Chapter 1
Bioinspired Metal Nanoparticles with Special Reference to Mechanism

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Abstract The interest in metallic nanoparticles and their synthesis has greatly increased over the past decades. Several physical and chemical methods for synthesis of nanoparticles have been developed. However, involvement of toxic chemicals, high-energy consumption, and costly equipments are the drawbacks to their wide use. Therefore, "green" approach for the synthesis of metallic nanoparticles by using plants and their extracts, algae, fungi, and bacteria, including actinomycetes as well as viruses and biomolecules, is promising way, which is quick, low-cost, and eco-friendly. The mechanism of synthesis of metal nanoparticles by living organisms has not been fully explained, to date. However, the bioreduction process with involvement of NADP-dependent nitrate reductase is considered as a main step. The physical (e.g., morphology, zeta potential) and chemical (composition of capping agents) properties of nanoparticles, which effect on their activity, can be controlled during biosynthesis process. There are several factors such as temperature, time, pH, and concentration of reagents used, which influence the biological synthesis of metallic nanoparticles, mainly the size and yield of synthesized nanoparticles.

Keywords Metallic nanoparticles · Biosynthesis · Bioreduction mechanisms
Reaction parameters · Physical factors · Chemical factors

Abbreviations

Ag	Silver
Ag^+	Silver ion
Ag^0	Metallic state of silver
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
Al	Aluminum

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As	Arsenic
Au	Gold
BSA	Bovine serum albumin
Cd	Cadmium
CdS	Cadmium sulfide
C-O	Carbon oxygen bond
Cu	Copper
Fe	Iron
Fe_2O_3	Iron oxide
FTIR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography–mass spectrometry
$HAuCl_4$	Chloroauric acid
Hg	Mercury
NADP	Nicotinamide adenine dinucleotide phosphate
$NADP^+$	Nicotinamide adenine dinucleotide phosphate ion
NADPH	Nicotinamide adenine dinucleotide phosphate reduced form
N-H	Nitrogen hydrogen bond
NP	Nanoparticle
NPs	Nanoparticles
OH^-	Hydroxide ion
Pb	Lead
-SH	Sulfhydryl group
TMV	Tobacco mosaic virus
Zn	Zinc
ZnS	Zinc sulfide

1.1 Introduction

Nanoparticles (NPs) are those particles, which have two or more than two dimensions and are in the size range of 1–100 nm (Alanazi et al. 2010). Due to the increased demand for various metallic and nonmetallic nanoparticles over the past two decades, a wide range of physical and chemical techniques have been developed to produce nanoparticles of different sizes, shapes, and compositions. Nanoparticles can be synthesized and stabilized via physical, chemical, and biological techniques. The physical approach includes techniques such as laser ablation, lithography, and high-energy irradiation (Joerger et al. 2001), while the chemical methods use chemical reduction, electrochemistry, and photochemical reduction (Rajput 2015). Physical and chemical methods for synthesis of metal nanoparticles are often extremely expensive and non-environmentally friendly due to the use of toxic, flammable, and hazardous chemicals, which may pose potential environmental and biological risk and high-energy requirement (Awwad et al. 2013). Additional drawbacks of chemical and physical approaches to nanoparticle synthesis are low production rate, structural particle deformation, and inhibition of particle growth (Keat et al. 2015). Thus, one of the primary goals of nanotechnology is to develop an eco-friendly production method that can provide nanoparticles with low toxicity. Because physical and chemical methods use high radiation or highly concentrated reductants and stabilizing agents that are harmful to environment and to human health, the researchers have turned to biological systems for inspiration of synthesis of metal nanoparticles, as these methods are rapid, cost-effective, and eco-friendly. Biosynthesis of metal nanoparticles mediated by living organisms such as bacteria, fungi, algae, plants or viruses and plant products such as their enzymes, proteins, or carbohydrates becomes an important field of nanotechnology (Iravani 2011, 2014). The green methods employ biological systems to fabricate nanostructures, which have the benefit of improving the biocompatibility of the nanomaterial (Xie et al. 2007). Prokaryotic and eukaryotic organisms are considered as excellent candidates to be used to synthesize metallic nanostructures by a purely enzymatic process (Ahmad et al. 2002). The important advantage of using biological methods is that newly formed nanostructures are stabilized by proteins, which act as capping agents and are also assumed to be responsible for the bioreduction metal ions to metal nanoparticles (Ahmad et al. 2002; Xie et al. 2007; Mukherjee et al. 2008). Such stabilization protects nanoparticles from aggregation and affects on their physical and chemical properties (Gole et al. 2001). It is known that any synthesis process, including synthesis of metallic nanoparticles, depends on many physical and chemical factors such as pH, reagent concentrations, temperature, and time (Joerger et al. 2001; Quester et al. 2016). The reaction conditions effect on nanoparticle morphology (Gericke and Pinches, 2006). Therefore, it is necessary to control reaction parameters resulting in desired nanoparticles (Sanghi and Verma, 2010; Quester et al. 2016).

Mechanisms of biological synthesis of metal nanoparticles have not been fully explained. It is claimed that NADH-dependent nitrate reductase enzyme is an important factor in biogenic synthesis of metal nanoparticles. The proposed mechanism is that bioreduction of metal ions (e.g., Ag^{+}) is initiated by electron transfer from NADH by NADH-dependent reductase as electron carrier. The metal ions which receive electrons are reduced to metal (e.g., Ag^{0}) and then to metal nanoparticles (e.g., AgNPs) (Duran et al. 2011; Duran and Seabra 2012). However, other non-enzymatic mechanisms are suggested to be involved in biogenic synthesis of metal nanoparticles where proteins, amino acids, and sugars play a major role in the reduction of metal ions (Mukherjee et al. 2001, 2008; Duran and Seabra 2012).

