Instead, probiotics or food-related bio-systems, such as

eatable mushroom, are preferable production systems (Philip, 2009).

Silver nanoparticles are important catalysts for the oxidation of olefins, such as styrene, to epoxides and aldehydes (Chimentao et al., 2004). On the other hand, an unexplored field for silver catalysts could be the removal of pollutants from wastewater or air. Although it might be less important for a catalyst if biogenic silver is produced with a pathogenic microorganism or not, the choice of the biomass type is still crucial. The reason can be found in the shape- and size-dependent activity of the catalyst (Chimentao et al., 2004; Xu et al., 2006). Moreover, it is essential that the nanoparticles are synthesized extracellularly in the supernatant or on the cell wall. In the case of intracellular nanoparticles, the nanoparticle-surface is covered in layers of organic material and inaccessible for the substrates, preventing catalysis by the nanoparticles.

As noble metals, silver nanoparticles interact with visible light creating a plasmon band. Due to this optical feature, nanosilver can be used in biosensing and imaging. Since the plasmon resonance depends on the size and shape of the nanoparticles, the biomass type used for the production of biogenic silver will also be important in this case (Mock et al., 2002; Mulvaney, 1996).

All of the above examples indicate that a application-directed production is needed in the future, in which the biological system to produce biogenic silver is chosen in function of the application. This will have many advantages in terms of biocompatibility and activity.

Conclusions

Biogenic silver is due to its green synthesis and stable nanoparticles an interesting alternative for chemically produced nanoparticles. However, the large scale production of biogenic silver has not been explored so far. To be able to compete with chemically produced nanosilver on the market, biogenic silver needs to have the same price or be cheaper. This can be only realized by choosing carefully the biological system—bacteria, fungi, algae or plant—which delivers the best yield during the production of biogenic silver. On the other hand, the biomass type or biological system will determine the shape, size and functionalization of the nanoparticles which at their turn will influence the antimicrobial, toxicological, catalytic and optical features of the nanoparticles. Hence, the choice of the biomass type for production will specify the range of applications in which biogenic silver can be implemented. Therefore, the true challenge for biogenic silver is finding the balance between scalability, price, and applicability.

This work was supported by a project grant 71333 (L. Sintubin) of the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT). It was part of project no. G.0808.10N (2010-2013), funded by the FWO.

References

Abid JP, Wark AW, Brevet PF, Girault HH. 2002. Preparation of silver nanoparticles in solution from a silver salt by laser irradiation. Chem Commun (7): 792–793. DOI: 10.1039/B200272H

Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, Sastry M. 2002. Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, *Fusarium oxysporum*. J Am Chem Soc 124(41):12108–12109.

Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf B Biointerfaces 28(4): 313–318.

Ankamwar B, Damle C, Ahmad A, Sastry M. 2005. Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. J Nanosci Nanotechnol 5(10):1665–1671.

Babu MMG, Gunasekaran R. 2009. Production and structural characterization of crystalline silver nanoparticles from *Bacillus cereus* isolate. Colloids Surf B Biointerfaces 74(1):191–195.

Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A. 2009. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf B Biointerfaces 68(1):88–92.

Bali R, Siegele R, Harris AT. 2010. Biogenic Pt uptake and nanoparticle formation in *Medicago sativa* and *Brassica juncea*. J Nanoparticle Res 12(8):3087–3095.

Bansal V, Rautaray D, Ahmad A, Sastry M. 2004. Biosynthesis of zirconia nanoparticles using the fungus *Fusarium oxysporum*. J Mater Chem 14(22):3303–3305.

Bar H, Bhui DK, Sahoo GP, Sarkar P, Pyne S, Misra A. 2009. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surf A Physicochem Eng Aspects 348(1–3):212–216.

Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH, Venkataraman A. 2008. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. Mater Res Bull 43(5):1164–1170.

Bfilainsa KC, D'Souza SF. 2006. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids Surf B Biointerfaces 47(2):160–164.

Bhambure R, Bule M, Shaligram N, Kamat M, Singhal R. 2009. Extracellular biosynthesis of gold nanoparticles using *Aspergillus niger*—its characterization and stability. Chem Technol 32(7):1036–1041.

