Chapter 12
Biotechnological Routes to Metallic Nanoparticles Production: Mechanistic Aspects, Antimicrobial Activity, Toxicity and Industrial Applications

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12.1 Introduction

This chapter will describe the biogenic metallic nanoparticles synthesis and their mechanistic aspects in the syntheses and in their antimicrobial activities.

12.1.1 General Aspects of Biotechnological Processes Applied to Biogenic Synthesis of Nanoparticles

Nanobiotechnology has appeared as one of the most promising areas in nanotechnology, since nanoparticles, specifically in special nanometals, as carriers of drugs to treat and diagnose important diseases, form a major topic in nanomedicine. Among the metal nanoparticles, silver nanoparticles have been extensively studied The most common synthetic methods of silver nanoparticle production are chemical reduction, photochemical methods, ultrasonic-assisted reduction, electrochemical methods, templates, and irradiating reduction (Zhang et al. 2007).

As indicated previously, there are many processes for metallic nanoparticle production, but the biological one is playing an important role in order to minimize the environmental pollution caused by physical and chemical procedures. In this

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N. Cioffi and M. Rai (eds.), *Nano-Antimicrobials*,
DOI 10.1007/978-3-642-24428-5_12,

regard, fungi, bacteria, algae, yeasts, and plants have inherent capacities to reduce metal through their specific metabolic pathway. In fact, some biomimetic (peptides) procedures are also used in their study. Different types of metallic nanoparticles like copper, zinc, titanium, gold, and silver, among others, have been produced by biological methods. However, the interest in silver nanoparticles has been increasing due to their high antimicrobial activity against bacteria, viruses and eukaryotic microorganisms (Rai et al. 2009). The synthesis of metallic nanoparticles using a green procedure is of great interest in terms of introducing environmentally friendly synthesis procedures (Durán et al. 2010a; Marcato and Durán 2011). The importance of these nanoparticles is testified by the publication of many reviews on the biosynthesis and properties of metallic nanoparticles in the last 4 years (Rai et al. 2008; Mohanpuria et al. 2008; Bhattacharya and Mukherjee 2008; Chen and Schluesener 2008; Sharma et al. 2009; Singh et al. 2009; Sinha et al. 2009; Korbekandi et al. 2009; Hennebel et al. 2009; Krumov et al. 2009; Kumar and Yadav 2009; Das et al. 2009; Durán et al. 2010b, 2011; Narayanan and Sakthivel 2010; Popescu et al. 2010; Gade et al. 2010a; Thakkar et al. 2010; Marambio-Jones and Hoek. 2010; Arya 2010; Blanco-Andujar et al. 2010; Zhang et al. 2011). For example, Sinha et al. (2009) reviewed the knowledge and production by bioreductive approaches of Ag, Au, Cd, Pt, and Pd nanoparticles and oxides by bacteria, actinomycetes, yeasts, algae, fungi, higher angiospermic plants and their perspectives. Biosynthesis of Au, Ag, Au–Ag alloy, Se, Te, Pt, oxides and quantum dots by bacteria, actinomycetes, fungi, yeasts and viruses have been discussed as well as the current status of microbial synthesis and applications of metals (Narayanan and Sakthivel 2010).

The new uses of metallic nanoparticles require techniques to synthesize nanoparticles that are both cost-effective and environmentally benign. As discussed before, many processes for metallic nanoparticle synthesis have been reported; however, the synthesis of nanoparticles with precise control over size distribution, shape selectivity, and stability remains a challenge. Why is it important to synthesize these metallic nanoparticles? The small size and large surface area of metallic nanoparticles makes them useful for many different applications. The importance of these nanoparticles has been described as follows:

- Ag^o. The small size and large surface area makes it useful for applications in nonlinear optics, spectrally selective coatings for solar energy absorption, optical receptors, catalysts in chemical reactions, biolabeling, antistatic materials, cryogenic superconducting materials, and biosensor materials, and as antibacterials.
- Au^o. Gold nanoparticles are used in opto-electronic devices, catalysis, biomedicals including DNA labeling and drug delivery, cell imaging, immunostaining, and biosensors.
- Cd^o. Cadmium is extremely toxic to the reproductive system of humans (accumulating in the body, particularly in the kidneys and the liver). Cadmium is widely used in alloys, pigments, solid-state batteries, metal coatings for protective coatings on steel, plastics and fertilizers.

- Cu^o. Due to copper toxicity as ions, the biological system attempts to prevent copper toxicity by its reduction. Common plants can transform copper into metallic nanoparticles inside and near roots, with evidence of assistance by endomycorrhizal fungi when grown in contaminated soil in the natural environment.
- Fe^o. Nano-scale zero-valent iron (nZVI) is of increasing interest for use in a variety of environmental remediations. In comparison to larger-sized ZVI particles, nZVI has a greater reactivity due to a greater surface area to volume ratio.
- Pd^o. Palladium in terms of market prices is both economically and environmentally important due to their high global level. Palladium nanoparticles possess higher catalytic properties than their macro-scale counterparts.
- Pt^o. Platinum exhibits excellent catalytic properties. The traditional methods are environmentally negative, and now the development of non-toxic, clean, eco-friendly and inexpensive synthetic protocols is needed ('green' processes).
- Se^o. The reduction of the soluble oxyanions selenate [Se(VI)] or selenite [Se(IV)] to the elemental state [Se(0)] represents a method of removing elements from wastewaters.
- Te^o. Nano-scale tellurium compounds, such as CdTe, have significant potential as solar-cell materials and are currently under intensive research scrutiny.
- Ti^o. Titanium, by weight, is one of the strongest metals readily available with a wide range of practical applications. Titanium is suggested for use in desalinization plants because of its strong resistance to corrosion from sea water. In medical applications, titanium pins are used because of their non-reactive nature when contacting bone and flesh.

12.2 Biogenic Synthesis of Metallic Nanoparticles

In this section of the chapter, selected cases of biogenic synthesis will be discussed in which some types of mechanism was proposed.

12.2.1 Silver Nanoparticles

12.2.1.1 Amino Acids and Peptides

In a biomimetic synthesis of silver nanoparticles through silver-binding peptides were incubated in an silver nitrate solution and, after a few hours, the solution turned reddish and a reddish-colored precipitate was obtained by centrifugation. No change in the color or precipitate was observed with non-specific peptides (Naik et al. 2002).

The silver-binding peptides (selected from the PhD-12C phage display peptide library) interact with preformed nanoclusters of silver metal present in the aqueous silver nitrate solution and this cluster gives the reducing moiety to obtain the silver nanoparticles. It has been demonstrated that the selected peptides containing specific amino acids permits the recognition and reduction of silver ions (Asn-Pro-Ser-Ser-Leu-Phe-Arg-Tyr-Leu-Pro-Ser-Asp), since the pure amino acids (e.g., proline, arginine or serine) or other non-silver binding peptides were unable to reduce the silver ion to silver nanoparticles. The amino acids, which are probably important in this process, are arginine, cysteine, lysine, and methionine, all known to interact with silver ions (Naik et al. 2002).

Aqueous silver sulfate solution and aqueous solution of tyrosine in basic medium at boiling temperature changed into a yellow solution, indicating the formation of silver nanoparticles. Under neutral and acidic pH conditions, this reaction did not occur. This result indicated the crucial role of tyrosine as a pH-dependent reducing agent (Selvakannan et al. 2004a). Silver–peptide nanoconjugate (peptide–SNPs) was formed from an aqueous solution of silver nitrate and a mixture of peptide NH_2-Leu-α-aminoisobutyric acid {Aib}-Trp-OMe). In this case, a color change from colorless to yellow was observed at pH 11. Similarly, peptide Tert-butyloxy-carbonyl {Boc}-Leu-Aib-Trp-OH) was used for synthesizing silver nanoparticles. The tryptophan residue was responsible for the reduction of metal ions to the respective metal atoms by donation of an electron to the metal ion and is itself converted to a transient tryptophyl radical, and then finally to finishing in a native tryptophan or in a ditryptophan/kynurenine form of the peptide (Si and Mandal 2007).

12.2.1.2 Fungi

Many of the *Fusarium oxysporum* strains studied were able to produce silver nanoparticles; however, this reduction was dependent on the nitrate reductase/ 2-acetyl-3,8-dihydroxy-6-methoxy anthraquinone or its isomers at 2-acetyl-2,8-dihydroxy-6-methoxy anthraquinone (Durán et al. 2005).

Silver nanoparticles were obtained from the fungal mycelia of *Trichoderma asperellum* that was resuspended in water in order to obtain a fungal filtrate in which aqueous solution of silver nitrate was added. The possible mechanism of the biosynthesis of silver nanoparticles was studied by FTIR. The *β*-carbon in cysteine loses one hydrogen radical and one electron in a concerted step, or two consecutive steps, and reduces Ag + ion to Ag nanoparticles, probably through NADPH-dependent dehydrogenase. During the process, other changes show that this process follows the hypothetical mechanisms shown in Fig. 12.1.

FTIR analysis showed that there is a decrease of the amide linkage in the aqueous solution after the formation of silver nanoparticles, suggesting that the peptide linkage undergoes a hydrolysis to generate free carboxylic acid and amino groups which can act as capping agent to the silver nanoparticles and not the –SH group as in other fungi. The surface-enhanced resonance Raman spectroscopy

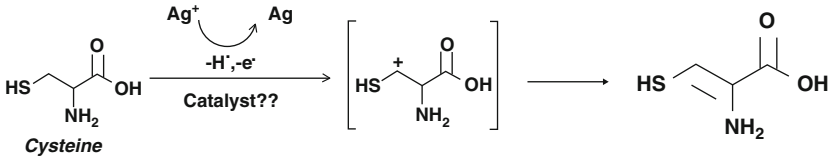

Fig. 12.1 Schematic representation of the mechanism for the formation of silver nanoparticles (Modified from Mukherjee et al. 2008)

showed this clearly since a very strong chemical bond between silver nanoparticles and nitrogen of the amino group in the amino acid appeared (Ag-N bond) (Mukherjee et al. 2008).