There is a long list of organisms (plants or plant extracts, micro- and macroalgae, fungi, yeasts, bacteria, and actinomycetes) that have been used to synthesize various metal nanoparticles such as silver, gold, platinum, lead, iron, titanium, cadmium (Bhau et al. 2015; Zahir et al. 2015; Kumar and Kathireswari, 2016; Ali et al. 2016; de Aragao et al. 2016; Quester et al. 2016; Składanowski et al. 2016; Wypij et al. 2017). However, synthesis of silver and gold nanoparticles has been mostly reported to date.

In this chapter, the use of prokaryotic (bacteria and actinomycetes) and eukaryotic (algae, fungi, yeast, and plants) organisms as well as viruses and biomolecules to the biosynthesis of metal NPs with special reference to mechanisms is presented.

1.2 Fabrication of Metal Nanoparticles

Metal nanoparticles can be synthesized by using physical, chemical, or biological methods (Iravani et al. 2014). From the structural point of view, methods of synthesis of metal nanoparticles can be divided to "bottom-up" or "top-down" approach (Keat et al. 2015). Both chemical and biological syntheses of nanoparticles rely on the bottom-up approach, which is based on the assembly of atom or molecules to molecular structure in nanoscale range. Physical approaches to synthesize metallic nanoparticles mostly use the top-down technique, which is based on the mechanical method of size reduction by breaking down the bulk materials gradually to nanoscale structure (Schröfel and Kratošová 2011).

The "bottom-up" technique is more preferable for the fabrication of nanoparticles. This approach involves a homogeneous system wherein catalysts such as reducing agent and enzymes synthesize nanostructures that are controlled by catalyst properties, reaction media, and conditions (e.g., solvents, stabilizers, and temperature) (Keat et al. 2015).

1.2.1 Physical Methods

Physical approaches to synthesize metallic nanoparticles include plasma arcing, ball milling, thermal evaporation, spray pyrolysis, ultra-thin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer–by-layer growth, molecular beam epistaxis, and diffusion flame synthesis of nanoparticles (Joerger et al. 2001).

The advantage of physical methods is narrow particle size distribution of the produced NPs. The nanoparticles with high purity and desired size can be selectively synthesized by using these methods (Sardar et al. 2014). The major drawback of this approach is often imperfection of the obtained structure. The surface defects significantly impact on physical and chemical properties of synthesized nanoparticles (Schröfel and Kratošová 2011). The disadvantage of these methods is also the need for expensive equipment (e.g., lasers) and higher-energy consumption for maintaining the pressure and temperature conditions to obtain nanoparticles, which may result in high operating costs. The lower productivity of physical methods for nanoparticle synthesis compared to chemical ones is an additional limitation (Schröfel and Kratošová 2011; Sardar et al. 2014).

1.2.2 Chemical Methods

The chemical methods such as electrodeposition, sol–gel process, chemical solution deposition, chemical vapor deposition, and precipitation are used to synthesize metallic nanoparticles (Rajput 2015).

Chemical reduction method is the most common synthetic pathway for metal nanoparticle synthesis (Lal et al. 2011). This method is carried out based on the reduction of aqueous metal ions (e.g., silver nitrate) in an appropriate operating medium using chemical reductants such as sodium citrate or branched polyethylenimine (Note et al. 2006; Nguyen et al. 2010). The use of sodium citrate as a reductant leads to synthesis of negatively charged silver nanoparticles, while the use of branched polyethylenimine to positively charged NPs (Moghaddam 2010). There are also other reducing agents reported as a suitable reducing agents for metallic NP preparation, viz. methoxypolyethylene glycol (Mallick et al. 2004), ascorbic acid (Wagner and Köhler 2005), stannous chloride (Vaskelis et al. 2007), $NaBH_4$ (Wagner et al. 2008), amine or hydroxyl-containing molecules (Note et al. 2006), azacryptand, amino acid (Selvakannan et al. 2004), or chitosan (Shih et al. 2009).

Chemical methods take a very short period of time for the synthesis of large quantity of nanoparticles (Sardar et al. 2014). Chemical techniques, similar to physical ones, allow to produce well-defined nanoparticles. Thus, the physiochemical properties, surface, and morphological characteristics of nanoparticles can possibly be controlled depending on the subsequent application through variation in precursor concentrations, reduction agent usage, and reaction conditions (Shah et al. 2015). However, in this method, capping agents are necessary for size stabilization of the nanoparticles (Lalitha et al. 2013; Shah et al. 2015). These processes are relatively inexpensive for large scale but involve the use of toxic chemicals, which is hazardous to the environment (Schröfel and Kratošová 2011). The chemicals used for synthesis of nanoparticles may lead to the presence of some toxic compounds being adsorbed on the surface of nanoparticles. These toxic chemicals may effect on adverse effects of use of metal nanoparticles in medical applications (Sardar et al. 2014; Shah et al. 2015).

1.2.3 Biological Methods

Biological methods use less energy, non-toxic compounds, and environmentally friendly resources such as plants, bacteria, fungi, micro- and microalgae (Sathishkumar et al. 2009a, b; Iravani 2011). In all biological systems used for synthesis of metal nanoparticles, the plant extract potential has been found to be comparatively higher than the microbial culture (Keat et al. 2015). Moreover, the microbial-based methods can be more harmful to the environment depending on the type of microbes involved in the synthesis (Moghaddam 2010).

However, on reproducibility and stability of biogenic synthesis, as well, the rate of bioreduction of metal ions using biological systems depends on synthesis conditions, which are detailed afterward in this chapter.