Binupriya AR, Sathishkumar M, Vijayaraghavan K, Yun SI. 2009. Bioreduction of trivalent aurum to nano-crystalline gold particles by active and inactive cells and cell-free extract of *Aspergillus oryzae* var. *viridis*. J Hazard Mater 177(1–3):539–545.

Binupriya AR, Sathishkumar M, Yun SI. 2010. Myco-crystallization of silver ions to nanosized particles by live and dead cell filtrates of *Aspergillus oryzae* var. *viridis* and its bactericidal activity toward *Staphylococcus aureus* KCCM 12256. Ind Eng Chem Res 49(2):852–858.

Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, Rai MK. 2009. Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lett Appl Microbiol 48(2):173–179.

Botes M, Cloete TE. 2010. The potential of nanofibers and nanobiocides in water purification. Crit Rev Microbiol 36(1):68–81.

CALS. 2008. Development of in vitro susceptibility testing criteria and quality control parameters. Approved guideline M23-A3, Wayne, PA: Clinical and Laboratory Standards Institute.

Castro-Longoria E, Vilchis-Nestor AR, Avalos-Borja M. 2011. Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus *Neurospora crassa*. Colloids Surf B Biointerfaces 83(1):42–48.

Chaloupka K, Malam Y, Seifalian AM. 2010. Nanosilver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol 28(11): 580–588.

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. 2006. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Progr 22(2):577–583.

Chen JC, Lin ZH, Ma XX. 2003. Evidence of the production of silver nanoparticles via pretreatment of *Phoma* sp.3.2883 with silver nitrate. Lett Appl Microbiol 37(2):105–108.

Chen P, Wu QS, Ding YP. 2008. Facile synthesis of monodisperse silver nanoparticles by bio-template of squama inner coat of onion. J Nanoparticle Res 10(1):207–213.

Chimentao RJ, Kirm I, Medina F, Rodriguez X, Cesteros Y, Salagre P, Sueiras JE, Fierro JLG. Sensitivity of styrene oxidation reaction to the catalyst structure of silver nanoparticles; 2004 Aug 30–Sep 03; Gdansk, Poland: Elsevier Science Bv. p. 793–800.

Cho KH, Park JE, Osaka T, Park SG. 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta 51(5):956–960.

De Gusseme B, Sintubin L, Baert L, Thibo E, Hennebel T, Vermeulen G, Uyttendaele M, Verstraete W, Boon N. 2010. Biogenic silver for disinfection of water contaminated with viruses. Appl Environ Microbiol 76(4):1082–1087.

De Gusseme B, Hennebel T, Christiaens E, Saveyn H, Verbeken K, Fitts JP, Boon N, Verstraete W. 2011. Virus disinfection in water by biogenic silver immobilized in polyvinylidene fluoride membranes. Water Res 45(4):1856–1864.

Deshpande R, Bedre MD, Basavaraja S, Sawle B, Manjunath SY, Venkataraman A. 2010. Rapid biosynthesis of irregular shaped gold nanoparticles from macerated aqueous extracellular dried clove buds (*Syzygium aromaticum*) solution. Colloids Surf B Biointerfaces 79(1):235–240.

Du LW, Jiang H, Liu XH, Wang EK. 2007. Biosynthesis of gold nanoparticles assisted by *Escherichia coli* DH5 alpha and its application on direct electrochemistry of hemoglobin. Electrochem Commun 9(5):1165–1170.

Du LW, Xian LA, Feng JX. 2010. Rapid extra-/intracellular biosynthesis of gold nanoparticles by the fungus *Penicillium* sp. J Nanoparticle Res 13(3):921–930.

Duran N, Marcato PD, De Souza GIH, Alves OL, Esposito E. 2007. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol 3(2):203–208.

Duran N, Marcato PD, Duran M, Yadav A, Gade A, Rai M. 2011. Mechanistic aspects in the biogenic synthesis of extracelular metal nanoparticles by peptides, bacteria, fungi and plants. Appl Microbiol Biotechnol 90:1609–1624.