12.2.1.3 Bacteria

Lactic acid bacteria, such as *Lactobacillus* spp., *Pediococcus pentosaceus, Enterococcus faecium*, and *Lactococcus garvieae*, were able to reduce silver ions to silver nanoparticles (Sintubin et al. 2009). The mechanism of silver ions biosorption by resting cells of *Lactobacillus* sp. strain A09 and reduction to silver nanoparticles was studied at the molecular level using spectroscopic techniques. The silver ions in the presence of *Lactobacillus* sp biomass were rapidly absorbed and reduced by the free aldehyde group of the hemiacetalic hydroxyl group from glucose that was oxidized to the carboxyl group. Assays with free glucose demonstrated this process. The redox reaction of glucose on silver ions was proposed as follows (Lin et al. 2005):

$$HOCH_2(CHOH)_4CHO + 2Ag^+ + H_2O \rightarrow HOCH_2(CHOH)_4COOH + 2Ag^0 + 2H^+$$

In an other study, different lactic acid bacteria (*Lactobacillus* spp., *Pediococcus pentosaceus, Enterococcus faecium*, and *Lactococcus garvieae*) were studied to produce silver nanoparticles. Mainly the size distribution was species dependent and the final yields of nanoparticles were pH dependent (Sintubin et al. 2009). The authors suggest that exopolysaccharides from lactic acid bacteria (glucose, galactose and rhamnose) might serve in the reduction of silver ions to silver nanoparticles. Similar results were also reported by Lin et al. (2005). A reduction mechanism for these results is demonstrated in Fig. 12.2. The observation that the bisorption of silver nanoparticles decrease the pH is a good indication that silver ions compete with protons (Fig. 12.2a, −RH). At high pH, the aldehyde form (Fig. 12.2c) exerts its reducing power forming silver nanoparticles (Fig.12.2c).

It has been suggested that the enzyme involved in the intracellular synthesis of silver nanoparticles from *B. licheniformis* (Kalimuthu et al. 2008) could be NADH-nitrate reductase dependent and mediated by an electron shuttle, in a similar way as has been suggested in fungi (Durán et al. 2005). *B. licheniformis* is known to secrete

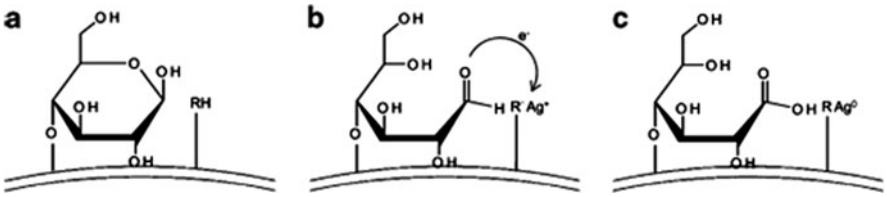

Fig. 12.2 Hypothetical mechanisms of bacterial reduction of silver ions to silver nanoparticles (From Sintubin et al. 2009. By permission of Springer)

the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, present in the membrane. The purified nitrate reductase was shown to have these properties confirming the possible mechanism (Kumar et al. 2007a).

A possible mechanism of silver nanoparticle production by *Morganella* sp., via extracellular synthesis, has been studied. In order to understand the silver resistance, it was studied at phenotypic and genotypic levels. In this case, three gene homologues, silE, silP and silS, were identified. The homologue of silE from *Morganella* sp. showed similarity with the gene which encodes a periplasmic silver-binding protein (Gupta et al. 1999). It was a good indication that silver-resistance machinery might have a similar role to play in *Morganella* sp. and could be associated with silver nanoparticle synthesis. A mechanistic speculation was proposed whereby an extracellular microenvironment created by the bacterium with several silver-specific proteins is secreted outside the cell during the growth. These proteins might specifically interact with silver ions and reduce it to silver nanoparticles stabilized by these proteins (Parikh et al. 2008).

12.2.1.4 Plants

Silver nanoparticles were efficiently synthesized by reacting silver ions with *Capsicum annuum* L. extract. In order to explain the mechanisms of the nanoparticles formation, Li et al. (2007) applied the model of recognition–reduction–limited nucleation and growth in the reaction solutions. In the first stage of the recognition process, the silver ions were trapped on the surface of proteins through electrostatic interactions. In the second stage, silver ions were reduced by proteins from the extract, and the secondary structures of the proteins changed with subsequent formation of silver nuclei. In the third stage, the silver nuclei grew by continuous reduction of silver ions and accumulation on these nuclei. The final stage is where the flexible linkage of the proteins and the large numbers of biomolecules coexist in the reaction solutions leading to an isotropic growth and the formation of the spherical silver nanoparticles. In the fifth stage, with an increase in aging time, large-sized silver nanoparticles formed and the crystalline phase changed from polycrystalline to single crystalline through Ostwald ripening (Li et al. 2007).

In another work, silver nanoparticles were prepared by reacting an aqueous solution containing silver ions, geraniol oil from Geranium leaves (*Pelargonium*

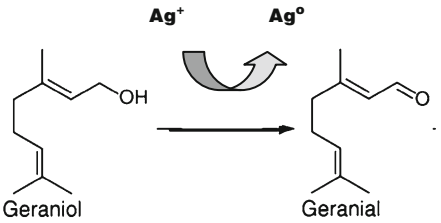

Fig. 12.3 Hypothetical mechanisms of silver nanoparticles prepared from geraniol (Modified from Safaepour et al. 2009)

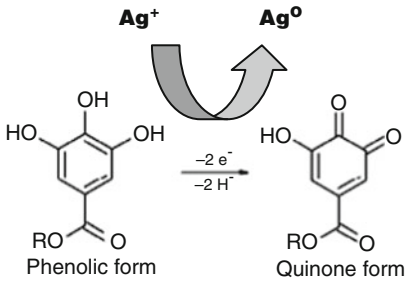

Fig. 12.4 Oxidation of phenolic group to quinones (Modified from Sivaraman et al. 2009)

graveolens) and an aqueous solution of polyethylene glycol 4,000 at pH >10 in a microwave for 40 s (Safaepour et al. 2009). The hypothetical mechanism of this reduction is illustrated in Fig. 12.3, with the genera *Pelargonium* exhibiting NADPH-dehydrogenases. The conversion of geraniol into geranial is catalyzed by $NADP^+$-geraniol dehydrogenase in *Polygonum minus* (Maarof et al. 2010).

Tannic acid, a polyphenolic compound derived from tea or oak wood extracts, was used as the reducing agent for silver nanoparticles synthesis (Fig. 12.4). Silver nanoparticle sizes were controlled by molar ratio variation of tannic acid to silver nitrate. Tannic acid were used as a reducing and stabilizing agent to synthesize silver nanoparticles for an extremely short period of time (seconds). An increase in particle size with increasing molar ratio of tannic acid/silver nitrate indicates that tannic acid acts as an organizer for facilitating nucleation (Sivaraman et al. 2009).

Isolation of phyllanthin from leaf powder of the plant *Phyllanthus amarus* has been used in the synthesis of silver nanoparticles. After the addition of phyllanthin extract to the aqueous silver nitrate, the solution changed from colorless to pale orange which is indicative of the formation of silver nanoparticles. At different concentrations of phyllanthin, different silver nanoparticle morphologies were obtained. At low concentration, quasispherical forms were exhibited, and at high concentration, ellipsoidal nanoparticles were formed. On the basis of UV-vis spectroscopy, transmission electron microscopy, FT-IR spectrometry, cyclic voltametry and thermogravimetric analyses, a mechanism was proposed as shown in Fig. 12.5 (Kasthuri et al. 2009).

Green synthesis of silver nanoparticles using silver nitrate and different volume fractions of latex from *Jatropha curcas* has been studied (Bar et al. 2009a). The TEM analysis showed two broad size distributions of particle, one having diameter in the range 20–30 nm and others with some larger diameters and uneven shapes. It is known

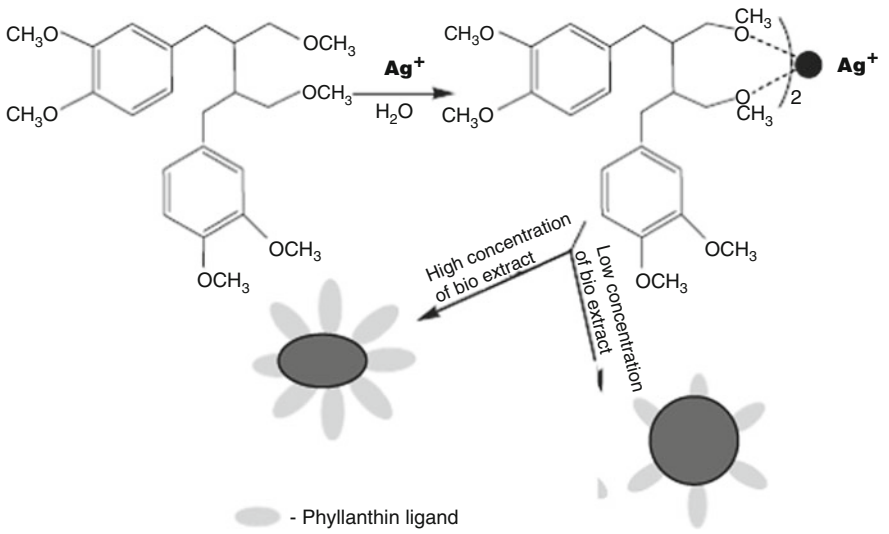

Fig. 12.5 Schematic diagram of the formation of phyllanthin-stabilized silver nanoparticles (Modified from Kasthuri et al. 2009)

that major components of *J. curcas* latex are curcacycline A (an octapeptide), curcacycline B (a nonapeptide) and curcain (an enzyme) (Bar et al. 2009a and references therein). Calculations on curcacycline A and curcacycline B using a semi-empirical AM1 method suggested that there are a number of different size pockets within the cavity in both structures having radii in the range ~20–35 nm. The authors suggested that silver ions were first entrapped into the core structure of the cyclic protein, and then reduced and stabilized in situ by the amide group of the host peptide under the present experimental conditions. This results in the formation of silver nanoparticles having a radius similar to that of the cavity of cyclic peptides, and probably, the enzyme, curcain, in the latex with its large folded structure helps to stabilize the silver nanoparticles with a larger size and having irregular shapes. Then, the most probable mechanisms in this case might be as shown in Fig. 12.6.

In a similar way, Bar et al. (2009b) synthesized silver nanoparticles using the aqueous seed extract of *J. curcas*. The particles were spherical and the size of the particles was controlled by varying the silver nitrate concentration. These nanoparticles were stable for several months due to protein capping.

Silver nanoparticles were synthesized in a short period of time (30 min) using leaf extract of *Acalypha indica*. Probably, quercetin and polysaccharides present in *A. indica* may be responsible for the nanoparticles synthesis. These molecules belong to a group of plant pigments called flavonoids, indicating that the plants with these compounds are able to reduce silver ions to silver nanoparticles (Krishnaraj et al. 2010).

