Chapter 5
Microbially Synthesized Nanoparticles: Scope and Applications

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Abstract The critical need for development of reliable and eco-friendly processes for synthesis of metallic nanoparticles has recently been realized in the field of nanotechnology. Increasing awareness toward green chemistry and biological processes has elicited a desire to explore environmentally friendly approaches for the synthesis of nanoparticles as a safer alternative to physical and chemical methods, which involves harsh conditions and use of hazardous chemicals. Therefore, the use of natural resources, including bacteria and fungi, has been exploited for cost-effective and environmentally nonhazardous nanoparticle synthesis. The rich microbial diversity of bacteria and fungi contains the innate potential for the synthesis of nanoparticles and may be regarded as potential biofactories. In fact, microbial synthesis of nanoparticles has emerged as an important branch of nanobiotechnology. The synthesis of inorganic materials by biological systems occurs through remarkable processes at ambient temperature and pressures and neutral pH. Among the various biological systems, bacteria are relatively easy to manipulate genetically, whereas fungi have an advantage of easy handling during downstream processing and large-scale production. In spite of the successes achieved in biological synthesis of nanoparticles, there is still a need to improve the rate of synthesis and monodispersity of nanoparticles. Also, microbial cultivation and downstream processing techniques must be improved, and more efficient methods should be developed. Furthermore, in order to exploit the system to its maximum potential, it is essential to understand the biochemical and molecular mechanisms involved in nanoparticle synthesis. Delineation of specific genomic pathways and characterization of gene products involved in biosynthesis of nanoparticles are required. The underlying molecular mechanisms that mediate microbial synthesis of nanoparticles will help in understanding the molecular switches and factors necessary to control the size and shape, as well as crystallinity of nanoparticles. Indeed, biological systems are still relatively unexplored, and therefore, the opportunities are

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open for budding nanobiotechnologists to utilize nonpathogenic biological systems for metallic nanoparticle synthesis with commercial perspectives.

5.1 Introduction

Nanotechnology is a fascinating area that is emerging as a cutting-edge technology encompassing interdisciplinary subjects such as physics, material science, chemistry, biology, and medicine. The prefix "nano" in the term nanotechnology is derived from a Greek word *nanos*, which means "dwarf". It refers to any engineered matter that is one billionth (10^{-9} m) in size and expressed as nanometer (nm) or roughly the length of three atoms side by side. Comparative analysis of nanoparticle size with that of other molecules indicates that the DNA molecule is 2.5 nm wide, a protein is approximately 50 nm in length, and a flu virus is about 100 nm vis-à-vis a human hair, which is approximately 10,000 nm thick. The concept of nanotechnology was first presented by Richard Feynman in 1959 through his famous lecture, at the American Institute of Technology, entitled "There's plenty of room at the bottom". Nanotechnology is a multidisciplinary field that has attracted the attention of material scientists, mechanical and electronics engineers, medical researchers, biologists, physicists, and chemists. With advancements in nanoparticle synthesis, many new applications of nanomaterials are emerging rapidly. In fact, the synthesis of nanoparticles is regarded as a cornerstone of nanotechnology. Developing new methods for nanoparticle synthesis is an active research area. The surge of interest in this field is due to the distinctness of nanoparticles in their physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical, and biological properties as compared with the characteristics of bulk materials (Schmid 1992; Daniel and Astruc 2004).

Diminution of particle size exerts pronounced effects on the physical properties of nanoparticles. The change in their physical properties is due to the large surface area, large surface energy, spatial confinement, and reduced imperfections. Nanoparticles are significantly different from bulk materials owing to their surface plasmon resonance, enhanced Rayleigh scattering, surface enhanced Raman scattering, quantum size effect, and supermagnetism. Therefore, they serve as basic units for next-generation electronics, optoelectronics, and a range of chemical and biochemical sensors, based on their size, shape, and crystallinity (Ramanavicius et al. 2005). Typically, nanoparticles measure 0.1–100 nm in each spatial dimension and are commonly synthesized using top-down and bottom-up strategies (Fendler 1998). In the top-down approach, the bulk materials are gradually broken down to nano-sized materials by machining and etching techniques. In contrast, the atoms or molecules are assembled into molecular structures in the nanometer range in the bottom-up approach, which is commonly applied for chemical and biological synthesis of nanoparticles.

Generally, the methods used for nanoparticle synthesis follow chemical routes that are not environmentally friendly and often generate hazardous by-products,

which could potentially pollute the environment. Chemical synthesis involves conditions such as high temperature, high pressure, and environmental inertness; such synthesis reactions are also cost-intensive (Rao et al. 2003). Furthermore, the use of toxic chemicals and organic solvents during nanoparticle synthesis and their occurrence on the surface of nanoparticles limit their applications. Such drawbacks necessitate the development of clean, biocompatible, nonhazardous, and eco-friendly methods for nanoparticle synthesis. Consequently, biological systems have been focused on and exploited as a preferred, green alternative for synthesis of nanoparticles. In nature, living organisms from bacteria to beetles rely on protein-based nanomachines, which perform excellent jobs from whipping flagella to flexing muscles. Indeed, the molecular machinery evolved by nature surpasses everything that mankind knows and designs with conventional manufacturing technology (Lowe 2000). Undoubtedly, biological systems have a unique ability to control the structure, phase, orientation, and nanostructural topography of inorganic crystals (Cui and Gao 2003).

It is well known that microbes such as bacteria (Beveridge and Murray 1980; Brierley 1990), yeast (Huang et al. 1990), fungi (Frilis and Myers-Keith 1986), and algae (Sakaguchi et al. 1979; Darnall et al. 1986) are capable of adsorbing and accumulating metals. These microorganisms could be used for recovery of metals and reduction of environmental pollution (Klaus et al. 1999; Sharma et al. 2000; Mukherjee et al. 2001a; Nair and Pradeep 2002; Oremland et al. 2004). The potential of microbes to reduce metals has provided another new dimension of "Quantum Dots" or bimetallic nanoparticles with immense use in semiconductor devices (Dameron et al. 1989). A well-known example of reduction of metals includes the magnetotactic bacteria that synthesize magnetic nanoparticles (Schuler and Frankel 1999) with widespread applications (Safarik and Safarikova 2002). Also, lactic acid bacteria in whey of buttermilk exhibit the capability of producing gold–silver composite materials when challenged with a mixture of the two metal ions. Fungi, due to their tolerance to metals and metal bioaccumulation ability, are well-suited for metal nanoparticle generation (Sastry et al. 2003). Based on their enormous biotechnological applications, microorganisms such as bacteria, fungi, and yeast are now regarded as possible eco-friendly "nano-factories" (Ahmad et al. 2002).