Dwivedi AD, Gopal K. 2010. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Colloids Surf A Physicochem Eng Aspects 369(1–3):27–33.

Edwards-Jones V. 2009. The benefits of silver in hygiene, personal care and healthcare. Lett Appl Microbiol 49(2):147–152.

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ. 2005. Interaction of silver nanoparticles with HIV-1. J Nanobiotechnol 3(6):6.

Evanoff DD, Chumanov G. 2005. Synthesis and optical properties of silver nanoparticles and arrays. Chemphyschem 6(7):1221–1231.

Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. 2010a. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: A study against gram-positive and gram-negative bacteria. Nanomed Nanotechnol Biol Med 6(1):103–109.

Fayaz M, Tiwary CS, Kalaichelvan PT, Venkatesan R. 2010b. Blue orange light emission from biogenic synthesized silver nanoparticles using *Trichoderma viride*. Colloids Surf B Biointerfaces 75(1):175–178.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res 52(4):662–668.

Fesharaki PJ, Nazari P, Shakibaie M, Rezaie S, Banoee M, Abdollahi M, Shahverdi AR. 2010. Biosynthesis of selenium nanoparticles using *Klebsiella pneumoniae* and their recovery by a simple sterilization process. Braz J Microbiol 41(2):461–466.

Fu MX, Li QB, Sun DH, Lu YH, He N, Deng X, Wang HX, Huang JL. 2006. Rapid preparation process of silver nanoparticles by bioreduction and their characterizations. Chin J Chem Eng 14(1):114–117.

Gade AK, Bonde P, Ingle AP, Marcato PD, Duran N, Rai MK. 2008. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobased Mater Bioenergy 2(3):243–247.

Gade A, Ingle A, Whiteley C, Rai M. 2010. Mycogenic metal nanoparticles: Progress and applications. Biotechnol Lett 32:593–600.

Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. 2009. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomed Nanotechnol Biol Med 5(4):382–386.

Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Troiani HE, Santiago P, Yacaman MJ. 2002. Formation and growth of Au nanoparticles inside live alfalfa plants. Nano Lett 2(4):397–401.

Goodman CM, McCusker CD, Yilmaz T, Rotello VM. 2004. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjug Chem 15(4):897–900.

Govindaraju K, Basha SK, Kumar VG, Singaravelu G. 2008. Silver, gold and bimetallic nanoparticles production using single-cell protein (*Spirulina platensis*) Geitler. J Mater Sci 43(15):5115–5122.

Gurunathan S, Kalishwaralal K, Vaidyanathan R, Deepak V, Pandian SRK, Muniyandi J, Hariharan N, Eom SH. 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Sur B Biointerfaces 74(1):328–335.

Hennebel T, De Gusseme B, Boon N, Verstraete W. 2009. Biogenic metals in advanced water treatment. Trends Biotechnol 27(2):90–98.

Huang HZ, Yang XR. 2004. Synthesis of polysaccharide-stabilized gold and silver nanoparticles: A green method. Carbohydr Res 339(15):2627–2631.

Huang JL, Li QB, Sun DH, Lu YH, Su YB, Yang X, Wang HX, Wang YP, Shao WY, He N, Hong JQ, Chen CX. 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnology 18(10):11.

Huang JL, Lin LQ, Li QB, Sun DH, Wang YP, Lu YH, He N, Yang K, Yang X, Wang HX, et al. 2008. Continuous-flow biosynthesis of silver nanoparticles by lixivium of sundried *Cinnamomum camphora* leaf in tubular microreactors. Ind EnginChem Res 47(16):6081–6090.

Ingle A, Rai M, Gade A, Bawaskar M. 2009. *Fusarium solani*: A novel biological agent for the extracellular synthesis of silver nanoparticles. J Nanoparticle Res 11(8):2079–2085.

International Organization of Standardization. 2007. Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices. Part 2: evaluation of performance of antimicrobial susceptibility test devices. Geneva.

Jain PK, Huang XH, El-Sayed IH, El-Sayed MA. 2008. Noble metals on the nanoscale: Optical and photothermal properties and some applications in imaging, sensing, biology, and medicine. Acc Chem Res 41(12):1578–1586.