Medicago sativa seeds were used to obtain silver nanoparticles from silver nitrate solution. The silver ion concentration, amount of exudate and pH determined the

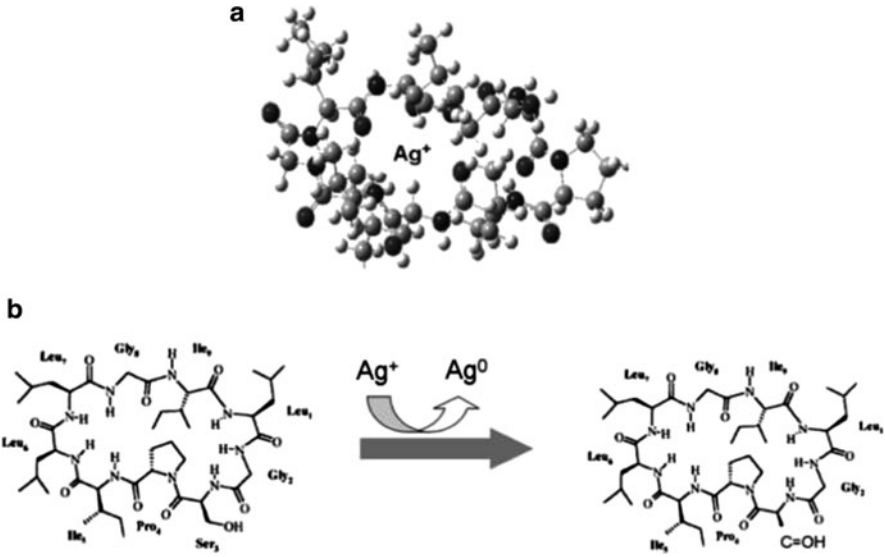

Fig. 12.6 Schematic representation of silver ion stabilization and reduction by curcacycline B. (**a**) Stabilization of silver ions in the curcacycline B pocket (Modified from Bar et al. 2009a) and (**b**) possible redox mechanism of the silver nanoparticles production, as suggested by Durán et al. 2011. By permission of Springer

particle size and morphology. At low silver ion concentration, largely spherical nanoparticles were produced, and at high concentration, flower-like clusters were observed. Pre-dilution of the exudate induced the formation of silver nanoplates, forming hexagonal particles and nanotriangles. These data are indicative that the formation of particles appears to follow a clear pathway, and the authors hypothesized that there were different primary growth mechanisms and combinations of these mechanisms, by which the particles (in particular, the nanotriangles) self-assembled in the silver exudate system. This exudate contained a large amount of quercetin, and the proposed oxidized structure for quercetin is that of a ketone, as quercetin possesses the readily oxidized hydroxyl group in the C ring next to the carbonyl group (Fig. 12.7). This further supports that this oxidized form of quercetin may dictate the growth of the Ag nanotriangles along the {111} direction and cap the Ag nanoparticles in the *M. sativa* seed exudate system.

This biogenic synthesis by *M. sativa* was mimicked by commercial quercetin and showed similar results to those observed in the biological systems (Fig. 12.8) (Lukman et al. 2011). These facts showed that the quercetin acts as an intermediate compound in the biosynthesis of metallic nanoparticles in plants (Egorova and Revina 2000; Materska 2008).

It is known that plants growing under different ecological conditions (e.g., xerophytes, mesophytes, and hydrophytes) are able to metabolize metallic nanoparticles (Jha et al. 2009). In the case of xerophytes (e.g., *Bryophyllum* sp.), during night-time CO_2 enters the leaf tissue and then the cells, CO_2 reacts with

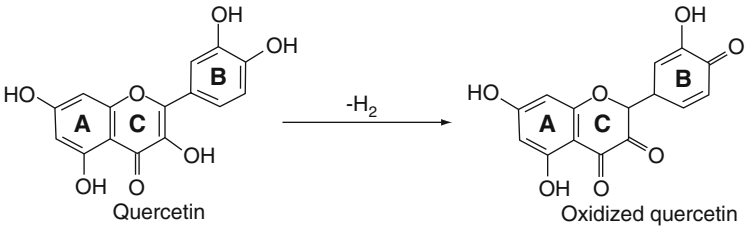

Fig. 12.7 Quercetin oxidation

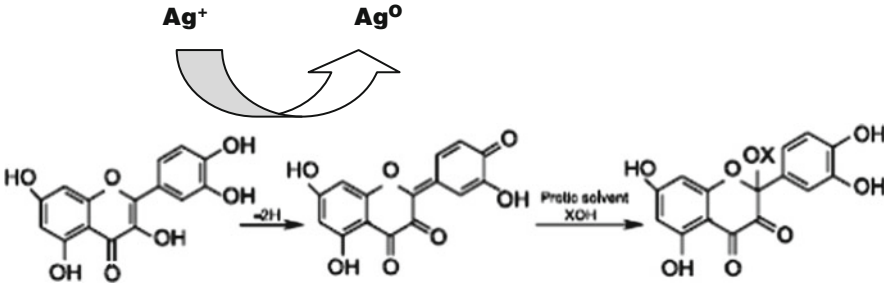

Fig. 12.8 Pathway of oxidative changes in quercetin reaction with silver nanoparticles in protic solvents (Modified from Materska 2008)

phosphoenol pyruvate (PEP) under PEP carboxylase to form oxalo-acetic acid which is reduced by Malate dehydrogenase/NADH to malic acid (by night). During the day, malic acid is oxidized to pyruvaye, regenerating PEP by PEP carboxykinase at pH variations (pool of organic acids). These redox reactions indicate that the changes must be due to redial transformation of silver. The authors suggested that emodin (anthraquinones, similar to Durán et al. 2005) can undergo a redial tautomerization producing the silver nanoparticles (Fig. 12.9).

In mesophytes. it appeared that nano-transformation is produced by the tautomerization of quinones. The candidate mesophytic genera, *Cyperus* sp., contained three types of benzoquinones, e.g., cyperoquinone (type I), dietchequinone (type II) and remirin (type III) were able to produced silver nanoparticles via redial tautomerization (Fig. 12.10).

Hydrophytes (*Potamogeton* sp.) exhibit a translocation of ammonia and this is dissolved in water to give its hydroxide. This plant due to high salinity must be protected from the levels of reactive oxygen species (ROS), since an excessive level of ROS leads to oxidative damage to cellular molecules, aging and cell death. Due to this fact, the antioxidative species must be kept at appropriate levels (ascorbic acid). Dehydroascorbate (DHA) reductase (DHAR) catalyze the re-reduction of DHA to ascorbate. In addition, this presence of catechol and protocatecheuic acid have also been reported along with other important phytochemicals in *Hydrilla*. In both the cases, reactive hydrogen gets liberated which participates in the synthesis of silver nanoparticles (Fig. 12.11).

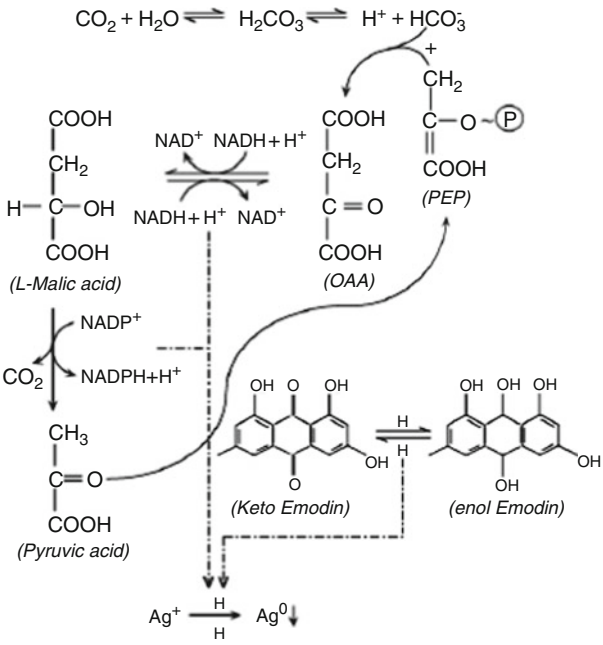

Fig. 12.9 Proposed mechanism of silver nanoparticles using xerophytes (From Jha et al. 2009. By permission of Elsevier)

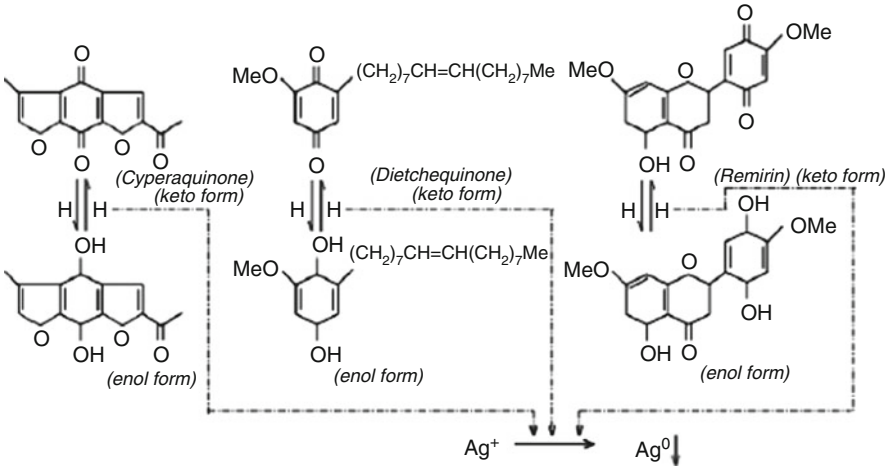

Fig. 12.10 Proposed mechanisms of silver nanoparticles using mesophytes (From Jha et al. 2009. By permission of Elsevier)

However, another intermediate for biosynthesis of silver nanoparticles has been proposed using xerophytic plant *O. ficus-indica*, commonly known as Opuntia. In this mechanism, quercetin was proposed as intermediate. This compound is the main bioactive products in this plant, thus probably this metabolite plays a major

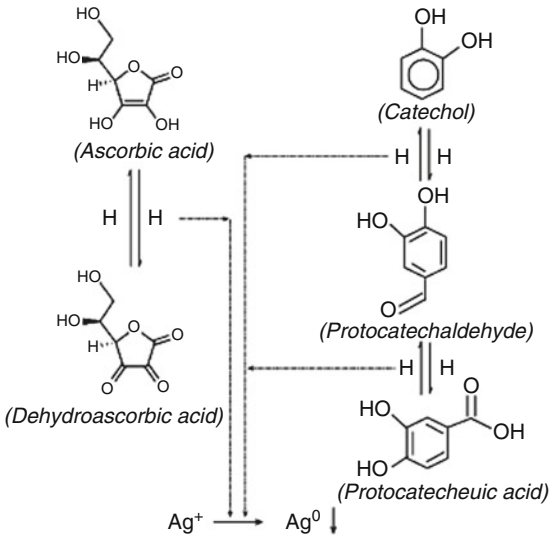

Fig. 12.11 Proposed mechanism of silver nanoparticles using hydrophytes (From Jha et al. 2009. By permission of Elsevier)