Many microorganisms are known to be employed in the remediation of toxic metals (Pérez-de-Mora et al. 2006). Therefore, fungi and bacteria were found as possible nanofacilities for NP fabrication. Metallic nanoparticles can be synthesized intra- or extracellularly (Ahmad et al. 2003, 2005; Golinska et al. 2014; Wypij et al. 2017). Fungi as a biological system for synthesis of metal nanoparticles possess some additional attributes when compared to bacteria. They secrete large amounts of proteins and enzymes per unit of fungal biomass, which results in larger amounts of nanoparticles being produced (Narayanan and Sakthivel 2010). However, the culture conditions can significantly influence the extra- or intracellular biosynthesis process (Ahmad et al. 2005). Authors who studied gold nanoparticle synthesis using *Trichothecium* sp. biomass observed that the reduction of Au ions performed under stationary conditions led to extracellular synthesis of nanoparticles, but agitation of the biomass tended to produce intracellular nanoparticles. It was concluded that non-agitation promoted the release of enzymes and proteins, while agitation prevented their release (Ahmad et al. 2005). The fluorescence spectra studies of fungal mediated nanoparticles revealed that extracellular synthesis resulted from the action of bioactive reducing agents secreted from the fungal cell wall and fabricated nanoparticles were protein-stabilized. The same proteins released by the fungal cells were present in the solution and bound to the surfaces of nanoparticles (Kumar et al. 2003). The extracellular fabricated NPs are readily recovered as they are fabricated at the cell surface or at the periphery of the cell. In contrast, intracellular synthesis needs extraction process of nanoparticles from cells which influence on low yield of obtained NPs (Dhillon et al. 2012).

Biological synthesis of metal nanoparticles is also a chemical approach as the living cells are highly complex system with thousands of molecules. These molecules with varied functional groups such as hydroxyl, amine can possibly facilitate metal reduction (Schröfel and Kratošová 2011). Hence, it is very difficult to determine a specific place of the cell or process responsible directly for NP synthesis. The resulting product is usually mixture of cells or cell debris and NPs, and separation of thousands of metabolic products or other molecules, which accompany to biosynthesized nanoparticles, is extremely hard. Moreover, surrounding matrix and capping proteins are responsible for stability of biosynthesized NPs (Lynch and Dawson 2008) and can affect their properties.

The mechanisms of the synthesis of metal nanoparticles in biological systems have not been fully described. However, the bioreduction is considered as a key step (Mukherjee et al. 2008; Duran et al. 2011). Although mechanisms of biogenic synthesis of nanoparticles have been mostly explained for silver and gold nanoparticles, it is supposed that for other metal nanoparticles similar mechanisms are involved (Golinska et al. 2016).

The NADH-dependent reductase from *Fusarium oxysporum* responsible for the reduction of Ag ions and the subsequent formation of silver nanoparticles was the first time proposed mechanism for the synthesis of silver nanoparticles both intraand extracellularly (Ahmad et al. 2003) (Fig. 1.1). The NADH-dependent reductase enzyme was also proposed by Juibari et al. (2011) as a mechanism for the reduction of silver ions to silver nanoparticles in *Pseudomonas stutzeri* AG259. The biogenic AgNPs were also recorded when NADPH-dependent nitrate reductase, anthraquinone, or hydroxyquinoline were present during biosynthesis process. Both, quinones and NADPH, were donors of electron generated during the reduction of silver ions to Ag^0. The reduction of NADPH to $NADP^+$ is required in this process (Kumar et al. 2007). It is claimed that a non-enzymatic reduction mechanism was involved in nanoparticle formation when *Corynebacterium* sp. was used for synthesis (Sneha et al. 2010). The reduction of nanoparticles is believed to be the result of a combination of several factors such as the presence of some organic functional groups at the cell wall that induce reduction and the appropriate environmental parameters such as pH and temperature that influence synthesis (Lin et al. 2001). The action of functional groups present on the cell wall to produce silver nanoparticles was supposed when the dried biomass of *Lactobacillus* sp. A09 and *Bacillus megaterium* D01 was used (Fu et al. 2000).

One of the hypothetical mechanisms responsible for mycosynthesis of metal nanoparticles is the presence of proteins with amino acid possessing -SH bonds (e.g., cysteine). Such biomolecules undergo dehydrogenation on reaction with silver nitrate, which leads to silver nanoparticle fabrication. The free amino acid groups possibly serve as a capping agent for silver nanoparticles (Mukherjee et al. 2008; Duran and Seabra 2012; Golinska et al. 2016).

Chan and Mashitah (2012) who studied mycosynthesis of silver nanoparticles suppose that in the reduction of silver ions to silver nanoparticles the diketone compound was involved which was also confirmed by GC–MS analysis.

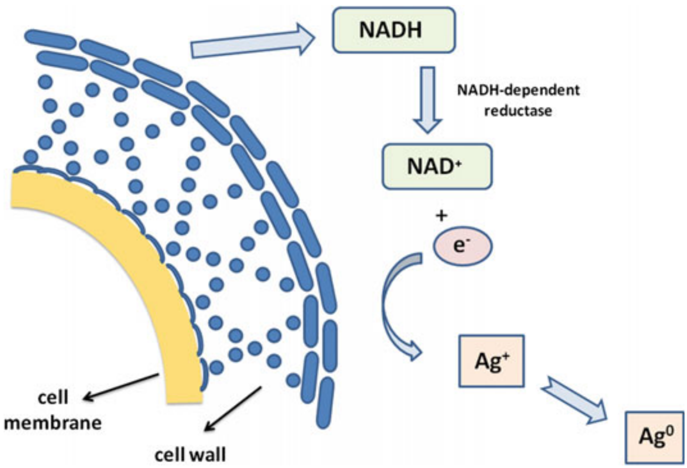

Fig. 1.1 A schematic diagram of a proposed mechanism for biomediated fabrication of metal nanoparticles based on fungal biosynthesis of silver nanoparticles

The Michaelis–Menten type of mechanism for the synthesis of metal nanoparticles has been also suggested (Mukherjee et al. 2008).

In intracellular synthesis, metal ions are firstly entrapped on the surface of cell, which occurs due to the electrostatic interaction between lysine residues and metal ions (Riddin et al. 2006), and then reduced below the cell surface by enzymes present in the cell membrane, which leads to aggregation and formation of nanoparticles (Mukherjee et al. 2001). It is also supposed that the cell wall sugars play a major role in the reduction of metal ions (Mukherjee et al. 2001).