Jain D, Kachhwaha S, Jain R, Srivastava G, Kothari SL. 2010. Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*. Indian J Exp Biol 48(11):1152–1156.

Jha AK, Prasad K, Kulkarni AR. 2009. Plant system: Nature's nanofactory. Colloids Surf B Biointerfaces 73(2):219–223.

Jiang ZJ, Liu CY, Sun LW. 2005. Catalytic properties of silver nanoparticles supported on silica spheres. J Phys Chem B 109(5):1730–1735.

Juibari MM, Abbasalizadeh S, Jouzani GS, Noruzi M. 2011. Intensified biosynthesis of silver nanoparticles using a native extremophilic *Ureibacillus thermosphaericus* strain. Mater Lett 65(6):1014–1017.

Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, Sangiliyandi G. 2008. Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. Mater Lett 62(29):4411–4413.

Kalishwaralal K, Deepak V, Pandian SRK, Gurunathan S. 2009. Biological synthesis of gold nanocubes from *Bacillus licheniformis*. Biores Technol 100(21):5356–5358.

Kalishwaralal K, Deepak V, Pandian SRK, Kottaisamy M, BarathManiKanth S, Kartikeyan B, Gurunathan S. 2010. Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. Colloids Surf B Biointerfaces 77(2):257–262.

Kasthuri J, Kathiravan K, Rajendiran N. 2009. Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: A novel biological approach. J Nanoparticle Res 11(5):1075–1085.

Kathiresan K, Manivannan S, Nabeel MA, Dhivya B. 2009. Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. Colloids Surf B Biointerfaces 71(1):133–137.

Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K. 2010. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. Spectrochim 79(3):594–598.

Kennedy DC, McKay CS, Tay LL, Rouleau Y, Pezacki JP. 2011. Carbon-bonded silver nanoparticles: Alkyne-functionalized ligands for SERS imaging of mammalian cells. Chem Commun 47(11):3156–3158.

Khlebtsov NG, Dykman LA. 2010. Optical properties and biomedical applications of plasmonic nanoparticles. J Quan Spectrosc Rad Transf 111(1):1–35.

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, et al. 2007. Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med 3(1):95–101.

Klasen HJ. 2000a. Historical review of the use of silver in the treatment of burns. I. Early uses. Burns 26(2):117–130.

Klasen HJ. 2000b. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns 26(2):131–138.

Klaus T, Joerger R, Olsson E, Granqvist CG. 1999. Silver-based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci USA 96(24): 13611–13614.

Kononova ON, Kholmogorov AG, Danilenko NV, Goryaeva NG, Shatnykh KA, Kachin SV. 2007. Recovery of silver from thiosulfate and thiocyanate leach solutions by adsorption on anion exchange resins and activated carbon. Hydrometal 88(1–4):189–195.

Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, Paknikar KM. 2003. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology 14(1):95–100.

Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 76(1):50–56.

Kumar V, Yadav SK. 2009. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol 84(2):151–157.

Kumar VS, Nagaraja BM, Shashikala V, Padmasri AH, Madhavendra SS, Raju BD, Rao KSR. Highly efficient Ag/C catalyst prepared by electrochemical deposition method in controlling microorganisms in water; 2003 Feb 06–08, Hyderabad, India: Elsevier Science Bv. p. 313–319.

Kumar SA, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, Khan MI. 2007. Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnol Lett 29(3):439–445.

Lansdown ABG. 2007. Critical observations on the neurotoxicity of silver. Crit Rev Toxicol 37(3):237–250.

Ledwith DM, Whelan AM, Kelly JM. 2007. A rapid, straight-forward method for controlling the morphology of stable silver nanoparticles. J Mater Chem 17(23):2459–2464.

Lengke MF, Fleet ME, Southam G. 2006a. Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from gold(I)-thiosulfate and gold(III)-chloride complexes. Langmuir 22(6):2780–2787.

Lengke MF, Fleet ME, Southam G. 2006b. Synthesis of platinum nanoparticles by reaction of filamentous cyanobacteria with platinum(IV)-chloride complex. Langmuir 22(17):7318–7323.