Microbial resistance to toxic heavy metals is due to the chemical detoxification and energy-dependent ion efflux from the cell by membrane proteins that function as either ATPase or chemiosmotic cation or proton anti-transporters. The detoxification of metal ions occurs by reduction and/or precipitation of soluble toxic inorganic ions to insoluble nontoxic metal nanoclusters. Such processes could be accomplished by either extracellular biomineralization, biosorption, complexation, precipitation or intracellular bioaccumulation. Microbes produce the inorganic materials either intra- or extracellularly in nanoscale dimensions. In the case of intracellular production, the accumulated particles are of relatively smaller dimension with low polydispersity. Since polydispersity is a major concern for practical commercial nanoparticle synthesis, it is important to optimize the conditions for monodispersity in biological processes (Bao et al. 2003). For controlling the size and shape of biological nanoparticles, genetically engineered microbes capable of

producing specific reducing agents can be developed. Nevertheless, the combinatorial approach such as photobiological methods, as demonstrated in the case of *Fusarium oxysporum*-mediated silver nanoparticle production (Mohammadian et al. 2007), could be helpful in increasing the rate of production. Moreover, there are certain advantages to fungal synthesis of nanoparticles such as (1) economic viability, (2) ease in scale-up as in the thin solid substrate fermentation method, (3) ease in handling biomass, and (4) large-scale secretion of extracellular enzymes. Although biological methods are regarded as safe, cost-effective, sustainable, and environmentally friendly, they still have some drawbacks in terms of culturing of microbes, which is time-consuming and difficult in providing optimal control over nanoparticle size distribution, shape, and crystallinity. However, proper strain selection and optimization of conditions such as pH, incubation temperature and time, concentration of metal ions, and amount of biological material can help in successful implementation of biological and biomimetic approaches for large-scale nanoparticle production for commercial applications.

5.2 Nanoparticle Synthesis by Bacteria

Bacteria are among the most extensively exploited natural resources for synthesis of metallic nanoparticles. The key reason for bacterial preference for nanoparticle synthesis is their relative ease of manipulation. While exploring the secrets of microbial synthesis of nanoparticles, the formation of magnetite particles was documented in magnetotactic bacteria (Lovley et al. 1987; Dickson 1999), siliceous materials by diatoms (Pum and Sleytr 1999), and gypsum and calcium layers by S-layer bacteria (Milligan and Morel 2002). The interactions between metals and microbes have been exploited for various biological applications in the fields of bioremediation, biomineralization, bioleaching, and biocorrosion (Klaus-Joerger et al. 2001). Lately, the microbial synthesis of nanoparticles has emerged as a promising field of research as has nanobiotechnology. The status of research on biosynthesis of some generally studied and commonly used metal nanoparticles by different bacteria, actinomycetes, and cyanobacteria is discussed below.

5.2.1 Silver Nanoparticles

A plethora of methods, namely, photocatalytic reduction (Chang et al. 2006), chemical reduction (Yu 2007), radiation–chemical reduction, metallic wire explosion, sonochemistry, polyol process (Nersisyan et al. 2003), photoreduction (Courrol et al. 2007), reverse micelle-based methods (Xie et al. 2006), matrix chemistry (Ayyad et al. 2010), and biological synthesis (Zeiri et al. 2002; Shahverdi et al. 2007; Durán et al. 2007; Sathishkumar et al. 2009; Kalishwaralal et al. 2010) have been employed for production of silver nanoparticles. Klaus et al. (1999)

reported generation of silver crystals using silver-resistant bacterium *Pseudomonas stutzeri* AG259 isolated from silver mines. This bacterium has been found to generate pyramidal and hexagonal silver nanoparticles measuring up to 200 nm in size, embedded in the organic matrix of the bacterial cell. Similarly, *Morganella* sp. RP-42, an insect midgut isolate, upon exposure to silver nitrate ($AgNO_3$), produced extracellular crystalline nanoparticles measuring 20 ± 5 nm. Three gene homologues (*sil*E, *sil*P, and *sil*S) have been identified in silver-resistant *Morganella* sp. The homologue of *sil*E from *Morganella* sp. showed 99% nucleotide sequence similarity with the previously reported gene, *sil*E, encoding a periplasmic silver-binding protein. Also, the cells of *Corynebacterium sp.* SH09 have been shown to produce silver nanoparticles at 60°C within 72 h on the cell wall in the size range of 10–15 nm with diamine silver complex $[Ag(NH_3)^2]^+$ (Zhang et al. 2005). The silver-binding proteins provide amino acid moieties that serve as nucleation sites for the formation of silver nanoparticles. Silver precipitating peptides (AG3 and AG4) have also been found with the capability of precipitating silver from aqueous solution of silver ions and form face-centered cubic (fcc) structured silver crystals (Naik et al. 2002). Under normal conditions, the small periplasmic silver-binding proteins bind silver at the cell surface and by efflux pumps propels the incoming metals and protects the cytoplasm from metal toxicity (Li et al. 1997; Gupta and Silver 1998). An airborne *Bacillus sp.* reduced Ag^+ ions to Ag^0 and accumulated metallic silver nanoparticles of 5–15 nm size in the periplasmic space of the cell (Pugazhenthiran et al. 2009). Silver nanoparticles of diameter 6.4 nm have also been produced by dried cells of *Aeromonas sp.* SH10, which reduced $[Ag(NH_3)^2]^+$ to Ag^0 within 4 h. These particles were monodispersed and uniform in size and remained stable for more than 6 months without aggregation and precipitation (Mouxing et al. 2006). Culture supernatants of *Enterobacteriaceae* (*Klebsiella pneumoniae, Escherichia coli,* and *Enterobacter cloacae*) also rapidly synthesize silver nanoparticles in sizes ranging from 28.2 to 122 nm with an average size of 52.5 nm by reducing Ag^+ to Ag^0. Addition of piperitone partially inhibited silver ion reduction, which suggested the involvement of nitroreductase enzymes in the reduction process (Shahverdi et al. 2007). Similarly, the culture supernatant of nonpathogenic bacterium *B. licheniformis* has been used for the extracellular synthesis of silver nanoparticles of ~50 nm size (Kalishwaralal et al. 2008). Barud et al. (2008) demonstrated the formation of homogeneous silver containing bacterial cellulose membranes obtained from hydrated membranes of *Acetobacter xylinum* cultures soaked on silver ion with triethanolamine (Ag^+-TAE) solution. Recently, Musarrat et al. (2010) have reported the biosynthesis of silver nanoparticles in the size range of 5–27 nm, produced by an industrially important fungal strain KSU-09, isolated from the roots of date palm (*Phoenix dactylifera*). It has been demonstrated that mycelia-free water extracts obtained from mycelia suspended in water for 72 h facilitated the production of stable, predominantly monodispersed, and spherical nanoparticles upon addition of 1 mM silver nitrate, as determined by UV–visible spectroscopy, XRD, AFM, and TEM (Figs. 5.1–5.4). The infrared spectrum revealed the presence of fungal proteins in the medium, plausibly responsible for nanoparticle stability (Fig. 5.5). Thus, bacteria from the