Interestingly, biologically synthesized nanoparticles tend to have higher antimicrobial activity when compared with those obtained by using physical and chemical methods. The higher antimicrobial activity is believed to be the result of the action of synergistic proteins involved in capping and stabilizing the nanoparticles (Kumar et al. 2013).

1.2.4 Synthesis by Plants

In recent years, biosynthesis of metal nanoparticles, especially silver and gold nanoparticles, using plant extracts as nanofactories, is an important subject of research in the field of bionanotechnology (Iravani 2011).

Plants have several cellular structures and physiological processes to combat the toxicity of metals and maintain homeostasis. They also possess dynamic solutions to detoxify metals, and hence, scientists have now turned into phytoremediation (Abboud et al. 2013). The mechanisms of detoxification include immobilization, exclusion, chelation, and compartmentalization of the metal ions and the expression of more general stress response mechanisms, such as ethylene and stress proteins (Sánchez et al. 2011). Heavy metals can be restricted by mycorrhizal association, binding with plant cell wall and root excretions, metal efflux from the plasma membrane, metal chelation by phytochelatins and metallothioneins, and compartmentalization within the vacuole (Shadid et al. 2017). The ability of plants to accumulate high concentrations of essential metals, such as copper (Cu), iron (Fe), zinc (Zn), as well as nonessential metals, such as cadmium (Cd), mercury (Hg), lead (Pb), aluminum (Al), and arsenic (As), was observed (Sahayaraj et al. 2012; Oves et al. 2016; Shadid et al. 2017).

Biosynthesis of nanoparticles can occur either on living or inactivated plant biomasses. In plants or plant extracts, a wide range of metabolites with redox potentials are present. These metabolites can act as reducing agents for the reduction of metal ions to metal nanoparticles in the process of biogenic synthesis of nanoparticles. Several bioorganic compounds in plant systems such as flavonoids, terpenoids, proteins, reducing sugars, and alkaloids were suggested to be involved as either reducing or capping agents during the formation of nanoparticles (Zhou et al. 2010; Duran et al. 2011). The plant-mediated synthesis of metal nanoparticles using plants or plants-derived materials has been found to be most effective, and produced nanoparticles are more stable when compared with microbial synthesis (Ahmad and Sharma 2012). The aqueous plant extracts are more frequently used for biogenic synthesis of metal nanoparticles than whole plants as the availability of reducing agents is higher in the extracts than the whole plants (Huang et al. 2007).

It is claimed that synthesis of metal nanoparticles in plants and plant extracts includes three main phases: firstly, the activation phase in which the reduction of metal ions and nucleation of the reduced metal atoms occur; secondly, the growth phase, referring to the spontaneous coalescence of the small adjacent nanoparticles into particles of a larger size, accompanied by an increase in the thermodynamic stability of nanoparticles, or a process referred to as Ostwald ripening; and finally, the termination phase in which the final shape of the nanoparticles is formed (Makarov et al. 2014).

To date, a large number of plants and plants extracts have been used for biosynthesis of various metal nanoparticles (e.g., silver, gold, platinum, lead, iron, titanium) (Joglekar et al. 2011; Bhau et al. 2015; Naseem and Farrukh 2015; Zahir et al. 2015; Ali et al. 2016; Kumar and Kathireshwari 2016). Plant-fabricated nanoparticles were found in different sizes and shapes, which are detailed in Table 1.1.

1.2.5 Synthesis by Algae

Algae are aquatic microorganisms and similar to plants not only accumulate heavy metals, but also used them for synthesis of metallic nanoparticles (Shah et al. 2015).

Mainly gold and silver nanoparticle synthesis has been reported using both micro- and macroalgae such as *Chlorella vulgaris, Sargassum wightii, Kappaphycus alvarezii* and *Fucus vesiculosus*, *Chondrus crispus*, *Spyrogira insignis, Tetraselmis kochinensis, Enteromorpha flexuosa* (Singaravelu et al. 2007; Xie et al. 2007; Govindaraju et al. 2009; Mata et al. 2009; Rajasulochana et al. 2010; Luangpipat et al. 2011; Senapati et al. 2012; Castro et al. 2013; Yousefzadi et al. 2014) (Table 1.1). However, synthesis of Au/Ag bimetallic nanoparticles was also observed from marine algae *Sargassum wightii* (Govindaraju et al. 2009). Most of algae-fabricated nanoparticles have been synthesized extracellularly (Govindaraju et al. 2009). Intracellular synthesis of gold nanoparticles was performed using *Tetraselmis kochinensis* (Senapati et al. 2012).

1.2.6 Microbial Synthesis of Metal Nanoparticles

Microorganisms are frequently exposed to various and sometimes extreme environmental factors. Their survival in such conditions depends on their ability to deal with environmental stresses such as high concentration of metal ions (Dhillon et al. 2012; Shah et al. 2015).

Table 1.1 Plant, plant extract, and algae used for synthesis of various metal nanoparticles