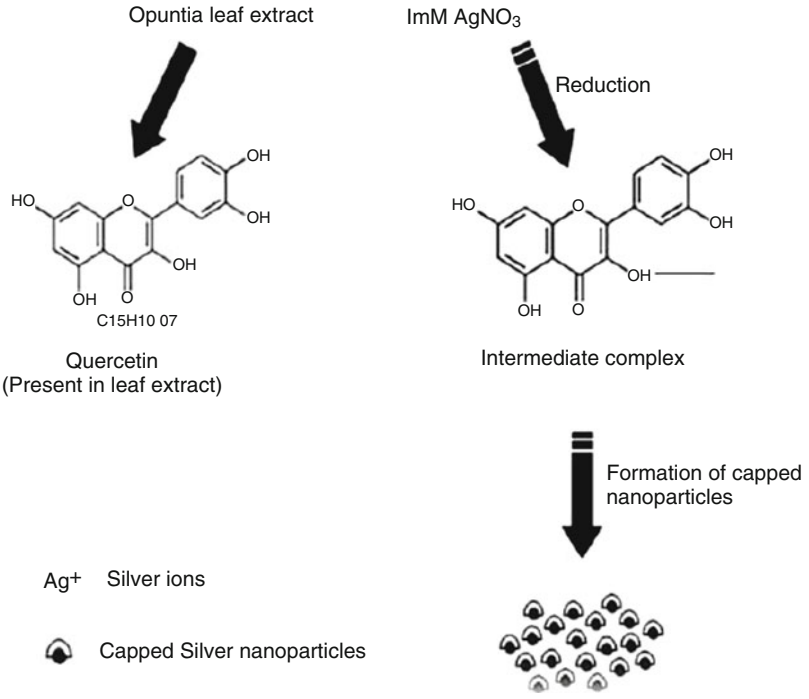

Fig. 12.12 A two-step hypothetical mechanism of biosynthesis of silver nanoparticles (From Gade et al. 2010b. By permission of Bentham Science)

role in the reduction of silver ions and formation of an intermediate complex which is further responsible for the formation of silver nanoparticles (Fig. 12.12) (Gade et al. 2010b).

Finally, the silver nanoparticle production by plants depends on their metabolism where different metabolites such as organic acids or quinones or flavonoids such as quercetin, or metabolic fluxes and other redox systems such as ascorbates or catechol/protocatacheuic acid play important roles in the mechanism of silver nanoparticle formation (Jha et al. 2009).

12.2.2 Gold Nanoparticles

There are very few publications on the mechanistic aspect of gold biosynthesis.

12.2.2.1 Aminoacids and Peptides

Gold nanoparticles can be produced by using peptide and aminoacid. It has been reported that some short model peptides can be self-assembled into fibrous structures under appropriate conditions (this was not observed in peptide–silver ions interaction). Bhattacharjee et al. (2005) used the capacity of self-assembly of surface-adsorbed fibril-forming peptides. The authors mentioned that similar kinds of large aggregated structures have also been prepared by the self-assembly of gold nanoparticles using other biological interactions (e.g., Brown 2001). Previously, it was reported that tyrosine was redox active and the probable mechanism suggested was spin delocalization that was in agreement with a role for the peptide bond in tyrosyl radical-based electron-transfer reactions (Pujols-Ayala et al. 2003). Bhattacharjee et al. (2005) showed that the tripeptide (H_2N-Leu-α-aminoisobutyric acid {Aib}-Tyr-OMe-1) containing a C-terminally located tyrosine residue reduced gold salt to metallic gold nanoparticles in situ (Fig. 12.13). Stable nanoparticles were obtained due to the presence of tripeptide on the gold nanoparticles surface as self-assembled into three-dimensional structures.

In 2005, analyses were carried out of the reduction potential of cowpea chlorotic mottle viruses of unmodified SubE (plasmid expressed in yeast), (HRE)-SubE engineered with interior HRE peptide epitopes (Ala-His-His-Ala-His-His-Ala-Ala-Asp) and wild-type as viral templates for synthesis of gold nanoparticles (Slocik et al. 2005). SubE, (HRE)-SubE, and wild-type were added to water with

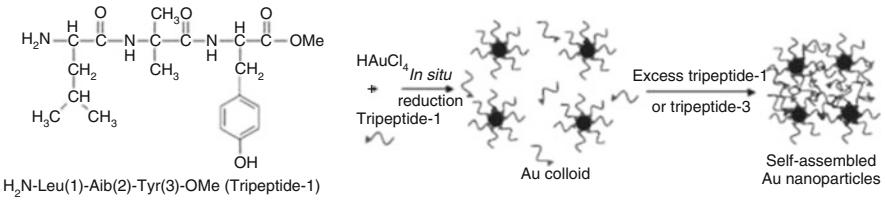

Fig. 12.13 Structure of tripeptide-1 and the self-assembled process for gold nanoparticles (Modified from Bhattacharjee et al. 2005 and from Durán et al. 2011. By permission from Springer)

$AuClP(CH_3)_3$ leading to the rapid reduction to form gold nanoparticles (either aerobically or anaerobically). Tyrosine played an important role in the gold reduction and it was demonstrated by the decay of the tyrosine fluorescence and correlated with an increase of the plasmon absorbance of gold nanoparticles. In addition, a sequence analysis of cowpea chlorotic mottle virus demonstrated that there were four tyrosine residues in close proximity to the C-terminus of each subunit that presented multiple reduction sites. This research showed a unique surface chemistry of viruses with a large potential in many nanomaterials (Slocik et al. 2005).

In another work (Selvakannan et al. 2004b), it was demonstrated that tryptophan is a reductant agent of gold ions and that the temperature can impact on the synthesis process by increasing the reduction rate and favoring the formation of gold nanoparticles in anisotropic shapes (triangles and hexagons). Through surface-enhanced Raman scattering (SERS), it was demonstrated how tryptophan caps the gold nanoparticles after the reduction process, stabilizing the nanoparticles. Figure 12.14 shows the SERS spectra from the tryptophan-reduced gold nanoparticles synthesis (tryptophan alone did not exhibit Raman signal). Furthermore, the influence of temperature in the SERS spectra can be seen in this figure. Gold nanoparticles synthesized at 60°C and 80°C (spectra b and c) provide higher Raman enhancement compared to the particles synthesized at 20°C (spectrum a). The SERS is expected to be higher for anisotropic nanoparticles, because symmetry breaking allows for plasmon localization and more intense electromagnetic field generation at the edge and tips of nanoparticles. The SERS spectrum also demonstrated that the band at 1,123 cm^{-1}, which corresponded to NH_2 twisting vibration mode, is relating to the positive charge of the secondary amino group (glycine moiety of tryptophan), interacting with the negative charge of the gold nanoparticles surface by electrostatic interaction. Another observation by SERS

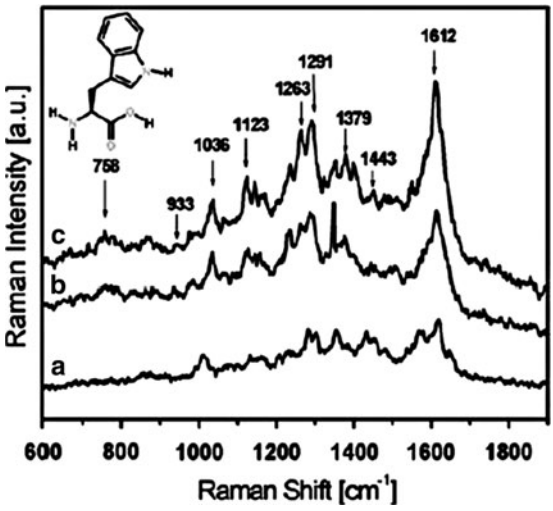

Fig. 12.14 SERS spectra of tryptophan-protected gold nanoparticles as synthesized at (*a*) 22°C, (*b*) 60°C, and (*c*) 80°C. The *inset* shows the molecular structure of tryptophan (From Iosin et al. 2010. By permission from Springer)

spectrum shows an enhancement of the band at 1,612 cm^{-1} (asymmetric stretching of the COO– group). The authors concluded that the indole ring did not interact with the gold nanoparticles surface, while both amino and carboxy groups of tryptophan were the preferential terminal groups to attach onto the surface of the gold nanoparticles (Iosin et al. 2010).

12.2.2.2 Fungi

Biosynthesis of size-controlled gold nanoparticles using the fungus *Penicillium* sp. was carried out by bioreduction of $AuCl_4^-$ ions, and led to the assembly and formation of intracellular gold nanoparticles with spherical morphology and monodispersity index. Temperature was an important physiological parameter for growth of *Penicillium* sp., and to control the size of the biosynthesized gold nanoparticles. FTIR spectra analysis of the fungus exposed to gold ions solution indicated that the intracellular reducing sugar played an important role in the occurrence of intracellular gold ions reduction and in the gold nanoparticle growth (Zhang et al. 2009).

12.2.2.3 Bacteria

An intracellular biosynthesis of gold nanoparticles by *Stenotrophomonas maltophilia* isolated from soil samples was reported by Nangia et al. (2009). This study suggested that biosynthesis and stabilization of gold nanoparticles via charge capping involved a NADPH-dependent reductase enzyme through electron shuttle process. The reduction of gold ions solution was probably processed in two steps. At the first step, $AuCl_4^-$ ions were reduced to Au^+ species, and in a second step, the latter product was then reduced by NADPH to metallic gold and subsequently followed by the encapsulation of gold nanoparticles by charged $NADP^+$ species as shown in the equations below.

$$AuCl_4^- + NADPH \rightarrow Au^+ + 4Cl^- + NADP^+ + H^+$$

$$2Au^+ + NADPH \rightarrow 2Au^0 + NADP^+ + H^+$$

All the data in this study suggested a potential mechanism of gold nanoparticles synthesis by *Stenotrophomonas maltophilia* based on enzymatic reduction (Fig. 12.15).

12.2.2.4 Plants

A bioreductive approach of anisotropic gold using the phyllanthin extract with tunable optical properties has been studied (Kasthuri et al. 2009). The presence of

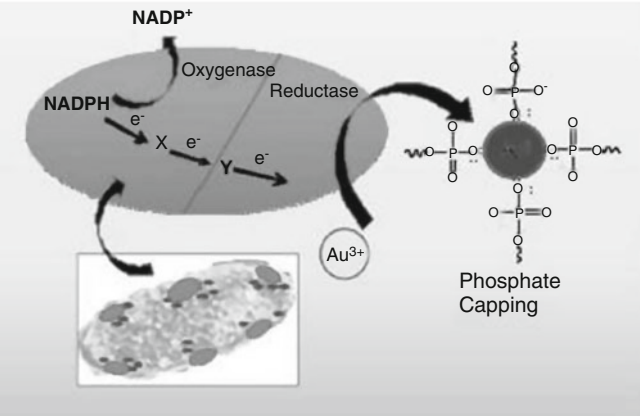

Fig. 12.15 Proposed synthesis mechanism of GNPs by *Stenotrophomonas maltophilia* through enzymatic reduction (From Nangia et al. 2009. By permission of American Institute of Physics)

a small amount of phyllanthin extract led to a slow reduction of $AuCl_4^-$ ions favoring the formation of triangular- or hexagonal-shaped nanoparticles. However, a large amount of phyllanthin extract leads to a higher population of spherical nanoparticles. As in the case of silver nanoparticles (see Fig. 12.5), the electron-donating nature of the $–OCH_3$ group of the phyllanthin extract plays an important role in this process (Kasthuri et al. 2009).