Lengke MF, Ravel B, Fleet ME, Wanger G, Gordon RA, Southam G. 2006c. Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold(III)—chloride complex. Environ Sci Technol 40(20):6304–6309.

Lengke MF, Fleet ME, Southam G. 2007a. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver(I) nitrate complex. Langmuir 23(5):2694–2699.

Lengke MF, Fleet ME, Southarn G. 2007b. Synthesis of palladium nanoparticles by reaction of filamentous cyanobacterial biomass with a palladium(II) chloride complex. Langmuir 23(17):8982–8987.

Li SK, Shen YH, Xie AJ, Yu XR, Qiu LG, Zhang L, Zhang QF. 2007. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. Green Chem 9(8):852–858.

Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. 1997. Interaction of silver nitrate with readily identifiable groups: Relationship to the antibacterial action of silver ions. Lett Appl Microbiol 25(4):279–283.

Lin ZY, Zhou CH, Wu JM, Zhou, JZ, Wang, L. 2005. A further insight into the mechanism of Ag^+ biosorption by Lactobacillus sp strain A09. Spectroc Acta Pt A-Molec Biomolec Spectr 61(6):1195–1200.

Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PKH, Chiu JF, Che CM. 2007. Silver nanoparticles: Partial oxidation and antibacterial activities. J Biol Inorg Chem 12(4):527–534.

Lukman AI, Gong B, Marjo CE, Roessner U, Harris AT. 2011. Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using *Medicago sativa* seed exudates. J Colloid Interface Sci 353(2):433–444.

Mafune F, Kohno J, Takeda Y, Kondow T, Sawabe H. 2000. Structure and stability of silver nanoparticles in aqueous solution produced by laser ablation. J Phys Chem B 104(35):8333–8337.

Malynych SZ, Chumanov G. 2003. Vacuum deposition of silver island films on chemically modified surfaces. J Vac Sci Technol A 21(3):723–727.

Marciano A, Chefetz B, Gedanken A. 2008. Differential adsorption of silver nanoparticles to the inner and outer surfaces of the *Agave americana* cuticle. J Phys Chem C 112(46):18082–18086.

Maynard AD. 2007. Nanotechnologies: Overview and issues. In: Simeonova PP, Opopol N, Luster MI, editors. Nanotechnology—toxicological issues and environmental safety, Dordrecht: Springer. p. 1–14.

Mock JJ, Barbic M, Smith DR, Schultz DA, Schultz S. 2002. Shape effects in plasmon resonance of individual colloidal silver nanoparticles. J Chem Phy 116(15):6755–6759.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. Nanotechnology 16(10):2346–2353.

Moyer CA, Brentano L, Gravens DL, Margraf HW, Monafo WW. 1965. Treatment of large human burns with 0.5% silver nitrate solution. Arch Surg 90(6):812.

Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, et al. 2001a. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett 1(10):515–519.

Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajayakumar PV, Alam M, et al. 2001b. Bioreduction of AuCl4-ions by the fungus, *Verticillium* sp. and surface trapping of the gold nanoparticles formed. Angew Chem Int Ed 40(19):3585.

Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M. 2002. Extracellular synthesis of gold nanoparticles by the fungus *Fusarium oxysporum*. Chembiochem 3(5):461–463.

Mulvaney P. 1996. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 12(3):788–800.

Nadworny PL, Wang JF, Tredget EE, Burrell RE. 2010. Anti-inflammatory activity of nanocrystalline silver-derived solutions in porcine contact dermatitis. J Inflam London 7:20.

Nair B, Pradeep T. 2002. Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. Cryst Growth Des 2(4):293–298.

Nakiboglu N, Toscali D, Nisli G. 2003. A novel silver recovery method from waste photographic films with NaOH stripping. Turk J Chem 27(1): 127–133.

Nanda A, Saravanan M. 2009. Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. Nanomed Nanotechnol Biol Med 5(4):452–456.

Narayanan KB, Sakthivel N. 2010. Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interfac 156:1–13.

Natarajan K, Selvaraj S, Murty VR. 2010. Microbial production of silver nanoparticles. Digest J Nanomater Biostruct 5(1):135–140.