Organism		Type of NPs	Size (nm)	Shape	Reference
Plants					
Nepenthes khasiana	Leaves	Au	50–80	Triangular and spherical	Bhau et al. (2015)
Grewia flaviscences	Leaf	Ag	50–70	Spherical	Sana et al. (2015)
Lawsonia inermis	Leaf	Ag	13–61	Spherical	Kumar and Kathireswari (2016)
Hydrastis canadensis	Whole plant	Ag	111	Spherical	Das et al. (2013)
Curcuma longa	Tuber	Pd	10–15	Spherical	Sathishkumar et al. (2009b)
Jatropha curcas	Latex	Pb	10–12.5	Spherical	Joglekar et al. (2011)
Glycine max	Leaf	Pd	15	Spherical	Petla et al. (2012)
Diopyros kaki	Leaf	Pt	15–19	Crystalline	Song et al. (2010)
Terminalia arjuna	Bark	Cu	23	Spherical	Yallappa et al. (2013)
Sterculia urens		CuO	4.8	Spherical	(Padil and Cerník 2013)
Annona squamosa	Peel	TiO_2	23	Spherical	Roopan et al. (2012)
Euphorbia prostrate	Leaf	TiO_2	83.22	Spherical	Zahir et al. (2015)
Psidium guajava	Leaf	TiO_2	32.58	Spherical	Santhoshkumar et al. (2014)
Calotropis procera		ZnO	5–40	Spherical	Singh et al. (2011)
Aloe barbadensis	Leaf	ZnO	15	Spherical	Ali et al. (2016)
Lawsonia inermis	Leaves	Fe	21	Hexagonal	Naseem and Farrukh (2015)
Gardenia jasminoides	Leaves	Fe	32	Shattered rock	Naseem and Farrukh (2015)
Euphorbia milii	Steam	Fe	13–21	Spherical	Shah et al. (2014)
Algae					
Chlorella vulgaris	Dried cell extract	Au	40–60	Tetrahedral, decahedral, icosahedral	Luangpipat et al. (2011)
Chondrus crispus	Dried algae	Au	30–200	Spherical, triangular and hexagonal	Castro et al. (2013)
Enteromorpha flexuosa	Seaweed	Ag	2–32	circular	Yousefzadi et al. (2014)
Spyrogira insignis	Dried algae	Ag	30	spherical	Castro et al. (2013)
Gracilaria birdiae		Ag			de Aragão et al. (2016)

Microbial strategies for dealing with high concentrations of metallic ions include changes in metal ion concentration via redox state changes, efflux systems, intracellular precipitation, accumulation of metals, and extracellular formation of complexes (Dhillon et al. 2012).

Although nanoparticles synthesized by microorganisms are very stable, there are drawbacks to microbial synthesis. A major problem is the difficulty in providing good control over size distribution, shape, and crystallinity of nanoparticles and the rates at which they are synthesized. The manipulation of reaction parameters such as pH and temperature might inactivate the microbes and suppress the bioreduction process. Specialized facilities and long incubation are required for maintaining the growth of microorganisms and subsequent formation of nanoparticles. Understanding the mechanism by which these microbes synthesize the nanoparticles at the cellular, biochemical and molecular level may provide information on how to improve the rate of synthesis, and the quality and intrinsic properties of the nanoparticles produced (Narayanan and Sakthivel 2010). In addition, special precautions must be taken when it is necessary to handle some bacteria or viruses that might be harmful to humans. Hence, the associated biological safety issue should also be addressed for the implementation of microorganism-mediated synthesis of nanoparticles on a large scale and for commercial applications (Sardar et al. 2014).

1.2.6.1 Synthesis by Fungi

A large number of different genera of fungi, including yeasts, have been investigated for the synthesis of metal nanoparticles. It has been found that fungi are extremely good candidates (Table 1.2). Nanoparticles synthesized by fungi are characterized by good monodispersity and well-defined dimensions (Mukherjee et al. 2001; Shah et al. 2015). To date, several types of metallic nanoparticles fabricated from different genera and species of fungi have been reported (Table 1.2) both intra- and extracellularly (Ahmad et al. 2005; Agnihotri et al. 2009; Pimprikar et al. 2009; Rai et al. 2015a; Golinska et al. 2016; Quester et al. 2016).

1.2.6.2 Synthesis by Bacteria

Many bacterial species, viz. *Escherichia coli, Serratia nematodiphila, Halomonas salina, Rhodopseudomonas* sp., *Lactobacillus* sp., *Bacillus* sp., *B. cereus*, *Pseudomonas putida*, *Myxococcus virescens*, *Rhodobacter sphaeroides*, *Shewanella algae*, including actinobacterial ones, namely, *Nocardiopsis valliformis, Streptomyces kasugaensis, Pilimelia columellifera* subsp. *pallida*, have been used for the synthesis of metallic nanoparticles, mainly of silver and gold, but also of lead, zinc, titanium, platinum, cadmium, copper etc. (Shankar et al. 2004; Bai et al. 2006; Deplanche et al. 2010; Rajamanickam et al. 2012; Sunkar and Nachiyar 2012; Das et al. 2014; Wrótniak-Drzewiecka et al. 2014; Rathod et al. 2016; Składanowski et al. 2016; Wypij et al. 2017) (Table 1.2). Bacteria are known to synthesize metallic

Table 1.2 Microbes used for synthesis of various metal nanoparticles

Organism	Type of NPs	Size (nm)	Shape	Reference
Fungi				
Alternaria alternata	Au	12	Spherical, triangular, hexagonal	Sarkar et al. (2012)
Aspergillus sydowii	Au	8.7–15.6	Spherical	Vala (2015)
Cylindrocladium floridanum	Au	19.05	Spherical	Narayanan and Sakthivel (2013)
Trichoderma koningii	Au	10–14	Spheres	Maliszewska (2013)
Penicillium nagiovense AJ12	Ag	25	Spherical	Maliszewska et al. (2014)
Phoma spp.	Ag	5–90	Spherical	Rai et al. (2015a)
Phoma gardeniae	Ag	10–30	Spherical	Rai et al. (2015b)
Neurospora crassa (FGSC 9013)	Ag	1–10	Spherical	Quester et al. (2016)
Fusarium oxysporum	Pt	70–180	Rectangular, triangular, spherical	Govender et al. (2009)
Aspergillus fumigatus	ZnO	1.2–6.8	Spherical, hexagonal	Raliya and Tarafdar (2013)
Candida albicans	Au	5	Spherical	Ahmad et al. (2013)
Pichia jadinii	Au	100	Triangles, hexagons, spheres and rods	Gericke and Pinches (2006)
Yarrowia lipolytica	Au	15	Cristalline	Agnihotri et al. (2009)
Yarrowia lipolytica	Au	7.5–27	Triangular, spherical, hexagonal	Pimprikar et al. (2009)
Yeast strain MKY3	Ag	2–5	Spherical	Kowshik et al. (2003)
Schizosaccharomyces pombe	CdS	–	–	Kowshik et al. (2002)
Torulopsis sp.	CdS	–	–	Kowshik et al. (2002)
Bacteria				
Halomonas salina	Au	30–100	Spherical, triangular	Shah et al. (2012)
Geobacillus sp.	Au	5–50	Quasi-hexagonal	Correa-Llanten et al. (2013)
Escherichia coli	Ag	8–9	Spherical	Mahanty et al. (2013)