12.2.2.5 Algae

Shewanella algae cell extract produced spherical, triangular, truncated triangular, and hexagonal nanoplates with an edge length of 100–200 nm. Through the FTIR technique, it was shown that there was no difference in the FTIR spectra between the *S. algae* extract and the spherical gold particle suspension in a short period, indicating that the components of the *S. algae* extract did not participate in the formation of spherical gold nanoparticles. The FTIR spectra of the gold particle suspension after longer periods of interaction showed a different peak shape for the carbonyl group (C=O) centered at 1,645 cm^{-1} as the gold particle morphology changed from spherical nanoparticles to nanoplates. This result indicates that the carbonyl group in the *S. algae* extract contributes to the formation of gold nanoplates (Ogi et al. 2010).

12.2.3 Cadmium Nanoparticles

In this specific case of cadmium nanoparticles, no mechanistic aspects were found with fungi, yeast or plants.

12.2.3.1 Bacteria

NAD-independent lactate dehydrogenases (iLDHs) are commonly responsible for lactate utilization during the stationary phase of aerobic growth in *Lactobacillus plantarum*. In *L. plantarum*, two stereospecific nLDHs are present (LdhD, responsible for D-lactate; and LdhL, responsible for L-lactate). Under the experimental conditions used, the NAD-independent lactate dehydrogenase activities do not seem to be involved in this process (Goffin et al. 2004). Then, cadmium nanoparticles were prepared using *L. plantarum* from *Lactobacillus* strains found in buttermilk using the procedure adopted by Nair and Pradeep (2002) with slight modifications (Jha and Prassada 2010). The filtrate from buttermilk was diluted and a suitable sugar solution was added to the culture solution and this was allowed to incubate overnight. Then, to each culture, cadmium carbonate solution was added. After 3–4 days, the culture solution was observed to have distinctly marked deposits at the bottom of the conical flask. A remarkable change in pH was observed at this stage, which is currently under standardization. A possible synthetic mechanism was suggested as follows:

$$CdCO_3 + 2HCl \Rightarrow CdCl_2 + H_2CO_3$$
$$CdCl_2 \Leftrightarrow Cd^{2+} + 2Cl^-$$
$$H_2CO_3 \Leftrightarrow 2H^+ + CO_3^{2-}$$
$$Cd^{2+} + 2e^- \Leftrightarrow Cd^0 \downarrow$$

Although the authors do not say which part was the reductant, the reduction agents should be lactate (oxidation to acetate). The mechanistic aspect in lactate to acetate as suggested by Goffin et al. (2004) is the following. Lactate is oxidized to acetate by the bacteria by requiring transfer of four electrons to an exogenous electron acceptor (Marsili et al. 2008). Then, the final equation summing up both semi-reductions should be:

$$\begin{array}{l} \text{Lactate}^- + 2H_2O \rightarrow \text{Acetate}^- + HCO_3^- + 5\ H^+ + 4\ e^- \\ 2Cd^{+2} + 4\ e^- \rightarrow 2\ Cd^o \\ \hline \text{Lactate}^- + 2H_2O + 2\ Cd^{+2} \rightarrow \text{Acetate}^- + HCO_3^- + 5\ H^+ + 2\ Cd^o \end{array}$$

12.2.4 Copper Nanoparticles

No mechanistic aspects with fungi or other biological systems were found.

12.2.4.1 Bacteria

Synthesis for copper nanoparticles using *Pseudomonas stutzeri* biomass in an aqueous $CuSO_4$ solution has been studied (Varshney et al. 2010). High resolution

transmission electron microscopy analysis showed that the particles produced were almost spherical, a thin coating layer can be observed on all particles and the thickness is a few nanometers. This was an indication that the bacterial surface acts both as a reducing as well as capping agent and that these micrographs also demonstrate that Cu nanoparticles were well dispersed and stable for months.

12.2.4.2 Yeast

Biocatalytic reduction and removal of copper ions was observed in the aqueous–organic phase by induction of copper reductase in yeast grown in catabolic repression conditions by addition of inducer (copper ions) during the initial growth phase of *Saccharomyces cerevisiae*. Excess of glucose under catabolic repression conditions was used to induce the Cyt P450 in the yeast cells. The harvested biomass was lysed using cell disrupter and the microsomes were obtained. The most important parameters in the biocatalytic reduction were initial substrate concentration, time of reaction, initial pH of the reaction mixture (optimization of the enzyme) and concentration and time of addition of the inducer (yeast growth) (Chandran et al. 2001).

12.2.5 Iron Nanoparticles

The biosyntheis and mechanistic views were only found with plant extracts.

12.2.5.1 Plants

A green single-step synthesis of iron nanoparticles using tea (*Camellia sinensis*) polyphenols has been described (Hoag et al. 2009). The rapid reaction between polyphenols and ferric nitrate occurred at room temperature. As was previously discussed (Fig. 12.4), the plant polyphenols act as a reducing and a capping agent, resulting in green synthesized nano-scale-sized zero-valent iron particles.

12.2.6 Palladium Nanoparticles

In this biosynthesis, no mechanistic data with fungi or other organisms were found.

12.2.6.1 Bacteria

Desulfovibrio fructosivorans mutants were constructed using marker exchange mutagenesis, and the *D. fructosivorans* wild-type strain and mutant strains with deletions of NiFe hydrogenase, Fe hydrogenase, and Fe and NiFe hydrogenases

were grown anaerobically. Pd^{o} was visible within the periplasmic spaces of the parent strain and the Fe hydrogenase mutants; however, Pd clusters (in wild-type and Fe hydrogenase-negative cells) were distributed randomly throughout the whole width of the periplasmic space, but Pd nanoparticles were localized on the cytoplasmic membrane in NiFe hydrogenase-negative and double-mutant cells. In both treated cells – double-mutant and wild-type periplasmically – or intact ones, Pd clusters were associated only with the inner cytoplasmic membrane. This result and the hydrogenase localization suggested that the enzyme serves as a nucleation site and assists initial Pd nanoparticle growth by supplying the electrons for Pd^{+2} reduction (Mikheenko et al. 2008). However, in another study using *Cupriavidus necator*, *Pseudomonas putida*, and *Paracoccus denitrificans* in palladium nanoparticle biosynthesis, no evidence was for the involvement of active hydrogenases or the participation of other active enzymes in nucleating Pd^{+2} reduction (Bunge et al. 2010). Pd^{o} was located in the periplasmic space, and the size of the nanoparticles was defined by the distance of inner and outer membranes. From this, the authors postulated a hydrogenase-independent mechanism, contrary to findings with *Desulfovibrio* spp (Mikheenko et al. 2008). Although active enzymes appear not to be required, it is possible that the coordination of Pd^{+2} to chemical groups on the cell surface contributes to nucleating the reduction process. This is an indication that the microbial surface operates as a scaffold for binding Pd^{+2} before reduction to Pd^{o}, with the cell surface acting as a type of biological stabilizer that prevents agglomeration (Bunge et al. 2010).

12.2.6.2 Plant

Palladium nanoparticles were obtained by a simple procedure using broth of *Cinnamomum camphora* leaf extract (Yang et al. 2010). According to the size distribution of the Pd nanoparticles, it was found that a higher concentration of Pd^{+2} in the solution produces smaller Pd nanoparticles. Polyols such as flavones, terpenoids, and polysaccharides in the broth played a critical role in reduction of Pd^{+2} ions. FTIR spectra of the reaction were indicative that polyols were oxidized to aldehydes or ketones confirming that the water-soluble fractions in the leaf broth played a leading role in bioreduction of the precursors. The proposed mechanism by Yang et al. (2010), in function of FTIR, was:

$$nPd^{+2} + 2R\text{-}(OH)_n \rightarrow nPd^{o} + 2nR{=}O + 2nH^{+}$$

Where 2R-(OH)n cold be polyol and R=O could be an aldehyde or ketone.

12.2.7 Platinum Nanoparticles

In this case, only the mechanistic aspects with bacteria, fungi and plants are discussed.

12.2.7.1 Bacteria

Platinum nanoparticles were produced using a consortium of sulfate-reducing bacteria (SRB) cells that were suspended in a buffer (pH 7.6) under both anaerobic and aerobic environments with platinum salt [Pt(IV)-H_2PtCl_6] or [(Pt(II)-Na_2PtCl_4]. On the basis of the results obtained, a probable mechanism for the platinum nanoparticles was proposed (Fig. 12.16) (Riddin et al. 2009). The endogenous production of hydrogen/electrons via the oxidation of organic compounds, evolved by the cytoplasmic hydrogenase, would be rapid (Fig. 12.16, step 1). The periplasmic hydrogenase (Fig. 12.16, step 2) acting as an electron donor for the Pt (II) ion (Fig. 12.16, step 2). No inhibition of copper ions exerted on the bioreduction of the Pt(IV) ion was observed, while the rate limiting step was that of Pt(II) to Pt(0) further supporting the hypothesis that a periplasmic hydrogenase was involved using endogenously produced hydrogen during the reduction of Pt(II). Thus, a mixed consortium of SRB was capable of reducing Pt(IV) to Pt(0) via the intermediate cation Pt(II) in a two-step process: (1) A cytoplasmic hydrogenase, that was oxygen sensitive and not inhibited by Cu(II), required no exogenous electron donors and produced hydrogen (and excess electrons) from metabolite oxidation and/or Pt(IV) reduction, and (2) a periplasmic hydrogenase that was oxygen-tolerant/protected, was inhibited by Cu(II) and used the endogenously formed hydrogen donors [and Pt(II) ions] to form Pt(0) nanoparticles (Fig. 12.16).

Later on, in a study with cell-free and cell-soluble protein extract, it was demonstrated that in order to form stable platinum nanoparticles the ratio of the initial concentration of the platinum salt and total protein were also critical to control the shape and size (Riddin et al. 2010).