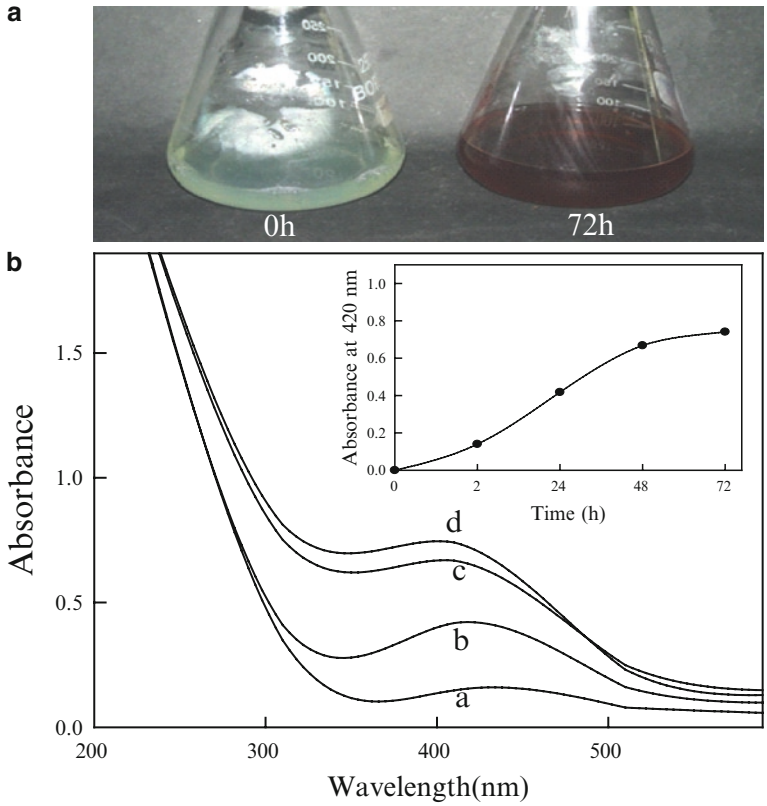

Fig. 5.1 Conical flasks containing fungal biomass in aqueous solution of 1×10^{-4} M $AgNO_3$ at the beginning and after 72 h of reaction. Panel (**b**) shows the UV–Visible absorption spectra of extracellularly synthesized silver nanoparticles at 420 nm exhibiting time-dependent increase in typical SPR bands upon (a) 2 h, (b) 24 h, (c) 48 h, (d) 72 h of incubation. The *inset* shows the change in SPR as a function of time (Adapted from Musarrat et al. 2010.)

environment could be exploited as a natural bioresource for simple, nonhazardous, and efficient synthesis of AgNPs for development of new generation nano-antimicrobials against multidrug-resistant microorganisms with a multitude of applications. This is discussed in a separate section of this chapter.

5.2.2 Gold Nanoparticles

Bacteria have been extensively used for the synthesis of gold nanoparticles. Ahmad et al. (2003a) demonstrated bacterial synthesis of monodispersed gold nanoparticles with extremophilic *Thermomonospora* sp. biomass via reduction of $AuCl_4$ ions through enzymatic processes. Konishi et al. (2004) reported gold

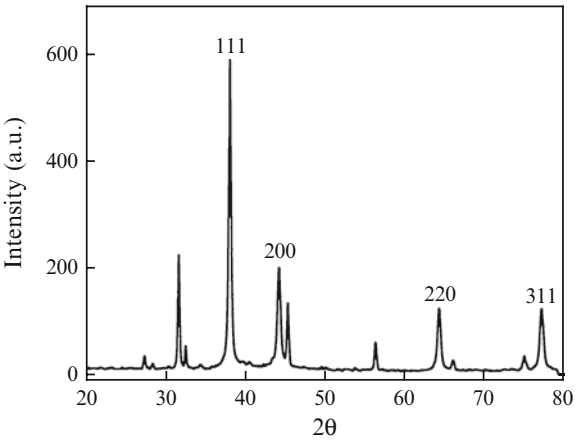

Fig. 5.2 XRD pattern depicting the crystalline nature of silver nanoparticles. Diffraction at 38.5°, 44°, 64.5°, and 72° 2θ indexed to the (111), (200), (220), and (311) planes of the face-centered cubic (fcc) silver, respectively. Particle size based on Scherrer's algorithm was 22 nm (Adapted from Musarrat et al. 2010.)

nanoparticle synthesis using the mesophilic bacterium *Shewanella*, with H_2 as an electron donor. Shiying et al. (2007) showed that the bacterium *Rhodopseudomonas capsulata* produced spherical gold nanoparticles in the range of 10–20 nm, upon incubation of bacterial biomass with aqueous chlorauric acid ($HAuCl_4$) solution at a pH range of 4.0–7.0. Solution pH is an important factor in controlling the morphology of biogenic gold particles and location of gold deposition in cells. Alkalotolerant *Rhodococcus* sp. produced more intracellular monodispersed gold nanoparticles on the cytoplasmic membrane than on the cell wall due to reduction of the metal ions by enzymes present in the cell wall and on the cytoplasmic membrane, but not in the cytosol (Ahmad et al. 2003b). Bacterial cell supernatants of *Pseudomonas aeruginosa* have been used for reduction of gold ions and for extracellular biosynthesis of gold nanoparticles (Husseiny et al. 2007). The exact mechanism leading to reduction of metal ions in organisms has not yet been elucidated. Nevertheless, gel electrophoresis observations revealed the presence of four different proteins ranging from 10 to 80 KDa, which could be responsible for reduction of the chloroaurate ions and capping of the gold nanoparticles. *Bacillus subtilis* 168 has been reported to reduce water-soluble Au^{3+} ions to Au^0 and produce nanoparticles of octahedral morphology and dimensions of 5–25 nm inside cell walls (Beveridge and Murray 1980). Heterotrophic sulfate-reducing bacterial enrichment from a gold mine has been exploited to destabilize gold (I)-thiosulfate complex $Au(S_2O_3)^2$ to elemental gold of 10 nm size in the bacterial envelope, releasing H_2S as an end product of metabolism (Lengke and Southam 2006). *E. coli* DH5α-mediated bioreduction of chloroauric acid to Au^0 resulted in accumulation of nanoparticles, mostly spherical and some triangles and quasi-hexagons, on the cell surface (Du et al. 2007). These cell-bound nanoparticles offer promising