(continued)

Table 1.2 (continued)

Organism	Type of NPs	Size (nm)	Shape	Reference
Serratia nematodiphila	Ag	10–31	Spherical and cristalline	Malarkodi et al. (2013)
Pseudomonas putida NCIM 2650	Ag	~70	Spherical	Thamilselvi and Radha (2013)
Pilimelia columellifera subsp. *pallida* SL19 and SL24	Ag	12.7, 15.9	Spherical	Golińska et al. (2015)
Nocardiopsis valliformis OT1	Ag	5–50	Spherical	Rathod et al. (2016)
Streptomyces sp. SH11	Ag	4–24	Spherical	Wypij et al. (2017)
Streptomyces sp. LK3	Ag			Karthik et al. (2014)
Streptomyces yanglinesis NH21	Ag and Au	8.4, 44 and 10	Spherical	Składanowski et al. (2016)
Rhodopseudomonas palustris	CdS	8.01	Crystalline, face-centered cubic	Bai et al. (2009)
Lactobacillus sp.	TiO_2	40–60	Spherical	Prasad et al. (2007)
Rhodobacter sphaeroides	ZnS	8	Spherical	Bai et al. (2006)
Shewanella algae	Pt	5	Elemental	Konishi et al. (2007)
Viruses				
Cowpea mosaic virus	Ni, Co, Pt, Fe, NiFe, CoPt	30.2–34.4	Spherical	Aljabali et al. (2010)
Tobacco mosaic virus	Au	4.78	Spherical	Kobayashi et al. (2012)
Tobacco mosaic virus	Pt	2.6–5	Spherical	Gorzny et al. (2010)

nanoparticles by either intracellular or extracellular mechanisms (Juibari et al. 2011; Abdeen et al. 2014; Golinska et al. 2014; Karthik et al. 2014; Wypij et al. 2017), with extracellular synthesis being the more common pathway. Intracellular reduction of metallic Au ions by the *Rhodococcus* sp. has revealed that Au nanoparticles were predominantly reduced on the cell membrane and cell wall, but not in the cytosol (Abdeen et al. 2014).

1.2.6.3 Synthesis by Viruses

The use of viruses in the synthesis of metallic nanomaterials such as cadmium sulfide (CdS), iron oxide (Fe_2O_3), zinc sulfide (ZnS), gold nanoparticles, and platinum nanotubes has been also reported over fifteen years (Lee et al. 2002; Mao et al. 2003; Gorzny et al. 2010; Kobayashi et al. 2012). An attractive feature of viruses is their dense surface covering of capsid proteins that form a highly reactive surface capable of interacting with metallic ions (Makarov et al. 2014). The capsid proteins can act as attachment points for the deposition of materials (Aljabali et al. 2010; Gorzny et al. 2010; Kobayashi et al. 2012). Love et al. (2014) who studied the influence of tobacco mosaic virus (TMV) on the synthesis of silver and gold nanoparticles showed that low concentrations of TMVs added to Ag or Au salts before mixing with plant extracts of *Nicotiana benthamiana* (round-leaved native tobacco) or *Hordeum vulgare* (Barley) decreased the size and highly increased the yield of the synthesized nanomaterials compared to the non-virus solutions. Their study also revealed that at higher virus concentrations fewer free nanoparticles were formed, and at the same time, the TMV acted as a biotemplate that underwent metallization to form nanowires (Love et al. 2014).

1.2.6.4 Synthesis by Biomolecules

It is claimed that different sugars such as monoses, dioses, and oligoses and proteins, including enzymes, can act as a reducing agents for synthesis of various metallic nanoparticles with different sizes and shapes (Panigrahi et al. 2004; Wangoo et al. 2008; Bar et al. 2009; Tan et al. 2010).

Panigrahi et al. (2004) demonstrated that the presence of fructose during synthesis process affects on the formation of uniform metallic nanoparticles, while glucose and sucrose on production of variable-sized nanoparticles. Authors studied sucrose as a non-reducing sugar for synthesis of silver and gold nanoparticles. The AgNPs were not formed when sucrose and $AgNO_3$ were present in the solution. However, in combination with $HAuCl_4$ as an Au precursor and sucrose, biosynthesized gold nanoparticles were observed. Obtained results suggested that sucrose was hydrolyzed by the chloroauric acid to glucose and fructose. Those compounds were responsible for the bioreduction of nanoparticles (Panigrahi et al. 2004).

The formation of Ag nanoparticles was demonstrated using succinoglycan, which is polysaccharide of *Sinorhizobium meliloti* consisting of one galactose and seven glucose residues (Panigrahi et al. 2004). The proposed mechanism of bioreduction was that one reducing sugar in the succinoglycan provided one electron to reduce Ag^+ to Ag^0. Some authors claimed that the aldehyde group of reducing sugar, which was oxidized to carboxyl group by nucleophile addition of OH^-, was responsible for the reduction of Ag^+ to Ag^0 (Kwon et al. 2009). Shankar et al. (2004) suggested that tetrachloroaurate ions could be bioreduced by the aldehyde or ketone groups of reducing sugars present in the lemongrass extract and lead to Au nanotriangles formation.