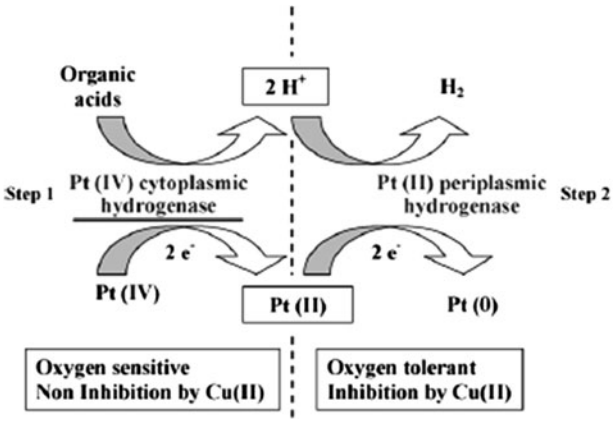

Fig. 12.16 Suggested mechanism for the double two-electron bioreduction of Pt(IV) into nanoparticles via an intermediary Pt(II) (From Riddin et al. 2009. By permission from Elsevier)

12.2.7.2 Fungi

The biosynthesis of platinum nanoparticles was obtained from cell-free extract of *Fusarium oxysporum* containing hydrogenase or purified enzyme and exposed to aqueous solutions of H_2PtCl_6 at the appropriate pH and temperature under an atmosphere of hydrogen (Govender et al. 2010). Experiments with cell-free extract from *F. oxysporum* with the platinum salts showed that there was a decrease in the concentration of the platinum salt after 1–3 h. Thereafter, there was a gradual increase in platinum salt concentration after 8 h incubation. This increase could have been as a result of, first, the bioreduction of H_2PtCl_6 to produce platinum with the release of HCl ions, followed by a reverse reaction and the in situ formation of $PtCl_4^{2-}$. By analogy, these authors with other results by Duff et al. (1995) proposed a two-stage reaction:

$$H_2PtCl_6^{2-} \rightarrow PtCl_4^{2-} + 2Cl^- + 2H^+$$
$$PtCl_4^{2--} \leftrightarrow Pt + 4Cl^-$$

However, the study with purified hydrogenase enzyme under conditions at its optimum pH and temperature, showed no bioreduction. The cell-free extract contained other factors that were contributing to the bioreduction at the same conditions. The authors pointed out that a continuing study must be necessary to identify this intermediate and understand more about the mechanism of the platinum bioreduction (Govender et al. 2010).

12.2.7.3 Plants

AQ leaf extract of *Diopyros kaki* was used as a reducing agent in the extracellular synthesis of platinum nanoparticles from an aqueous $H_2PtCl_6 \times 6H_2O$ solution. Results of this biosynthesis showed that platinum nanoparticles synthesized with *D. kaki* extract were surrounded by some metabolites like terpenoids, that have functional groups of amines, alcohols, ketones, aldehydes, and carboxylic acids, and not by proteins. The platinum nanoparticle synthesis using *D. kaki* is not an enzyme-mediated process because the rate of platinum nanoparticle synthesis was greatest at temperatures over 95°C and there were no peaks associated with proteins/enzymes on FTIR analysis. It appears more likely that the reduction of platinum ions and stabilization of synthesized platinum nanoparticles was the responsibility of many functional groups, which are present in various metabolites such as terpenoids and reducing sugars (Song et al. 2010).

12.2.8 Selenium Nanoparticles

The selenium nanoparticle biosynthesis and mechanistic views were only found with bacteria.

12.2.8.1 Bacteria

Bacillus arsenicoselenatis (strain E1H) and *Bacillus selenitireducens* (strain MLS10) isolated from a lake sediment were able to reduce oxyanions selenate-Se(VI) to selenite-Se(IV) and selenite-Se(IV) to selenium nanoparticles-Se(0), respectively, by a dissimilatory reduction with a concomitant of lactate to acetate and CO_2 (Blum et al. 1998). Growth of strain E1H in the oxidation of lactate to acetate consumed two electrons for reduction of Se(VI) to Se(IV); however, MLS10 consumed four electrons in the lactate oxidation for the Se(IV) reduction to Se(0). The co-culture of these two isolates was in agreement with the six-electron stoichiometry of complete selenate reduction to the elemental state as given by the equation (Oremland et al. 1994):

$$3\ \text{Lactate}^- + 2\ \text{SeO}_4^{2-} + \text{H}^+ \rightarrow 3\ \text{Acetate}^- + 2\ \text{Se}(0) + 3\ \text{HCO}_3^- + 2\ \text{H}_2\text{O}$$

$$\text{Selenate}\ \left(\text{SeO}_4^{2-}\right);\ \text{Selenite}\ \left(\text{SeO}_3^{2-}\right)$$

Species of selenate- and selenite-respiring bacteria, *Sulfurospirillum barnesii*, *Bacillus selenitireducens*, and *Selenihalanaerobacter shriftii*, growing with selenium oxyanions as the electron acceptor, formed extracellular granules of stable, uniform nanospheres of Se^o. Intracellular packets of Se^o were also noted, but the extracellular Se^o accumulation was more common than the intracellular one. Because the Se^o nanospheres eventually migrate from the individual cells, the Se^o particles correspond to all the Se(VI) added at the initial incubation time. This also suggests that the external and internal Se^o nanospheres arise independently of each other and by different mechanisms. The external nanosphere accumulation is probably directly tied to the respiratory Se reductases (Oremland et al. 2004).

The reduction of Se (IV) by *Klebsiella. pneumoniae* and the formation of intracellular selenium nanoparticles were investigated. The appearance of a red color in the culture flasks suggested the formation of elemental selenium. *K. pneumoniae* cells containing the red selenium particles which were disrupted using a wet heat sterilization process (Fesharaki et al. 2010). Probably, a similar mechanism as described for *Bacillus selenitireducens* (Oremland et al. 1994) is also applied.

Bacillus cereus generated selenium nanoparticles by transformation of toxic selenite $\left(\text{SeO}_3^{2-}\right)$ anions into red elemental selenium (Se^o) under aerobic conditions. Microscopic analysis showed spherical Se^o nanospheres adhering to bacterial biomass (intracellular) as well as free particles (extracellular). Data suggested a hypothetical mechanism for the biogenesis of selenium nanoparticles involving membrane-associated reductase enzyme(s) that reduces selenite $\left(\text{SeO}_3^{2-}\right)$ to Se^o through an electron shuttle enzymatic metal reduction process as shown in Fig. 12.17 (Dhanjal and Cameotra 2010).

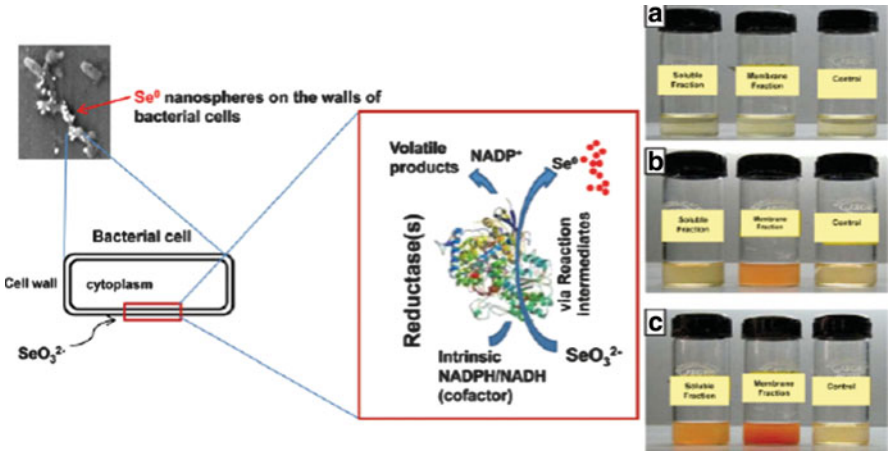

Fig. 12.17 Schematic representation of proposed mechanism of biogenesis of Selenium (Se0) nanospheres. (*a*) Selenite reduction at 0 h. (*b*) Formation of red elemental selenium in membrane fraction after 3–4 h of incubation. (*c*) Prolonged incubation of 12 h resulted also in formation of red elemental selenium in soluble fraction (From Dhanjal and Cameotra 2010. By permission from BioMed Central)

12.2.9 Tellurium Nanoparticles

In the case of tellurium nanoparticles, biosynthesis was only found the mechanistic aspect involving bacteria.

12.2.9.1 Bacteria

Tellurium occurs in nature in four oxidation states: 6, 4, 0, and 2. The first two form the partially soluble oxyanions tellurate [TeO_4^{2-}, or Te(VI)] and tellurite [TeO_3^{2-}, or Te(IV)], and with respect to the latter two, the occurrence of native tellurium [Te (0)] is rare. Two anaerobes previously shown to be capable of respiring oxyanions of selenium also achieve growth by reduction of either tellurate [Te(VI)] or tellurite [Te(IV)] to elemental tellurium [Te(0)]. Those tellurium nanoparticles formed by *Bacillus selenitireducens* were initially nanorods, which cluster together forming larger rosettes composed of numerous individual shards. In contrast, *Sulfurospirillum barnesii* forms extremely small, irregularly shaped nanospheres that coalesce into larger composite aggregates (Baesman et al. 2007). The linkage of lactate oxidation to these reductions demonstrated that these Te derivatives served as terminal electron acceptors for anaerobic respiration. The biological oxidation of organic matter using Te(IV) as an electron acceptor support the growth of *B. selenitireducens*, and following the stoichiometry:

$$\begin{aligned}\text{Lactate}^- + \text{TeO}_3^{2-} + \text{H}^+ \rightarrow &\text{Acetate}^{-1} + \text{Te}(0) \\ &+ \text{HCO}_3^- + \text{H}_2\text{O}\end{aligned}$$

Growth of *S. barnesii* on lactate and Te(VI) also following the stoichiometry:

$$3\ \text{Lactate}^- + 2\ \text{TeO}_4^{2-} + \text{H}^+ \rightarrow 3\ \text{Acetate}^- + 2\ \text{Te}(0) + 3\ \text{HCO}_3^- + 2\ \text{H}_2\text{O}$$

12.2.10 Titanium Nanoparticles

The biosynthesis and mechanistic aspect for titanium nanoparticles synthesis were only found with bacteria.

12.2.10.1 Bacteria

Titanium dioxide was added to curdled milk and whey from boiled milk that contained lactic acid bacteria, and with extra sugar after a few days the titanium nanoparticles were deposited in the flask. Nanoparticles containing the culture solution was filtered under the laminar flow through filter paper (Prasad et al. 2007). The nanotransformation from *Lactobacillus sporogens* was found superior to the *Lactobacilli* and was less expensive, more reproducible, emphatically least time-consuming and a truly green approach, and probably follows the same type of mechanisms as other metallic nanoparticles (Jha and Prasad 2010).

12.3 Antibacterial Potential of Metallic Nanoparticles

Silver nanoparticles exhibit special chemical and physical properties and have been appearing in the recent years as a possible new antibiotic or with other biological activities, since is possible to use them in wound dressing, medical tools, textiles, etc., and escape from antibiotic resistance (Durán et al. 2005, 2010a, b, c, 2011; Nair and Laurencin 2007; Singh et al. 2009; Rai et al. 2009; Dastjerdi and Montazer 2010; Vijayaraghavan and Nalini 2010; Marcato and Durán 2011). One important fact is that silver nanoparticles with different shapes and sizes exhibit variable antimicrobial activity. However, the mechanisms of antimicrobial activity of silver ions and silver nanoparticles, and their toxicity to human tissues, are not fully characterized. A recent review evaluated the potential use of silver nanoparticles to control pathogens with emphasis on their action against pathogenic bacteria, their toxicity and possible mechanisms of action (Durán et al. 2010b). In the last 3 years, many important results with silver nanoparticles on antibacterial activities have been published. Now we will discuss the most recent results on biogenic metallic nanoparticles and their antimicrobial activities.