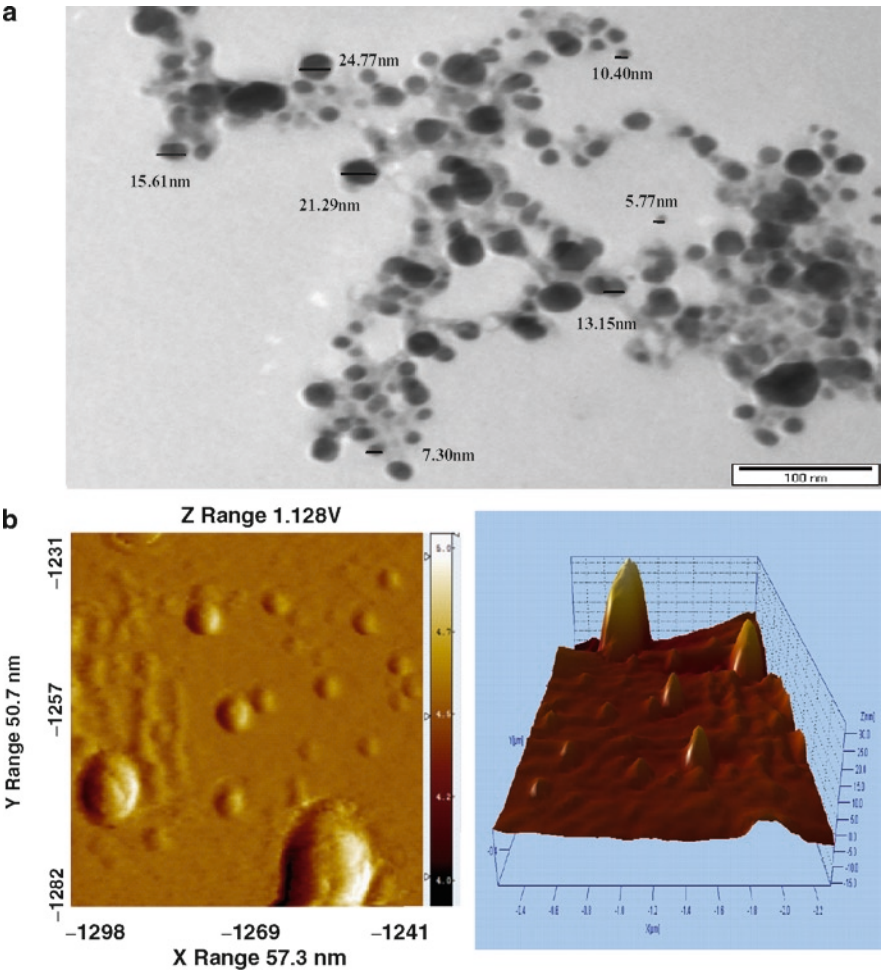

Fig. 5.3 The electron and atomic force microscopic analyses of AgNPs. Panel (**a**) shows the representative transmission electron micrograph recorded from a drop-coated film of the AgNPs produced by fungus on Morgagni™ 268 (d) instrument at a voltage of 80 kV. Panel (**b**) shows the 3D topography of nanoparticles in both the perspective and top views. Scan size is 5×5 μm. The intensity of color in side bar reflects the height of the particles (our unpublished data)

applications in electrochemistry of hemoglobin and other proteins (Du et al. 2007). Bioreduction of trivalent aurum has also been reported in the photosynthetic bacterium *Rhodobacter capsulatus*, which has a higher biosorption capacity of $HAuCl_4$ per gram dry weight in the logarithmic phase of growth. The carotenoids and NADPH-dependent enzymes embedded in the plasma membrane and/or secreted extracellularly have been found to be involved in the biosorption and bioreduction of Au^{3+} to Au^0 on the plasma membrane and also outside the cell (Feng et al. 2007).

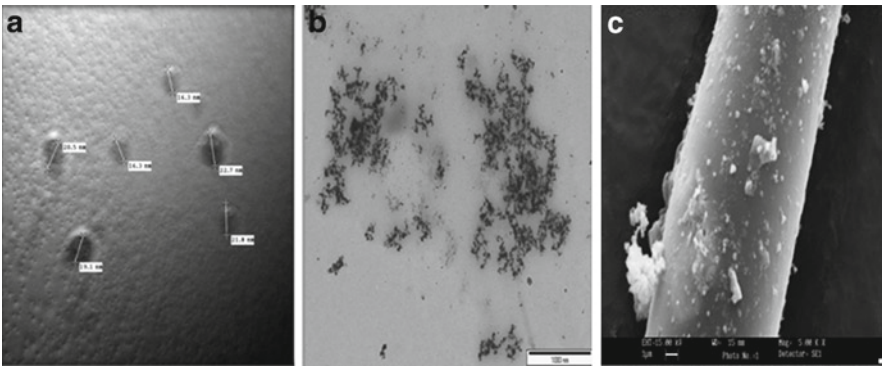

Fig. 5.4 Panel (**a**): Transmission electron-microscopic (TEM) image analysis of extracellularly produced silver nanoparticles in the size range of 16–23 nm, Panel (**b**): TEM image of intracellularly produced silver nanoparticles, Panel (**c**): Scanning electron-microscopic (SEM) image of fungus producing silver nanoparticles (our unpublished data)

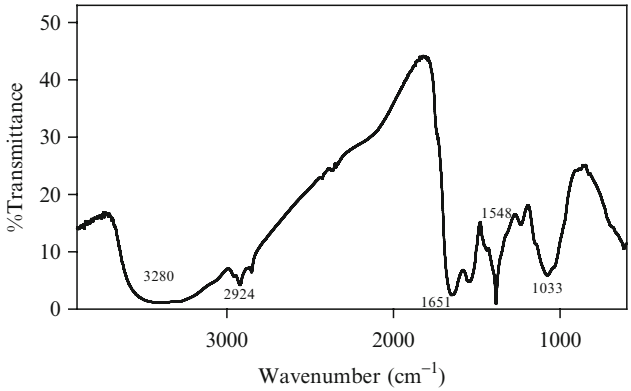

Fig. 5.5 FTIR spectrum of silver nanoparticles synthesized by fungus (our unpublished data)

5.2.3 Magnetic Nanoparticles

The synthesis of magnetic nanoparticles has been widely reported in magnetotactic bacteria, which are Gram-negative bacteria of diverse morphology and occur widely in marine and freshwater sediments. They are known to produce intracellular, membrane-bound magnetite (Blakemore et al. 1979), greigite, and pyrrhotite (Bazylinski et al. 1993). Mann et al. (1984) reported that a microaerophilic bacterium *Aquaspirillum magnetotacticum*, isolated from sediments, produces crystals of ordered single-domain magnetite (Fe_3O_4) particles with octahedral prism morphology of (111) faces truncated by (100) faces. The marine magnetotactic bacterium MV-1, isolated from sulfide-rich sediments of an estuarine salt marsh, anaerobically bioreduced nitrous oxide and ferric quinate to yield iron-rich magnetosomes.