NEN-EN. 1997. Chemical disinfectants and antiseptics–quantitative suspension test for the evaliation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas—test method and requirements (phase 2, step 1), Brussels: European Committee for Standardization.

Neouze MA, Schubert U. 2008. Surface modification and functionalization of metal and metal oxide nanoparticles by organic ligands. Monatsh Chem 139(3):183–195.

Nino-Martinez N, Martinez-Castanon GA, Aragon-Pina A, Martinez-Gutierrez F, Martinez-Mendoza JR, Ruiz F. 2008. Silver nanoparticles synthesized on titanium dioxide fine particles. In: Laudon M, Romanowicz B, editors. Nanotechnology 2008: materials, fabrication, particles, and characterization technical proceedings of the 2008 NSTI Nanotechnology Conference and Trade Show, Boston, June 1-5, 2008. Boca Raton: Crc Press—Taylor & Francis Group. p. 737–740.

Nithya R, Ragunathan R. 2009. Synthesis of silver nanoparticles using *Pleurotus sajor* caju and its antimicrobial study. Digest J Nanomater Biostruct 4(4):623–629.

Nowack B. 2010. Nanosilver revisited downstream. Science 330(6007): 1054–1055.

Nowack B, Krug HF, Height M. 2011. 120 years of nanosilver history: Implications for policy makers. Environ Sci Technol 45(4):1177–1183.

Pal S, Tak YK, Song JM. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol 73(6):1712–1720.

Parashar V, Parashar R, Sharma B, Pandey AC. 2009. *Parthenium* leaf extract mediated synthesis of silver nanoparticles: A novel approach towards weed utilization. Digest J Nanomater Biostruct 4(1):45–50.

Park K, Seo D, Lee J. 2008. Conductivity of silver paste prepared from nanoparticles. Colloids Surf A Physicochem Eng Aspects 313:351–354.

Park TJ, Lee SY, Heo NS, Seo TS. 2010. *In vivo* synthesis of diverse metal nanoparticles by recombinant *Escherichia coli*. Angew Chem Int Ed 49:7019–7024.

Philip D. 2009. Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract. Spectrochim Acta Part A Mol Biomol Spectrosc 73(2):374–381.

Philip D. 2010a. Green synthesis of gold and silver nanoparticles using *Hibiscus rosa sinensis*. Phys E Low-Dimensional Syst Nanostruct 42(5):1417–1424.

Philip D. 2010b. Honey mediated green synthesis of silver nanoparticles. Spectrochim Acta Part A Mol Biomol Spectr 75(3):1078–1081.

Prasad K, Jha AK, Kulkarni AR. 2007. Lactobacillus assisted synthesis of titanium nanoparticles. Nanoscale Res Lett 2(5):248–250.

Rai M, Yadav A, Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27:76–83.

Rajani P, SriSindhura K, Prasad T, Hussain OM, Sudhakar P, Latha P, Balakrishna M, Kambala V, Reddy KR. 2010. Fabrication of biogenic silver nanoparticles using agricultural crop plant leaf extracts. In: Giri PK, Goswami DK, Perumal A, Chattopadhyay A, editors. International conference on advanced nanomaterials and nanotechnology, Melville: Amer Inst Physics. p. 148–153.

Rajasekharreddy P, Rani PU, Sreedhar B. 2010. Qualitative assessment of silver and gold nanoparticle synthesis in various plants: A photobiological approach. J Nanoparticle Res 12(5):1711–1721.

Rajesh R, Jaya L, Niranjan K, Vijay M, Sahebrao K. 2009. Phytosynthesis of silver nanoparticle using *Gliricidia sepium* (Jacq.). Curr Nanosci 5(1):117–122.

Raskin I. 1996. Plant genetic engineering may help with environmental cleanup—commentary. Proc Nal Acad Sci USA 93(8):3164–3166.

Raskin I, Smith RD, Salt DE. 1997. Using plants to remove pollutants from the environment. Curr Opin Biotechnol 8(2):221–226.

Riddin TL, Gericke M, Whiteley CG. 2006. Analysis of the inter- and extracellular formation of platinum nanoparticles by *Fusarium oxysporum* f. sp. *lycopersici* using response surface methodology. Nanotechnology 17(14):3482–3489.