Many aspects of the inhibitory effect of silver ions have been discussed previously, but silver nanoparticles also have inhibitory and lethal effects on bacterial species such as *E. coli*, *S. aureus* and even against yeast (Durán et al. 2010b and references therein).

Raffi et al. (2008) observed that silver nanoparticles were completely cytotoxic to *E. coli* at a low concentration. Gade et al. (2008) produced silver nanoparticles by extracellular biosynthesis using *Aspergillus niger* isolated from soil and these were cytotoxic to *E. coli*. A recent synthesis of silver nanoparticles using a reduction of aqueous silver ions with the culture supernatants of *Staphylococcus aureus* found them to be active against a methicillin-resistant *Staphylococcus aureus* (MRSA) followed by methicillin-resistant *Staphylococcus epidermidis* (MRSE) and *Streptococcus pyogenes*. However, when *Salmonella typhi* and *Klebsiella pneumoniae* were treated with silver nanoparticles, a moderate antimicrobial activity was observed (Nanda and Saravanan 2009). The synthesis of silver nanocrystals encapsulated in mesoporous silica nanoparticles showed a complete inhibition of bacterial growth (Liong et al. 2009).

The effect of shape on the antibacterial activity of silver nanoparticles was recently discussed studying the different synthesis of the silver nanoparticles (Sharma et al. 2009 and references therein). Silver nanoparticles with different shapes (triangular, spherical and rod) were tested against *E. coli*. The truncated triangular silver nanoplates displayed the strongest biocidal action when compared with spherical, rod-shaped nanoparticles and with silver ions. Furthermore, the antibacterial activity was also particle size-dependent (Sharma et al. 2009 and references therein). The influence of size was also related to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have greater antibacterial activity (Choi and Hu 2008).

The chemically synthesized silver nanoparticles exhibited high antibacterial activity against both Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria. TEM images showed the interaction and bactericidal mechanism of silver nanoparticles. After interacting with bacteria, silver nanoparticles had adhered to the cell wall and penetrated the cell membrane, resulting in the inhibition of bacterial cell growth and multiplication due to their well-developed surface (Le et al. 2010). In this direction, on the basis of new research by Li et al. (2010), the action model of silver nanoparticles on *E. coli* was described as first making a break through the permeability of the outer membrane by lysis of cellular components. Then, second, the silver nanoparticles enter the inner membrane and inactivate respiratory chain dehydrogenases inhibiting respiration and growth of cells. Probably, silver nanoparticles could also affect some proteins and lipids and induce a collapse of the membrane, resulting in cell death (Le et al. 2010). This is very similar to that observed in biogenic silver nanoparticles acting as antimicrobial agents.

The availability of biosynthetically produced silver nanoparticles opened the possibility to investigate the association of these biological particles with antibiotics (Basavaraja et al. 2008; Parikh et al. 2008). A recent study was carried out with clindamycin associated to silver nanoparticles produced chemically or biosynthetically (Durán et al. 2008). Results with methicillin-resistant *S. aureus*

strains (MRSA) and *Staphylococcus epidemidis* showed a significant bactericidal activity of both formulations with slightly lower MIC values observed with the biosynthetic nanoparticles. In addition, a study on the effect of silver nanoparticles associated with clindamycin on leishmaniasis was published (Marcato et al. 2008). The effect of a combination of silver nanoparticles with different antibiotics was investigated against several bacterial strains. Silver nanoparticles were associated with penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin, etc. The association of antibiotics with silver nanoparticles increased the antibacterial activity against all bacteria strains studied (Durán et al. 2010b and references therein). Ampicillin, gentamycin, kanamycin, streptomycin and vancomycin were tested with silver nanoparticles produced from *Phoma glomerata*, and a synergistic activity was observed with *E. coli* and *Pseudiomonas aeruginosa* (Birla et al. 2009).

In another study, silver nanoparticles as active carriers for chloramphenicol in polyvinylpyrrolidone showed substantially enhanced activity against clinically isolated *S. typhi* (Patil et al. 2009).

Silver ions exposed to a filtrate of *T. viride* produced silver nanoparticles and the antibacterial activities of a combination of these nanoparticles with ampicillin, kanamycin, erythromycin, and chloramphenicol showed an increase of the antibiotic effect. A mechanistic suggestion on the function of the synergistic effect between silver nanoparticles and ampicillim has been proposed (Fig. 12.18). First, the antimicrobial groups came into contact with silver nanoparticles forming a core with ampicillin molecules. The ampicillin molecules act on the cell wall producing a lysis increasing the penetration of the silver nanoparticles into the bacterium. Finally, the silver nanoparticles–ampicillin complex interacts with DNA which produced serious damage to the bacterial cells (Fayaz et al. 2010).

Revision of all the data published suggests possible mechanisms of the silver nanoparticles as antimicrobial activity, and a recent review discussed the three most common mechanisms of toxicity proposed to date which are: (1) the uptake of free silver ions followed by disruption of ATP production and DNA replication, (2) the silver nanoparticle and silver ion generation of reactive oxygen species (ROS), and (3) silver nanoparticle direct damage to cell membranes (Marambio-Jones and Hoek 2010 and references therein). Figure 12.19 summarizes all these hypotheses in one scheme.

Recently, a review focuses on the work done in the last few years developing gold nanoparticles as therapeutics and diagnostic agents (Arvizo et al. 2010). These particles also exhibit antibacterial activity. Das et al. (2009) studied the antimicrobial activity of gold nanoparticles, produced by a native *Rhizopus oryzae* strain, against *P. aeruginosa*, *E. coli*, *B. subtilis*, *S. aureus*, *Salmonella* sp., *S. cerevesiae*, and *C. albicans* by the cup-plate method. Exposure of microbial cells to gold nanoparticles resulted in a significant decrease in cell viability compared to the control cells. The SEM images of cells exposed to gold nanoparticles showed morphological changes compared to that of the control. After incubation with the nanoparticles, the integrity of most of the microbial cells was lost, indicating irreversible cell damage and ultimate cell death.

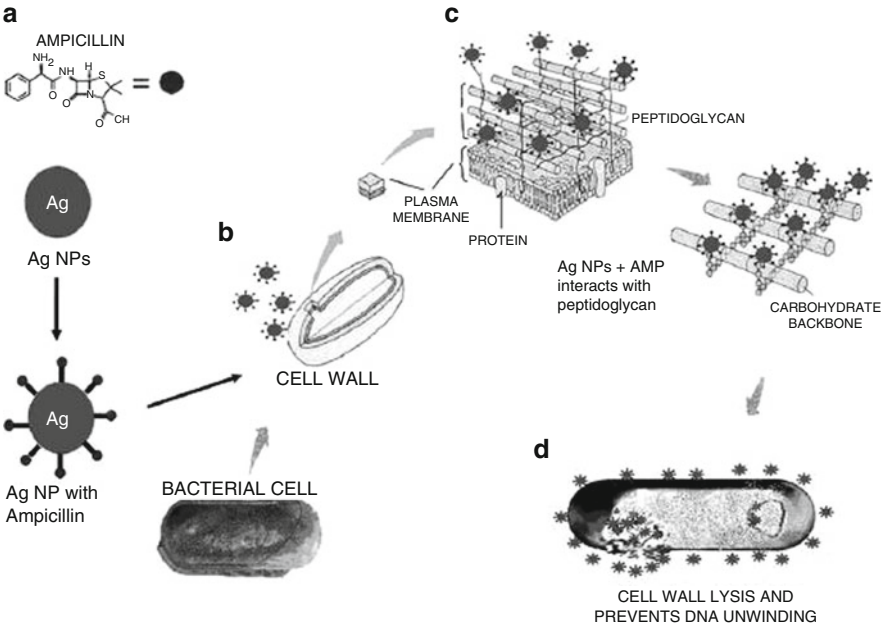

Fig. 12.18 Synergistic activity of silver nanoparticles with ampicillin (*Amp*) against bacteria. (**a**) Formation of core silver nanoparticles with ampicillin. (**b**) Interaction of silver nanoparticles complex over the cell wall of bacteria. (**c**) Silver nanoparticles–Amp complex inhibits the formation of cross-links in the peptidoglycan layer (which provides rigidity to the cell wall), leading to cell wall lysis. (**d**) Silver nanoparticles–Amp complex prevents the DNA unwinding (From Fayaz et al. 2010.. By permission from Elsevier)

The antimicrobial activity of silver nanoparticles and platinum nanoparticles, stabilized with sodium dodecyl sulfate and poly-(*N*-vinyl-2-pyrrolidone), against *S. aureus* and *E. coli* was studied. The growth of Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria wase inhibited by silver nanoparticles, but the silver nanoparticles either stabilized with sodium dodecyl sulfate or the platinum nanoparticles with any stabilizer did not show antimicrobial activity (Cho et al. 2005).

The selenium nanoparticles prepared by *Klebsiella pneumoniae* exhibited a good antimicrobial effect on fungi such as *Aspergilluis terreus*, *A. niger*, *A. flavus*, *A. fumigatus*, *Malassezia sympodialis* and *M. furfur*, with a MIC ranging from 10 to 260 μg mL^{-1} for all isolate microorganisms (Shakibaie et al. 2010).

The antibacterial activity of silver and copper nanoparticles (both synthesized chemically) was compared for various strains of microorganisms (*Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*). The effectiveness of silver nanoparticles was higher than those observed with copper nanoparticles for all *E. coli* and *S. aureus* strains selected for this study. However, copper nanoparticles showed a significantly higher activity than silver nanoparticles against *B. subtilis* (Ruparelia et al. 2008).