Each magnetite (Fe_3O_4) particle is a parallelepiped with dimensions $40 \times 40 \times 60$ nm, with a single magnetic domain (Bazylinski et al. 1988). Similarly, magnetotactic bacteria isolated from brackish and marine sulfide-rich water and sediments intracellularly deposited single crystals of ferromagnetic iron sulfide, greigite (Fe_3S_4), reportedly associated with nonmagnetic iron pyrite (FeS_2) and aligned in chains. Each chain contains approximately ten nanoparticles measuring 75 nm in size. Most of the particles have irregular shape, whereas some exhibit octahedral and cubo-octahedral symmetry with strong diffraction contrast (Mann et al. 1990). Bacteria such as *Magnetospirillum magneticum* have demonstrated the ability to synthesize fine (50–100 nm) intracellular membrane-bound ferromagnetic particles composed of magnetite (Fe_3O_4) or greigite (Fe_3S_4). These particles are surrounded by an intracellular phospholipid membrane forming structures called magnetosomes (Schuler and Frankel 1999). Each bacterial cell contained from 0 to 45 nanoparticles with polydispersity. Magnetosomes comprise both crystallite and noncrystallite magnetic crystals. In *M. magnetotacticum* (MS-1), magnetite (Fe_3O_4) nanoparticles have been found assembled into single or multiple chains and anchored inside the cell, enabling the bacteria to passively orient themselves along the geomagnetic field. Each nanoparticle assembled in the bacterial phospholipid membrane has a cubo-octahedral crystal structure with a diameter of ~50 nm and magnetic moment of $\sim 6 \times 10^{-17}$ A m^2. Accumulation of magnetic iron mineral crystals into highly ordered chain-like structures was also evidenced in the magnetosomes of *M. gryphiswaldense* (Lang and Schuler 2006). Watson et al. (1999) demonstrated the synthesis of magnetic iron sulfide (FeS) nanoparticles of 2 nm on the surface of sulfate-reducing bacteria. Moreover, Bharde et al. (2005) studied magnetite nanoparticle synthesis by *Actinobacter*, a nonmagnetotactic bacterium. Lee et al. (2004) demonstrated that by manipulating magnetotactic bacteria in fluid using microelectromagnets, the assembly of magnetic nanoparticles inside the cell can be controlled. Furthermore, the multicellular magnetotactic bacterium *Candidatus Magnetoglobus multicellularis* has been reported to interact with the geomagnetic field on the basis of biomineralized magnetic nanocrystals (Perantoni et al. 2009). The magnetite nanoparticles formed by bacteria such as *A. magnetotacticum* (Mann et al. 1984), the magnetotactic bacterium MV-1 (Bazylinski et al. 1988), Sulfate-reducing bacteria (Watson et al. 1999), *M. magnetotacticum* (Lee et al. 2004), and *M. gryphiswaldense* (Lang and Schuler 2006) largely exhibited octahedral prism, parallelepipeds, cubo-octahedral, and hexagonal prism morphologies in the size range of 2–120 nm.

5.2.4 Uranium Nanoparticles

Cell-free extracts of *Micrococcus lactilyticus* have been reported to reduce uranium (VI) to uranium (IV) (Woolfolk and Whiteley 1962). Also, *Alteromonas putrefaciens* grown in the presence of hydrogen as electron donor and U (VI) as electron acceptor reduced U(VI) to U(IV) (Myers and Nealson 1988; Lovley et al. 1989).

Lovley et al. (1991) demonstrated that *G. metallireducens* GS-15, grown anaerobically in the presence of acetate and U(VI) as electron donor and electron acceptor, respectively, reduced soluble U(VI) to insoluble U(IV), oxidizing acetate to CO_2. The Gram-positive sulfate-reducing bacterium *Desulfosporosinus sp.*, isolated from sediments, has been found to reduce U(VI) to U(IV), which is precipitated to yield uraninite (UO_2) crystals of 1.5–2.5 nm size range, coated on the cell surface (Suzuki et al. 2002). Marshall et al. (2006) found that c-type cytochrome (MtrC) on the outer membrane of dissimilatory metal-reducing bacterium *S. oneidensis* MR-1 is involved in the reduction of U(VI), predominantly with extracellular polymeric substance as UO_2-EPS, both in cell suspension and periplasm.

5.2.5 *Cadmium Nanoparticles*

Cadmium is primarily used in the synthesis of particles called quantum dots (QDs), which are semiconductor metalloid-crystal structures of approximately 2–100 nm and containing about 200–10,000 atoms (Smith et al. 2008; Juzenas et al. 2008). Due to their small size, QDs have unique optical and electronic properties that impart the nanoparticles with a bright, highly stable, "size-tunable" fluorescence. The large surface area due to their small size also makes QDs easily functionalized with ligands for site-directed activity. Thus, QDs have potential applications in biological imaging at the cellular level, cancer detection, radio- and chemosensitizing, and targeted drug delivery (Juzenas et al. 2008; Alivisatos 2004; Smith et al. 2008; Hardman 2006). The active center of the QD demonstrated as the core consists of atoms from groups II to VI with CdSe and CdTe, most commonly used for biological applications (Smith et al. 2008). The significant characteristic of QDs is their size-tunable fluorescence. They are significantly brighter than organic fluorophores and far more stable. Since the fluorescence is size-dependent, a single light source can be used for excitation and emission, which is tuned via particle size to various wavelengths spanning the UV, visible, and near and mid-infrared regions of the electromagnetic spectrum. Unlike organic fluorophores, QDs are also much larger, permitting easy addition of targeting groups to the surface of the nanoparticle. CdSe and CdTe are important for optical, bioananalytic, and bioimaging applications, with CdSe fluorescence spanning the visible light region of the spectrum and CdTe utilizing the infrared regions. Since the QDs are hydrophobic, their functionalization with secondary coatings or "capping" materials such as mercaptopropionic acid and polyethylene glycol (PEG) is required to improve solubility and maintain them in a nonaggregated state. These coatings can be further conjugated with targeting molecules such as receptor ligands or antibodies, which guide the QD to a specific tissue or organ (Medintz et al. 2005; Smith et al. 2008). Thus, QDs have the potential to dramatically improve medical therapy with respect to cancer detection and treatment.

Among early reports of intracellular semiconductor nanoparticle synthesis, *E. coli* has been found to accumulate nanocrystals composed of wurtzite crystal in

the size range of 2–5 nm, with spherical and elliptical shapes when incubated with cadmium chloride and sodium sulfide. The production of nanocrystals is reported to be 20-fold higher when the *E. coli* cells are grown to the stationary phase as compared to late logarithmic phase. It has also been found that spherical aggregates of 2–5 nm diameter sphalerite (ZnS) particles are formed within natural biofilms dominated by aerotolerant sulfate-reducing bacteria of the family *Desulfobacteriaceae* (Labrenz et al. 2000). Among semiconductor nanocrystals, CdS synthesized by *C. thermoaceticum*, *Klebsiella pneumoniae* (Smith et al. 1998), and *E. coli* (Sweeney et al. 2004) showed spherical and elliptical shapes in the size range of 2–200 nm. Sharma et al. (2000) isolated a highly cadmium-resistant *Klebsiella planticola* strain Cd-1 from reducing salt marsh sediments. The strain could grow in up to 15 mM $CdCl_2$ under a wide range of NaCl concentrations and at pH values ranging from acidic to neutral. In growth media amended with thiosulfate, the strain precipitates significant amounts of cadmium sulfide (CdS), as confirmed by X-ray absorption spectroscopy. *Klebsiella aerogenes* synthesized CdS crystallites of spherical shape, bound to the cell wall as electron-dense particles in the size range of 20–200 nm, upon exposure to Cd^{2+} in the growth medium. Energy dispersive X-ray analysis has established that cadmium and sulfur occur in a 1:1 ratio (Holmes et al. 1995). Bai et al. (2009) showed that *Rhodopseudomonas palustris*, a purple nonsulfur, photosynthetic bacterium, produced CdS nanocrystals extracellularly at room temperature. TEM and electron diffraction analyses confirmed the spherical distribution of fcc structured nanoparticles of 8.01 ± 0.25 nm size. Cysteine desulfhydrase (C-S lyase) activity has been reported to be responsible for the formation of CdS nanocrystals. The bacterial cellulose isolated from the strain *Gluconoacetobacter xylinus* has also been used in the synthesis of 30-nm CdS nanoparticles (Li et al. 2009).