Many authors by FTIR analysis observed the amide I and amide II as well as the C–O-stretching bands and amino groups (N-H) on the surface of biosynthesized nanoparticles, which indicate the presence of protein functional groups (Golinska et al. 2015; Wypij et al. 2017). This suggests that Ag nanoparticles can bind to proteins through their free amine groups or carboxylate ions of the amino acid residues. It was reported that amino acids like phenylalanine, tyrosine, tryptophan, and histidine are the key players during synthesis of metallic nanoparticles as well as their stabilization (Das et al. 2009). Highly stable and monodispersed Au nanoparticles were obtained by electrostatic stabilization via surface-bound amino acids (Mandal et al. 2002). Cyclic peptides in the latex of *Jatropha curcas* were used as a bioreducing and biocapping agents to produce Ag nanoparticles (Bar et al. 2009). Similarly, in the presence of glutamic acid as both the reducing and the stabilizing agent, the Au nanoparticle formation was observed (Wangoo et al. 2008). The reduction and binding capabilities of 20 natural amino acids to Au ions were also screened by Tan and Lee (2010). It was found that the reduction process was determined by the extent of complexation between the peptide and metal ions. For synthesis of shape- and size-controlled Au nanoparticles, protein—bovine serum albumin (BSA)—rich with cysteine, tyrosine, and charged residues was used (Carter and Ho 1994; Xie et al. 2007, 2009).

Many authors found that several enzymes such as β-glucosidase (Govindaraju et al. 2011), trypsin (Li and Weng 2010), pepsin (Kawasaki et al. 2011), serrapeptase (Ravindra 2009), and lysozyme (Das et al. 2009) were successfully used for synthesis of gold nanoparticles. Rangnekar et al. (2007) found that the enzymes with free exposed thiol groups were able to catalyze the synthesis of gold nanoparticles when compared with those without free exposed thiol groups.

Similarly, silver nanoparticles were synthesized using various enzymes like lysozyme (Das et al. 2009), fibrinolytic enzyme produced by *Bacillus cereus* NK1 (Deepak et al. 2011), alpha amylase isolated from *Aspergillus oryzae* (Mishra and Sardar 2012). The nanosilver particles synthesized using alpha amylase were monodispersed, 22–44 nm in diameter, and with triangular and hexagonal shape. Moreover, hydrogenase enzyme was used for the fabrication of platinum nanoparticles (Govender et al. 2010).

The use of biomolecules to the biosynthesis leads to the production of high-quality metal nanoparticles (good control of shape and size) which can be use for a wide variety of applications.

1.3 Factors Affecting Biosynthesis of Metal Nanoparticles

There is a number of controlling factors involved in the nucleation and subsequent formation of stabilized metal nanoparticles and their size and shape during biological synthesis process. These factors include pH, reactant concentrations (e.g., dosage of plant biomass or salts), reaction time, and temperature as well as growth in the light or dark, and composition of the culture medium (Joerger et al. 2001; Quester et al. 2016). By establishing the relationship of these factors to the size and shape of nanoparticles, it is possible to produce nanoparticles with desired properties in a controlled manner (Shah et al. 2015).

1.3.1 pH

The pH value of the reaction medium plays a significant role during the formation of nanoparticles (Gardea-Torresdey et al. 1999). It has been reported that varying the pH of the reaction medium leads to the synthesis of nanoparticles with various shape and size. Generally, larger particles were produced at a lower acidic pH values compared to higher pH values. Gurunathan et al. (2009a, b) reported that at acidic pH the size of nanoparticles synthesized from *Escherichia coli* was of 45 nm, whereas at alkaline pH of 15 nm. Gericke and Pinches (2006) obtained different shape morphologies (triangle, hexagons, spheres, and rods) of metallic nanoparticles by modulating the pH of reaction mixture between 3 and 9.

Sathishkumar et al. (2009a) reported that the number of synthesized silver nanoparticles from *Cinnamon zeylanicum* bark extract increased with increasing concentrations of bark extract and at higher pH values (>pH 5). On the other hand, when palladium nanoparticles were synthesized from the bark extract of *Cinnamon zeylanicum* at various pH values, a slight increase in particle size was observed. At pH less than 5, the particles ranged from 15 to 20 nm, meanwhile at pH higher than 5 particles ranged from 20 to 25 nm (Sathishkumar et al. 2009b).

1.3.2 Temperature

This factor is important during any synthesis, including biological synthesis of metal nanoparticles, which determine the size, shape, and yield of biosynthesized nanoparticles (Gericke and Pinches 2006; Song et al. 2009). Generally, reaction rate and particle formation rate appear to become faster when reaction temperature increases; however, the average particle size decreases with increasing temperature (Shah et al. 2015). Some authors demonstrated that at higher temperature of 65 °C the yield of biosynthesized nanoparticles was lower when compared to synthesis process at 35 °C (Riddin et al. 2006). However, Gericke and Pinches (2006) who studied gold nanoparticle synthesis observed that higher temperatures promote the higher formation rate of nanoparticles. At lower temperatures, spherical-shaped Au nanoparticles were predominantly formed, while at higher temperatures rodlike and platelike nanoparticles were formed. It was observed that bigger particles (35 and 50 nm) were biosynthesized at room temperature of 25 °C than at 60 °C (10 and 15 nm) using both, bacteria (*E. coli*) and plants (*Citrus sinensis*) (Gurunathan et al. 2009a, b; Kaviya et al. 2011). However, Song et al. (2009) using leaf extract of *Diospyros kaki* were able to synthesize stable AgNPs over a reaction temperature ranging from 25 to 95 °C.

The size-controlled synthesis of silver nanoparticles by controlling the environment is due to the formation of many seed crystals. At acidic pH and lower temperatures, there will be less nucleation for silver crystal formation on which new incoming silver atoms deposit to form larger sized particles. But as the pH and temperature increase, the dynamics of the ions increase and more nucleation regions are formed due to the availability of −OH ions and increased temperature. The conversion of Ag^+ to Ag^0 increases followed by increase in the kinetics of the deposition of the silver atoms (Rai et al. 2011). However, studies by Quester et al. (2016) did not supported above statement. Authors observed synthesis of smaller nanoparticles (1–6 nm) at low temperature and pH (4 °C and pH 3, respectively) than at 25 °C and pH 6 or 10 (1–10 and 1–13 nm, respectively) using fungal extract of *Neurospora crassa*.