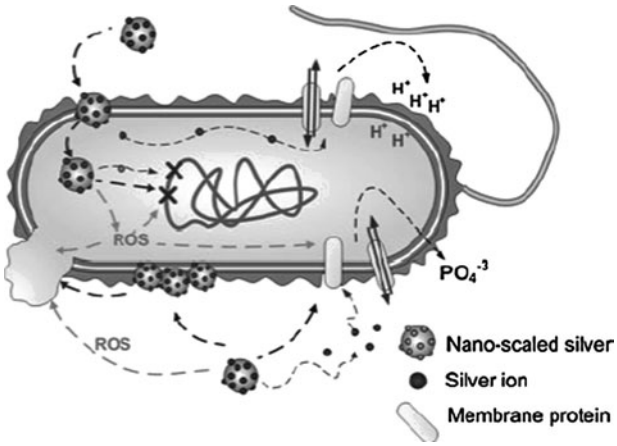

Fig.12.19 Diagram summarizing nano-scaled silver interaction with bacterial cells. Nano-scaled silver may (1) release silver ions and generate ROS; (2) interact with membrane proteins affecting their correct function; (3) accumulate in the cell membrane affecting membrane permeability; and (4) enter into the cell where it can generate ROS, release silver ions, and affect DNA. Generated ROS may also affect DNA, cell membrane, and membrane proteins, and silver ion release will likely affect DNA and membrane proteins (From Marambio-Jones and Hoek 2010. By permission from Springer)

The effective *E. coli* inactivation by Fe^{o} nanoparticles (chemically synthesized) was compared with several iron compounds. No significant inactivation of *E. coli* was achieved with iron oxide (magnetite) nanoparticles and microscale Fe^{o} powder. In contrast, Fe^{o} nanoparticles showed strong activity. These results suggest that the biocidal effect of Fe^{o} nanoparticles originates from both a size-related physical property of the nanoparticles and a chemical interaction of elemental iron with *E. coli*. Furthermore, Fe^{o} nanoparticles showed a higher bactericidal activity than silver nanoparticles (Lee et al. 2008).

12.4 Cytotoxicity and Toxicity of Metallic Nanoparticles

As described in the previous section, the beneficial effect on human health of metallic nanoparticles is clear but it does not mean that they are risk-free (El-Ansary and Al-Daihan 2009; Hussain and Schlager 2009) The small particle size possibly leads them to penetrate easily by inhalation, by oral ingestion and probably by contact with the skin, and disseminate throughout the body. In many cases, the cytotoxicity will depend on the support of the nanoparticles matrix or the concentration (Durán et al. 2010b).

Recently, Acticoat™ (commercial silver support) was used in post-cardiac surgery for mediastinitis, and in all four patients, negative cultures were obtained within a maximum of 72 h (Totaro and Rambaldini 2009). Acute toxicity of

different metallic nanoparticles in vitro using a rat liver-derived cell line or neural cells treated with different silver nanoparticle sizes resulted in dose-dependent cytotoxicity. The toxicity on the C18-4 cells of a mouse cell line with spermatogonial stem cell characteristics showed that silver nanoparticles were the most toxic among other metallic (molybdenum, and aluminum) nanoparticles tested. Toxicity and biocompatibility of silver nanoparticles were evaluated in vivo using zebrafish embryos, and the results showed the same as before, that is dose-dependence of silver nanoparticles. Another important fact was observed in an alga where it was found that silver nanoparticle toxicity was mediated by silver ions (Durán et al. 2010b and references therein). Some reports have summarized the hazardous effects of silver nanoparticles to human health through biodistribution, organ accumulation, degradation and possible adverse effects and toxicity (Panyala et al. 2008; Chen and Schluesener 2008).

A possible mechanism of toxicity using human lung fibloblast and glioblastomacells of silver nanoparticles has been proposed. The mechanism involves disruption of the mitochondrial respiratory chain leading to production of reactive oxygen species (ROS) and interruption of ATP synthesis, which in turn cause DNA damage (Fig. 12.20) (AshaRani et al. 2009).

The immunological response of macrophages to gold and silver nanoparticles showed that both nanoparticles enter the cells but only gold nanoparticles up-regulate the expressions of pro-inflammatory genes interlukin-1 (IL-1), interlukin-6 (IL-6), and tumor necrosis factor (TNF-a). Probably, the gold nanoparticles might adsorb serum protein due to partial negative charge and enter cells via a complex endocytotic pathway, resulting in higher cytotoxicity and immunological activity for gold rather than silver nanoparticles (Yen et al. 2009). Using of gene expression data, it was suggested that silver nanoparticles may produce neurotoxicity by generating free radical-induced oxidative stress (Rahman et al. 2009).

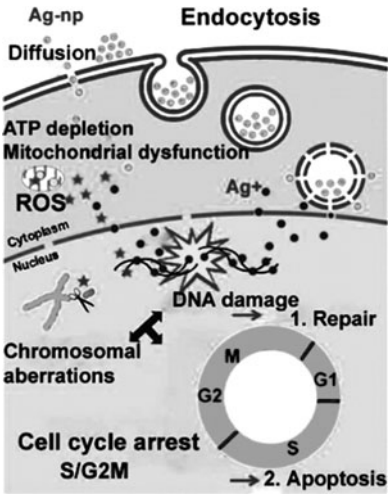

Fig.12.20 A possible mechanism of toxicity which involves disruption of the mitochondrial respiratory chain by silver nanoparticles leading to production of ROS and interruption of ATP synthesis, which in turn cause DNA damage (From AshaRani et al. 2009. By permission from ACS Publications)

Histopathological examinations of inflammatory response and pulmonary function changes in rats treated by inhalation exposure to silver nanoparticles indicated dose-dependent increases in lesions related to silver nanoparticle exposure, such as infiltrated mixed cell and chronic alveolar inflammation, including thickened alveolar walls and small granulomatous lesions (Sung et al. 2008, 2009).

In vitro interactions of 7–20 nm spherical silver nanoparticles with primary fibroblasts and primary liver cells remained unaltered up to 25 and 100 μg mL^{-1} silver nanoparticles, respectively. Many biological processes and morphological transformations have been suggested to the effect that, although silver nanoparticles seem to enter the eukaryotic cells, cellular antioxidant mechanisms protect the cells from possible oxidative damage (Carlson et al. 2008; Arora, et al. 2009).

Silver nanoparticles prepared by dispersing them in fetal bovine serum showed cytotoxicity to cultured RAW264.7 cells by cellular apoptosis, decreasing intracellular glutathione level, increasing NO secretion, increasing TNF-a in protein and gene levels, and increasing gene expression of matrix metalloproteinases. All the data suggested that silver nanoparticles were ionized in the cells to cause cytotoxicity by a Trojan-horse type mechanism (Park et al. 2010).

Investigations on the in vitro cytotoxicity of gold nanoparticles showed that they induced cytotoxicity in Cos-1 cells and in human dermal fibroblasts, but not in human leukemic cells or murine macrophages, where particles were taken up and stored in intracellular perinuclear vesicles. Gold nanoparticles induced a mild cytotoxicity in human alveolar type-II (ATII)-like cell lines A549 and NCIH441 (Uboldi et al. 2009, and references therein).

The cytotoxicity of the selenium nanoparticles prepared by *Bacillus* sp. MSh-1 was evaluated in vitro against the fibrosarcoma cell line (HT-1080), and the cytotoxicity analysis showed a direct dose–response effect; the higher the concentration, the higher the toxicity. The presence of a low dose level (10 μg ml^{-1}) showed a small cytotoxicity with a viability percentage of more than 80% (Shakibaie et al. 2010). Apoptosis had been shown previously with selenium nanoparticles (Chen et al. 2008).

Previous data of copper nanoparticles (chemically synthesized) showed, in vivo, a potent toxic effect (cytotoxic and genotoxic) (Chen et al. 2006). Earlier, the effect of copper nanoparticles inducing single-stranded breaks in cultured human lung cells was reported (Midander et al. 2009), and also, recently, in cultured cancer cell lines (Studer et al. 2010). The DNA degradation potential and anti-cancer activities of copper nanoparticles through singlet oxygen have been reported. The same authors observed that the copper nanoparticles exhibited a cytotoxic effect towards human histiocytic lymphoma cell (U937) and human cervical cancer (Hela cells) by inducing apoptosis (Jose et al. 2011).

As far as we know, no cytotoxic or toxic studies with nanoparticles of cadmium, copper, iron, palladium, platinum, tellurium or titanium have been found in the literature.

12.5 Industrial Applications

The strong antibacterial activity of silver nanoparticles discussed in this chapter can be as coatings and on certain implants and other applications such as treatment of wounds and burns, in contraception and also marketed as a water disinfectant and a room spray. Thus, the use of silver nanopaonicles appears to be an important issue in medicine and related fields. Many aspects related to biodistribution, organ accumulation, degradation, possible adverse effects and toxicity have been reviewed focusing on major questions associated with the increased medical use of silver nanoparticles and related nanomaterials (Chen and Schluesener 2008).

Metal nanoparticles have many physicochemical and opto-electronic properties. Due to all these diverse properties, nanoparticles have multiple applications in various fields like electronics, agriculture, and medicine. Some of the nanoparticles that can be produced by fungi may be of particular relevance to new and emerging technologies. The use of silver coatings in solar absorption systems has already been mentioned (Durán et al. 2010c). Other applications in such areas include the use of gold nanoparticles as precursors to coatings for electronic applications (Mukherjee et al. 2001) and platinum nanoparticles in the production of fuel cells (Riddin et al. 2006). Applications in the field of medicine includes the formulations of many potential antimicrobial agents, which are effective against many human pathogens including multidrug-resistant bacteria (Ingle et al. 2008).

Most studies in the literature on the production of sterile materials (fabrics, textiles), using metal nanoparticles involve production by chemical methods (Gao and Cranston 2008; Gupta et al. 2010). One which was biogenic produced silver nanoparticles (from *F.oxysporum*) for sterilized fabrics (Durán et al. 2007) by the impregnation of biogenic silver nanoparticles in cotton and polyester fabrics. The padding method appeared to yield more homogeneous impregnations than the centrifugation process (Huber et al. 2009) with high resistance to washing with maintainance of the antibacterial activity, demonstrating the large adhesion of these particles in the fabric fibers probably due to the fungal protein capping the silver nanoparticles (Durán et al. 2005). Many fabric materials having silver nanoparticles are commercially available but they release the silver nanoparticles to the environment at the first washing.

Silver nanoparticle applications in fabrics, cosmetics and agriculture were recently discussed by Rai et al. (2011).

12.6 Conclusions

This chapter has described biogenic metallic nanoparticle synthesis and their mechanistic aspects in the syntheses and in their antimicrobial activities. Although many aspects have already been studied with silver and gold nanoparticles, many questions are still open to discussion related to the final consequence of the other

metallic nanoparticles such as cadmium, copper, iron, palladium, platinum, tellurium or titanium.

In the applications dealt with, the use of silver nanoparticles appears to be an important issue in medicine and related fields, such as coatings on certain implants, treatment of wounds and burns, in contraception and as a water disinfectant and a room spray. Many aspects related to biodistribution, organ accumulation, degradation and adverse effects were reviewed focusing on major questions associated with the medical use of silver nanoparticles and related nanomaterials. The physicochemical and opto-electronic properties of metal nanoparticles have multiple applications in various fields like electronics, agriculture and medicine. The uses of metallic nanoparticles in coating in solar absorption systems, or for electronic applications and production of fuel cells were also discussed. Their applications as antimicrobials against multidrug-resistant bacteria, and production of sterile materials are no less important than the previous issues mentioned.

Acknowledgements Support from FAPESP, CNPq and DST/CNPq Brazil-India Project is acknowledged.

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