5.2.6 Selenium Nanoparticles

Considering selenium oxyanions as the electron acceptor, bacteria such as *Sulfurospirillum barnesii*, *B. selenitireducens*, and *Selenihalanaerobacter shriftii* form uniform and stable crystalline extracellular nanoparticles of Se nanoparticles measuring ~300 nm. The spectral properties of nanoparticles differ significantly from that of amorphous Se^0 formed by the chemical oxidation of H_2Se and the vitreous (black) Se^0 formed chemically by reduction of selenite with ascorbate. Oremland et al. (2004) reported the structural and spectral features of selenium nanospheres produced by Se-respiring bacteria. *Stenotrophomonas maltophilia* SELTE02, a strain isolated from rhizospheric soil of selenium hyperaccumulator legume *Astragalus bisulcatus*, showed promising transformation of selenite (SeO_3^{2-}) to elemental selenium (Se^0) and accumulation of selenium granules in either the cell cytoplasm or extracellular space (Gregorio et al. 2005). Also, the facultative anaerobic bacterium, *E. cloacea* SLD1a-1 (Losi and Frankenberger 1997), purple nonsulfur bacterium *Rhodospirullum rubrum* in oxic and anoxic conditions, and *Desulfovibrio*

desulfuricans (Tomei et al. 1995) are reported to bioreduce selenite to selenium both inside and outside the cell. *E. coli* has also been found to deposit elemental selenium both in the periplasmic space and cytoplasm (Gerrard et al. 1974; Silverberg et al. 1976). *P. stutzeri* is also known to aerobically reduce selenite to elemental selenium (Lortie et al. 1992). Recently, Yadav et al. (2008) have showed that *P. aeruginosa* SNT1, isolated from rhizospheric seleniferous soil, biosynthesized nanostructured selenium by biotransforming selenium oxyanions both intracellularly and extracellularly to spherical amorphous allotrophic elemental red selenium. Selenium has photo-optical and semiconducting properties and, therefore, has applications in photocopiers and microelectronic circuit devices.

5.2.7 Titanium, Platinum, and Palladium Nanoparticles

The extracellular culture filtrate of *Lactobacillus* sp. has been shown to produce titanium nanoparticles at room temperature in the form of spherical aggregates ranging in size from 40 to 60 nm (Prasad et al. 2007). Titanium dioxide (TiO_2) nanoparticles are lighter in weight and resistant to corrosion and, therefore, have widespread applications in automobiles, missiles, airplanes, submarines, cathode ray tubes, and in desalting plants, besides a promising role in gene delivery and cancer chemotherapy. TiO_2 nanoparticles also exhibit photocatalytic activities, and therefore, are recommended for use as antibacterial agents, UV protecting agents, water and air purifiers, and in gas sensors and high-efficiency solar cells. Its photoactivity is strongly related to its structure, microstructure, and powder purification. The three known crystalline structures for TiO_2 include the anatase (tetragonal, a = 0.3785 nm, c = 0.9514 nm, band gap = 3.2 eV, which is equivalent to a wavelength of 388 nm), rutile (tetragonal, a = 0.4593 nm, c = 0.2959 nm, band gap = 3.02 eV), and brookite (orthorhombic, a = 0.9182 nm, b = 0.5456 nm, c = 0.5143 nm, band gap = 2.96 eV). The anatase form of TiO_2 has more photocatalytic activity than rutile. The rutile is thermodynamically more stable than anatase and brookite.

The Gram-negative Cyanobacterium *P. boryanum* UTEX 485 has been reported to produce extracellular Pt (II)-organics and metallic platinum nanoparticles with spherical, bead-like chains, and dendritic morphologies in the particle size range of 30–300 nm. Stationary phase culture of metal ion-reducing bacterium *Shewanella algae* in aqueous solution of H_2PtC_{16}, under anaerobic conditions at room temperature and neutral pH, has been shown to reduce $PtCl_6^{2-}$ ions within 60 min to metallic platinum in the presence of lactate as electron donor. Platinum nanoparticles of ~5 nm size have been observed deposited in the periplasmic space between inner and outer membranes of the bacterial cell (Konishi et al. 2007). Also, the sulfate-reducing bacterium *D. desulfuricans* NCIMB 8307 anaerobically bioreduced and biocrystallized palladium (2+) ions to palladium nanoparticles on the cell surface in the presence of formate as an exogenous electron donor within minutes at neutral pH (Yong et al. 2002). De Windt et al. (2005) have demonstrated

that an iron-reducing bacterium, *S. oneidensis* MR-1, reduced Pd(II) to Pd(0) nanoparticles in the presence of lactate as electron donor on the cell wall and within the periplasmic space. This cell-associated nano-bioPd has an application as a catalyst in the dechlorination of polychlorinated biphenyls.

5.3 Nanoparticle Biosynthesis by Actinomycetes

Actinomycetes are generally considered as the primary source for the synthesis of secondary metabolites like antibiotics. However, screening of actinomycetes for their innate potential for nanoparticle synthesis is an area open for further exploration. An extremophilic actinomycete *Thermomonospora* sp. has been reported to synthesize extracellular monodispersed, spherical gold nanoparticles of average size 8 nm (Ahmad et al. 2003a). Fourier transform infrared spectroscopic (FTIR) analysis confirmed the presence of amide (I) and (II) bands of protein as capping and stabilizing agent on the surface of nanoparticles. Furthermore, an alkalotolerant actinomycete *Rhodococcus* sp. accumulated intracellularly gold nanoparticles of 5–15 nm. The available reductases on the cell wall reduced Au^{3+} and accumulated Au^{0} on the cell wall and cytoplasmic membrane.

5.4 Nanoparticle Biosynthesis by Cyanobacteria

The cyanobacterium *Plectonema boryanum* UTEX 485 has been found to produce silver nanoparticles. Also, this filamentous cyanobacterium upon incubation with aqueous $Au(S_2O_3)^2$ and $AuCl_4$ solutions produced cubic gold nanoparticles and octahedral gold platelets, respectively (Lengke et al. 2006a, b). The mechanism of gold bioaccumulation by cyanobacteria from gold (III)-chloride solution suggested that its interaction with cyanobacteria promotes the precipitation of nanoparticles of amorphous gold (I)-sulfide at the cell wall, and finally deposited metallic gold in the form of octahedral (III) platelets (10 nm to 6 μm) near cell surfaces and in solution (Lengke et al. 2006a, b). Some common *Anabaena*, *Calothrix*, and *Leptolyngbya* cyanobacteria have also been found to produce intracellular Au, Ag, Pd, and Pt nanoparticles, which naturally released in the culture medium and stabilized by algal polysaccharides for their easy recovery. Indeed, the size of the recovered particles and yield depend on the cyanobacteria genus (Brayner et al. 2007).

5.5 Nanoparticle Biosynthesis by Yeast

The yeast *Candida glubrata* has been used for the intracellular production of monodispersed spherical and peptide-bound CdS quantum dots measuring 2 nm, by forming a metal–thiolate complex with phytochelatins (Dameron et al. 1989).