1.3.3 Reagent Concentration

There are reports that the concentration of biomolecules found in plants or fungal extracts can significantly influence the formation of metallic nanoparticles (Chandran et al. 2006; Huang et al. 2007; Quester et al. 2016). It was observed that various amount of *Cinnamomum camphora* or *Aloe vera* leaf extract in the reaction medium significantly influenced the shape of the synthesized silver or gold nanoparticles (Chandran et al. 2006; Huang et al. 2007). When the precursor chloroauric acid was added to increasing concentrations of plant extract, the resulting nanoparticle shape changed from triangular to spherical. The study also found that the carbonyl compounds present in the extract assisted in shaping particle growth. Various extract concentrations modulated particle size between 50 and 350 nm. Quester et al. (2016) demonstrated that ratio of fungal extract to metal precursor ($AgNO_3$) influenced the size of biosynthesized silver nanoparticles. Smaller particles were formed at ratio 1:3 than 1:2, respectively.

It is known that higher concentrations of precursors for biosynthesis of metal nanoparticles such as $AgNO_3$ can be toxic for target organisms. Thus, the concentration of silver ions used for the nanoparticle synthesis should be lesser than concentration defined as a threshold level beyond which the cells die (Schröfel and Kratošová 2011).

Generally, for the synthesis of silver nanoparticles by biomass, the optimum concentration of silver salts that has been applied is 1 mM (Ahamd et al. 2003; Kalimuthu et al. 2008).

1.3.4 Time

Rection time is an important factor for synthesis of metal nanoparticles. Many authors showed various incubation time of reaction mixture essential for synthesis of metal nanoparticles such as silver or gold NPs using biological systems (Ahmad et al. 2003; Golińska et al. 2015; Rai et al. 2015a; Składanowski et al. 2016). Study by Ahmad et al. (2003) revealed that the reaction time to synthesize spherical Ag nanoparticles using *Ananas comosus* (Pineapple) extract observed by color change of reaction mixture was 2 min. However, Dwivedi and Gopal (2010) using *Chenopodium album* leaf extract fabricated spherical Ag and Au nanoparticles within 15 min and up to 2 h. After that time, very few new nanoparticles were produced (Dwivedi and Gopal 2010). Golinska et al. (2015), Składanowski et al. (2016), and Wypij et al. (2017) using various species of actinobacteria showed that time needed for good yield of biosynthesized silver and gold nanoparticles was 2–3 days. Rai et al. (2015a) using three strains of *Phoma* sp. observed synthesis of silver nanoparticles after 20 min of incubation of *Phoma* cell filtrate with $AgNO_3$. Incubation time was found to be one of the factors which effect on nanoparticle size (Quester et al. 2016). Authors demonstrated that bigger nanoparticles were formed from fungal extract after 24 h than after 12 h of incubation time. Similar observations were recorded by Prathna et al. (2011) who studied synthesis of silver nanoparticles from *Azadirachta indica* leaf extract. Authors demonstrated that increase in the reaction time tended to produce particles with bigger size. The reaction time was varied between 30 min and 4 h to produce a change in particle size ranging from 10 to 35 nm (Prathna et al. 2011).

1.4 Conclusion and Future Perspectives

This chapter encompasses the various methods of synthesis of metal nanoparticles which can be fabricated by physical, chemical, or biological approach. The green chemistry approach of using biological entities is in contrast with conventional physical and chemical processes that often are expensive and use toxic materials that have the potential to cause environmental toxicity, cytotoxicity, and carcinogenicity. Moreover, the chemically synthesized nanoparticles require another step for the prevention of aggregation of the particles by using stabilizers.

Biological approach includes either a cell-based system (living organisms or inactivated biomass) or a cell-free system (mixtures of biomolecules from the organisms or metabolic products secreted by the cells) which may be used for the metal nanoparticle synthesis. Bacteria are relatively cheap to cultivate and have a high growth rate compared to other biological systems such as fungi or plants. The another advantage of bacterial systems over plants and fungi is that bacteria are relatively easy for genetic manipulations that can lead to better yield of fabricated nanoparticles. Alternatively, fungi have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate. Many fungi have mycelia that provide a much higher surface area than bacteria, and it can effect on interaction of metal ions and fungal reducing agent, thus enhancing the reduction of ions to metallic nanoparticles. Finally, the use of plants offers a straightforward and clean procedure that does not need any special culture preparation or isolation techniques that are normally required for bacteria- and fungi-based techniques.

Many biosynthesized nanoparticles are identical or similar to the products of conventional chemical synthesis; however, some new architecture has been identified that is not found in chemical synthesis. Similar to chemical and physical methods, the shape and size distribution of biosynthesized metal nanoparticles can be controlled by modifying synthesis conditions, such as time, temperature, pH, or reagent concentration.

Although the diversity of biological entities ranges from microorganisms to plants, the cellular mechanism leading to the biosynthesis of metal nanoparticles is not yet fully understood and need to be studied comprehensively. Further research will therefore focus on the development of a fundamental understanding of the process mechanism on a cellular and molecular level, including isolation and identification of the compounds responsible for the reduction of metal ions. Stabilization and capping mechanisms of nanoparticles must be further investigated as well. The surface chemistry of biogenic nanoparticles should be properly recognized.

Nowadays, study of biological synthesis is focused on searching for a better understanding of the reaction pathways in both the cell-mediated and biomolecule-mediated formation of metal nanoparticles. The general detoxification process of metals by living organisms represents the most likely biological pathway for the reduction and deposition of metal nanoparticles in vivo. Proteins are the most active biomolecules involved in the synthesis of metal nanoparticles, because they can act either directly on the metal (as multifunctional reducing and capping agents) or through a mediated process, such as enzyme catalysis.

Although research in the field of nanotechnology has been dramatically increasing since two decades, biofabrication methods and approaches still need to be developed. This field will require great research efforts from biochemists, physicists, biologists, and materials scientists; however, it shows great potential in the biotechnology sector.

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