Rodriguez-Carmona E, Villaverde A. 2010. Nanostructured bacterial materials for inoovative medicins. Trends Microbiol 18:423–430.

Roy N, Mondal S, Laskar RA, Basu S, Mandal D, Begum NA. 2010. Biogenic synthesis of Au and Ag nanoparticles by *Indian propolis* and its constituents. Colloids Surf B Biointerfaces 76(1):317–325.

Saifuddin N, Wong CW, Yasumira AAN. 2009. Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation. E-J Chem 6(1):61–70.

Sanghi R, Verma P. 2009a. Biomimetic synthesis and characterisation of protein capped silver nanoparticles. Biores Technol 100(1):501–504.

Sanghi R, Verma P. 2009b. A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus. Chem Eng J 155(3):886–891.

Saravanana M, Nanda A. 2010. Extracellular synthesis of silver bionanoparticles from *Aspergillus clavatus* and its antimicrobial activity against MRSA and MRSE. Colloids Surf B Biointerfaces 77(2):214–218.

Sastry M, Ahmad A, Khan MI, Kumar R. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 85(2):162–170.

Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS. 2009. *Cinnamon zeylanicum bark* extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids Surf B Biointerfaces 73(2):332–338.

Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. 2010. Biosynthesis of silver nanoparticles using *Coriandrum Sativum* leaf extract and their application in nonlinear optics. Adv Sci Lett 3(2):138–143.

Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi AA. 2007. Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteria*: A novel biological approach. Process Biochem 42(5):919–923.

Shaligram NS, Bule M, Bhambure R, Singhal RS, Singh SK, Szakacs G, Pandey A. 2009. Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain. Process Biochem 44(8):939–943.

Shankar SS, Rai A, Ahmad A, Sastry M. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J Colloid Interface Sci 275(2):496–502.

Sharma VK, Yngard RA, Lin Y. 2009. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv Colloid Interface Sci 145(1–2):83–96.

Silver S, Phung le T, Silver G. 2006. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol 33(7):627–634.

Singh AK, Talat M, Singh DP, Srivastava ON. 2010. Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group. J Nanoparticle Res 12(5):1667–1675.

Sintubin L, De Windt W, Dick J, Mast J, van der Ha D, Verstraete W, Boon N. 2009. Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl Microbiol Biotechnol 84(4):741–749.

Sintubin L, Awoke A, Wang Y, Van der Ha D, Verstraete W. 2011a. Enhanced disinfection efficiencies of solar irradiation by biogenic silver. Ann Microbiol 62(1):187–191.

Sintubin L, De Gusseme B, Van der Meeren P, Pycke BFG, Verstraete W, Boon N. 2011b. The antibacterial activity of biogenic silver and its mode of action. Appl Microbiol Biotechnol 91(1):153–162.

Sondi I, Salopek-Sondi B. 2004. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram-negative bacteria. J Colloid Interface Sci 275(1):177–182.

Storm-Versloot MN, Vos CG, Ubbink DT, Vermeulen H. 2010. Topical silver for preventing wound infection. Cochrane Database Syst Rev (3): 108. DOI: 10.1002/14651858.CD006478.pub2

Thakkar KN, Mhatre SS, Parikh RY. 2010. Biological synthesis of metallic nanoparticles. Nanomed Nanotechnol Biol Med 6(2):257–262.

Tian J, Wong KKY, Ho CM, Lok CN, Yu WY, Che CM, Chiu JF, Tam PKH. 2007. Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem 2(1):129–136.

Tolaymat TM, El Badawy AM, Genaidy A, Scheckel KG, Luxton TP, Suidan M. 2010. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. Sci Total Environ 408(5):999–1006.

Toshima N. 2008. Capped bimetallic and trimetallic nanoparticles for catalysis and information technology. Macromol Symp 270: 27–39.

Toshima N, Yonezawa T. 1998. Bimetallic nanoparticles—novel materials for chemical and physical applications. New J Chem 22(11):1179–1201.