Schizosaccharomyces pombe also produced wurtzite-typed hexagonal lattice structured CdS nanoparticles in mid-log phase in the size range of 1–1.5 nm (Kowshik et al. 2002). The synthesis of fcc structured PbS nanocrystallites exhibiting quantum semiconductor properties by yeast *Torulopsis sp.* has been reported (Kowshik et al. 2002). The quantum dots are intracellularly produced in the vacuoles with a dimension of 2–5 nm spherical shape. These nanoparticles are used to fabricate diode heterojunction with poly (*p*-phenylenevinylene). In addition, Baker's yeast, *Saccharomyces cerevisiae*, has been reported to biosorb and reduce Au^{3+} to elemental gold in the peptidoglycan layer of the cell wall by the aldehyde group present in reducing sugars (Lin et al. 2005). Similarly, another yeast, *Pichia jadinii* (*Candida utilis*), intracellularly produced spherical, triangular, and hexagonal gold nanoparticles of 100 nm size within 24 h (Gericke and Pinches 2006). Another tropical marine yeast, *Yarrowia lipolytica* NCIM 3589, produced hexagonal and triangular gold crystals of average size ~15 nm, nucleated on the cell surfaces by reduction of gold ions at pH 2.0. Further, *S. cerevisiae* has been found to produce spherical antimony oxide (Sb_2O_3) nanoparticles in the size range of 2–10 nm at room temperature, exhibiting semiconductor properties. The plausible mechanism could be the radial tautomerization of membrane-bound quinines or by membrane bound/cytosolic pH-dependent oxidoreductases (Jha et al. 2009). Extracellular production of hexagonal silver nanoparticles 2–5 nm in size has also been reported in the silver-tolerant yeast strain MKY3 in the exponential growth phase (Kowshik et al. 2003).

5.6 Nanoparticle Biosynthesis by Fungi

Biosynthesis of metal nanoparticles using fungi such as *F. oxysporum* (Senapati et al. 2004; Bansal et al. 2004, 2005; Kumar et al. 2007), *Colletotrichum* sp. (Shankar et al. 2003), *Trichothecium* sp., *Trichoderma asperellum*, *T. viride*, (Ahmad et al. 2005; Mukherjee et al. 2008; Fayaz et al. 2010), *Phaenerochaete chrysosporium* (Vigneshwaran et al. 2006), *Fusarium solani USM3799* (Ingle et al. 2009), *Fusarium semitectum* (Basavaraja et al. 2008), *Aspergillus fumigatus* (Bhainsa and D'Souza 2006), *Coriolus versicolor* (Sanghi and Verma 2009), *Aspergillus niger* (Gade et al. 2008), *Phoma glomerata* (Birla et al. 2009), *Penicillium brevicompactum* (Shaligram et al. 2009), *Cladosporium cladosporioides* (Balaji et al. 2009), *Penicillium fellutanum* (Kathiresan et al. 2009), and *Volvariella volvacea* (Philip 2009) has been extensively studied. Indeed, fungi are regarded as more advantageous for nanoparticle biosynthesis as compared to other microorganisms because (1) fungal mycelial mesh can withstand flow pressure, agitation, and other conditions in bioreactors compared to bacteria, (2) they are fastidious to grow and easy to handle, and (3) they produce more extracellular secretions of reductive proteins and can easily undergo downstream processing. Moreover, the nanoparticles precipitated outside the cell can be directly used in various applications. The size limit of nanoparticles could be related to the fact that the particles nucleate within the organism. Such nanoparticles could be smaller

compared to extracellularly produced nanoparticles. Mukherjee et al. (2001b) demonstrated the biological synthesis of 20-nm gold nanoparticles using *Verticillium* sp. (AAT-TS-4). TEM analysis of ultrathin sections of fungal mycelia showed mostly spherical forms and few triangles and hexagonal nanoparticles on cell walls and quasi-hexagonal morphology on cytoplasmic membranes. In addition, *Verticillium luteoalbum* has been reported to produce spherical 10-nm gold nanoparticles within 24 h at pH 3.0. However, at pH 5.0, spheres and rods were formed along with triangular and hexagonal morphologies (Gericke and Pinches 2006). *Trichothecium* sp. has also been found to accumulate gold nanoparticles intracellularly (Dastjerdi et al. 2009).

Furthermore, *Verticillium* sp. Biomass, on exposure to aqueous silver nitrate solution, resulted in accumulation of silver nanoparticles beneath the fungal cell surface (Mukherjee et al. 2001a; Senapati et al. 2004). Phoma PT35 and Phoma sp.3.2883 have been shown to selectively accumulate silver nanoparticles (Pighi et al. 1989; Chen et al. 2003). Vigneshwaran et al. (2007) reported that *Aspergillus flavus* accumulated silver nanoparticles 8.9 nm in size on the surface of its cell wall when incubated with silver nitrate solution for 72 h. Since fungi are known to secrete much higher amounts of proteins compared to bacteria, it could be one of the contributory factors for significantly higher productivity of nanoparticles in this biosynthetic approach. In order to elucidate the mechanism of nanoparticle formation, species-specific NADH-dependent reductase, released by *F. oxysporum*, has been used to catalyze the reduction of $AuCl_4$ ions to gold nanoparticles. The trapping of $AuCl_4$ ions on the surface of fungal cells could occur by electrostatic interactions with positively charged lysine residues present in the mycelia cell wall. The gold ions could be reduced by enzymes within the cell wall, leading to aggregation of metal atoms; however, the exact mechanism of formation of the gold nanoparticles is still unknown. It has been suggested that extracellularly produced nanoparticles are stabilized by proteins and other reducing agents secreted by the fungus. Experimental data suggest the association of some high-molecular-weight proteins including the NADH-dependent reductase released by fungal biomass in nanoparticle synthesis and stabilization. Fluorescence emission spectra reveal that the native form of these proteins present in solution as well as bound to the surfaces of nanoparticles remains unaltered and the reduction of metal ions did not significantly influence protein tertiary structure (Macdonald and Smith 1996; Kumar and McLendon 1997).

Proteins isolated from fungal cultures have been successfully used to demonstrate nanoparticle production. For instance, nanocrystalline zirconia has been produced at room temperature by cationic proteins, similar in nature to silicatein, secreted by *F. oxysporum* and was capable of extracellularly hydrolyzing aqueous ZrF_6 ions (Bansal et al. 2004).

Growth conditions play an important role during biosynthesis of nanoparticles. *Trichothecium* sp. biomass under stationary conditions produced extracellular nanoparticles when incubated with gold ions. However, under agitation, the fungus produces intracellular gold nanoparticles. The plausible reason for this could be the release of enzymes and proteins responsible for nanoparticle synthesis in the

medium under stationary conditions and no release under shaking conditions (Ahmad et al. 2005). Bharde et al. (2006) reported the synthesis of magnetic nanoparticles by using *F. oxysporum* and *Verticillium* sp. at room temperature. Both fungi secreted the proteins capable of hydrolyzing iron precursors to form iron oxides extracellularly (Gericke and Pinches 2006). Bhainsa and D'Souza (2006) have reported the production of monodispersed silver nanoparticles within 10 min using *A. fumigatus*. Also, Bansal et al. (2006) demonstrated the production of tetragonal barium titanate ($BaTiO_3$) nanoparticles of <10 nm dimension using *F. oxysporum* under ambient conditions. The ferroelectric properties of these nanoparticles have tremendous potential for revolutionizing the electronics industries with their applications in preparing ultrasmall capacitors and ultrahigh density nonvolatile ferromagnetic memories. Furthermore, Bansal et al. (2005) and Kumar et al. (2007) have reported the synthesis of highly luminescent CdSe quantum dots, and silica and titania nanoparticles using the fungus *F. oxysporum.*

5.7 Scope and Applications of Nanoparticles

Production of inorganic and metal-based nanomaterials has stimulated the development of a new field linking many disciplines of sciences for the quest for different types of nanoparticles with unique properties. Designing and developing novel and affordable techniques for scale-up production of nanomaterials have not only provided an interesting area of study but will also address the expanding human requirements including health safety and environmental issues. In industry, the application of nanomaterials is increasingly adopted, and they will soon replace the harmful or toxic chemicals conventionally used as antimicrobial agents (Mucha et al. 2002). Application of nanoparticles and their nanocomposites offers a sound and relatively safer alternative (Chen et al. 2006; Dimitrov 2006) and, therefore, open up new opportunities for development of antimicrobials. Since ancient times, silver has been most extensively studied and used to fight against infection and prevent spoilage (Rai et al. 2009). It is a safer antimicrobial agent in comparison to certain organic antimicrobial agents (Dastjerdi et al. 2010). Silver has been described as being oligodynamic because of its ability to exert a bactericidal effect on products containing silver, principally due to its antimicrobial activities and low toxicity to human cells (Dastjerdi et al. 2009). Its therapeutic property has been proven against a broad range of microorganisms (Jeong et al. 2005; Lok 2006). Lately, Musarrat et al. (2010) demonstrated the broad-spectrum antimicrobial activity of biosynthesized AgNPs against several human and plant pathogenic bacteria and fungi such as *Shigella dysenteriae* type I, *Staphylococcus aureus*, *Citrobacter* spp., *E. coli*, *P. aeruginosa*, *B. subtilis*, *Candida albicans,* and *F. oxysporum*.

Similarly, ZnO nanoparticles and nanorods have remarkable applications in solar cells, sensors, displays, gas sensors, piezoelectric devices, electroacoustic transducers, photodiodes and UV light emitting devices, sunscreens, gas sensors, UV absorbers, antireflection coatings, photocatalysis, and chemical catalysts

(Pan et al. 2001; Xu and Xie 2003). Gold nanoparticles are also known for their potent antibacterial activity against acne or scurf and have commercial applications in soap and cosmetic industries. They can remove waste materials from skin and control sebum (Park et al. 2006; Zhang et al. 2008). Zhang et al. (2008) have reported Au nanoparticle-mediated growth inhibition of different Gram-positive and Gram-negative bacteria and fungi. Park et al. (2006) loaded gold nanoparticles inside the liposomes, which could be used as a controlled release delivery system.

Nanoparticles have enormous applications in biology and medicine. In a dynamic range of size <100 nm, they could be used as probes attached to peptides, antibodies, or nucleic acids for detection and quantification of molecular reactions in vivo (Niemeyer 2001). The potential for coating nanoparticles with antibodies, collagen, and other substances makes them biocompatible for detection and medical diagnosis. Bruchez et al. (1998) showed that nanoparticle-based fluorescent labeling is superior to the use of conventional fluorophores. Wu et al. (2003) observed that quantum-dot-based immunofluorescent labeling of the cancer marker Her2 is more efficient than normal fluorophores in labeling different target cell-surface receptors, nuclear antigens, the cytoskeleton, and other intracellular organelles. They also demonstrated that bioconjugated colloidal quantum dots were valuable in cell labeling, cell tracking, DNA detection, and in vivo imaging. Zhang et al. (2002) showed that surface modification of superparamagnetite nanoparticles with ethylene glycol and folic acid is effective in facilitating phagocytosis by cancer cells for potential cancer therapy and diagnosis. O'Neal et al. (2004) observed in mice that selective photothermal ablation of tumors using near infrared-absorbing polyethylene-coated gold nanoshells of 130 nm size inhibited tumor growth and increased survival of animals for up to 90 days compared with controls. Moreover, the antibody-coated magnetic iron nanoparticles reported by Perkel (2004) have been proven very effective to heat and virtually burn tumors. Gopalan et al. (2004) reported nanoparticle-based gene therapy using a novel tumor suppressor gene, FUSI, to be effective in systemic gene treatment of lung cancer. Dufes et al. (2005) reported gene therapy by intravenous administration of nanoparticle-based vector systems using tumor necrosis factor (TNF)-α expression plasmid and found increased transgene expression and long-term survival of rats with no toxicity. In vitro studies with breast cancer cells have shown the efficacy of nanoparticle-mediated gene delivery of the wild-type p53 gene. Cancer cells, upon nanoparticle-based gene delivery, exhibited an increased and sustained antiproliferative activity. Kaul and Amiji (2005) observed that PEG-modified gelatin nanoparticles used for tumor-targeted gene delivery have been highly effective, biocompatible, biodegradable, and long-circulating for systemic delivery to solid tumors.

Pathogen detection is another widely explored area in BioMEMS research. Culture and colony counting methods and PCR have been the two conventional and most selective/reliable methods in molecular biology laboratories, although they take hours to days to provide conformity. The emphasis of detection technologies has been moved to BioMEMS/sensor technology because this provides equally reliable results in a fraction of the time employed for conventional methods.

5.8 Conclusions

During the past several years, various methods based on chemical reduction, thermal treatment, irradiation and laser ablation, etc. have been used for synthesis of metal nanoparticles. Most of these methods rely heavily on the use of organic solvents and toxic reducing agents like sodium borohydride and N, N-dimethylformamide, which may pose severe environmental problems and biological risks. Therefore, biological and biomimetic approaches for green synthesis of nanomaterials are now highly appealing, utilizing the potential of bacteria, fungi, and even plants for nanoparticle synthesis as eco-friendly nanofactories. The cell mass and leached components from microorganisms have reportedly been utilized for the reduction of metal ions to nanoparticles, through enzymes such as oxidoreductases and a shuttle quinone extracellular process. Filamentous fungi possess some distinctive advantages over bacteria due to ease of handling, mass cultivation, high metal tolerance, wall-binding capacity, and intracellular metal uptake capabilities. Nanoparticles of noble metals like gold, platinum, palladium, and silver, etc. have attracted scientific attention in recent years due to their unique chemical and physical attributes that differ from the respective bulk substance. The extremely small size and large surface area relative to their volume make them useful for many applications viz. nonlinear optics, spectrally selective coatings for solar energy absorption, optical receptors, catalysis in chemical reactions, biolabelling, and as antibacterials. Thus, the use of biologically compatible materials for nanoparticle synthesis and stabilization could play a crucial role in medical diagnosis and therapeutics including the detection of genetic disorders by color-coded fluorescent labeling of cells using semiconductor quantum dots and cell transfection for gene therapy and drug delivery.

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