Varshney R, Bhadauria S, Gaur MS, Pasricha R. 2010. Characterization of copper nanoparticles synthesized by a novel microbiological method. JOM 62(12):102–104.

Vaseeharan B, Ramasamy P, Chen JC. 2010. Antibacterial activity of silver nanoparticles (AgNps) synthesized by tea leaf extracts against pathogenic *Vibrio harveyi* and its protective efficacy on juvenile *Feneropenaeus indicus*. Lett Appl Microbiol 50(4):352–356.

Veerasamy R, Xin TZ, Gunasagaran S, Xiang TFW, Yang EFC, Jeyakumar N, Dhanaraj SA. 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. J Saudi Chem Soc 15(2):113–120.

Veglio F, Beolchini F. 1997. Removal of metals by biosorption: A review. Hydrometal 44(3):301–316.

Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH. 2006a. Biomimetics of silver nanoparticles by white rot fungus, *Phaenerochaete chrysosporium*. Colloids Surf B Biointerfaces 53(1): 55–59.

Vigneshwaran N, Nachane RP, Balasubramanya RH, Varadarajan PV. 2006b. A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch. Carbohydr Res 341(12):2012–2018.

Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH. 2007a. Biological synthesis of silver nanoparticles using the fungus *Aspergillus* flavus. Mater Lett 61(6):1413–1418.

Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH. 2007b. Silver-protein (core–shell) nanoparticle production using spent mushroom substrate. Langmuir 23(13):7113–7117.

Vijayakumar PS, Prasad BLV. 2009. Intracellular biogenic silver nanoparticles for the generation of carbon supported antiviral and sustained bactericidal agents. Langmuir 25(19):11741–11747.

Wang TC, Rubner MF, Cohen RE. 2002. Polyelectrolyte multilayer nanoreactors for preparing silver nanoparticle composites: Controlling metal concentration and nanoparticle size. Langmuir 18(8):3370–3375.

Wang XW, Zhang L, Ma CL, Song RY, Hou HB, Li DL. 2009. Enrichment and separation of silver from waste solutions by metal ion imprinted membrane. Hydrometal 100(1–2):82–86.

Wijnhoven SWP, Peijnenburg W, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, Van de Meent D, et al. 2009. Nano-silver—a review of available data and knowledge gaps in human and environmental risk assessment. Nanotox 3(2):109–138.

Xu R, Wang DS, Zhang JT, Li YD. 2006. Shape-dependent catalytic activity of silver nanoparticles for the oxidation of styrene. Chem Asian J 1(6):888–893.

Yang X, Li QB, Wang HX, Huang JL, Lin LQ, Wang WT, Sun DH, Su YB, Opiyo JB, Hong LW. et al. 2009. Green synthesis of palladium nanoparticles using broth of *Cinnamomum camphora* leaf. J Nanoparticle Res 12(5):1589–1598.

Yoon KY, Byeon JH, Park JH, Hwang J. 2007. Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. Sci Total Environ 373(2–3):572–575.

Zeng F, Hou C, Wu SZ, Liu XX, Tong Z, Yu SN. 2007. Silver nanoparticles directly formed on natural macroporous matrix and their antimicrobial activities. Nanotechnology 18(5):8.

Zhang HR, Li QB, Lu YH, Sun DH, Lin XP, Deng X, He N, Zheng SZ. 2005. Biosorption and bioreduction of diamine silver complex by *Corynebacterium*. J Chem Technol Biotechnol 80(3):285–290.

Zhang HR, Li QB, Wang HX, Sun DH, Lu YH, He N. 2007. Accumulation of silver(I) ion and diamine silver complex by *Aeromonas* SH10 biomass. Appl Biochem Biotechnol 143(1):54–62.

Zhang ZL, Wen YQ, Ma Y, Luo J, Jiang L, Song YL. 2011. Mixed DNA-functionalized nanoparticle probes for surface-enhanced Raman scattering-based multiplex DNA detection. Chem Commun 47(26): 7407–7409.

Zhu JJ, Liu SW, Palchik O, Koltypin Y, Gedanken A. 2000. Shape-controlled synthesis of silver nanoparticles by pulse sonoelectrochemical methods. Langmuir 16(16):6396–6399.