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The use of microorganisms for the formation of metal nanoparticles and their application

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Abstract Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. The development of reliable experimental protocols for the synthesis of nanomaterials over a range of chemical compositions, sizes, and high monodispersity is one of the challenging issues in current nanotechnology. In the context of the current drive to develop green technologies in material synthesis, this aspect of nanotechnology is of considerable importance. Biological systems, masters of ambient condition chemistry, synthesize inorganic materials that are hierarchically organized from the nano- to the macroscale. Recent studies on the use of microorganisms in the synthesis of nanoparticles are a relatively new and exciting area of research with considerable potential for development. This review describes a brief overview of the current research worldwide on the use of microorganisms in the biosynthesis of metal nanoparticles and their applications.

Introduction

There is an enormous interest in the synthesis of nanomaterials due to their unusual optical (Krolikowska et al. 2003), chemical (Kumar et al. 2003), photoelectrochemical (Chandrasekharan and Kamat 2000), and electronic (Peto et al. 2002) properties. Impressive advances have been

observed in various aspects such as the synthesis of nanoscale matter and understanding/utilizing their exotic physicochemical and optoelectronic properties. Recent developments in the organization of nanoscale structures into predefined superstructures ensure that nanotechnology will play an increasingly crucial role in many key technologies of the new millennium. It is gaining importance in areas such as catalysis, optics, biomedical sciences, mechanics, magnetics, and energy science. The synthesis of nanomaterials over a range of chemical composition and high monodispersity is still challenging in material science. Several manufacturing techniques that usually employ atomistic, molecular, and particulate processing in a vacuum or in a liquid medium are in use (Daniel and Astruc 2004). Most of the techniques are capital intensive, as well as inefficient in materials and energy use. Hence, there is an ever-growing need to develop clean, nontoxic, and environmentally benign synthesis procedures. Consequently, researchers in nanoparticle synthesis have turned to biological systems for inspiration.

It is well known that many organisms can provide inorganic materials either intra- or extracellularly (Simkiss and Wilbur 1989; Mann 1996). For example, unicellular organisms such as magnetotactic bacteria produce magnetite nanoparticles (Lovley et al. 1987; Spring and Schleifer 1995; Dickson 1999), and diatoms synthesize siliceous materials (Mann 1993; Oliver et al. 1995; Kroger et al. 1999). Multicellular organisms produce hard inorganic-organic composite materials such as bones, shells, and spicules using inorganic materials to build a complex structure (Lowenstam 1981). These biominerals are composite materials and consist of an inorganic component and a special organic matrix (proteins, lipids, or polysaccharides) that controls the morphology of the inorganic compound. The surface layer bacteria produce gypsum and calcium carbonate layers (Pum and Sleytr 1999; Sleytr et al. 1999). Even though many biotechnological applications such as the remediation of toxic metals employ microorganisms such as bacteria (Stephen and Macnaughton 1999) and fungi (Mehra and Winge 1991), such microorganisms are recently found as possible eco-friendly nanofactories

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(Southam and Beveridge 1994; Beveridge and Murray 1980). Processes devised by nature for the synthesis of inorganic materials on nano- and microlength scales have contributed to the development of a relatively new and largely unexplored area of research based on the use of microbes in the biosynthesis of nanomaterials (Sastry et al. 2004).

In this article, we provide a brief overview of the current research worldwide on the use of microorganisms such as bacteria and actinomycetes (both prokaryotes), as well as algae, yeast, and fungi (eukaryotes) in the biosynthesis of metal nanoparticles and their applications.

Bacteria in nanoparticle synthesis

Among the microorganisms, prokaryotic bacteria have received the most attention in the area of biosynthesis of nanoparticles. Early studies reveal that *Bacillus subtilis* 168 is able to reduce Au^{3+} ions to produce octahedral gold particles of nanoscale dimensions (5–25 nm) within bacterial cells by incubation of the cells with gold chloride (Beveridge and Murray 1980; Southam and Beveridge 1994; Fortin and Beveridge 2000) under ambient temperature and pressure conditions. Organic phosphate compounds play a role in the in vitro development of octahedral Au (Southam and Beveridge 1996), possibly as bacteria–Au-complexing agents. Fe(III)-reducing bacteria *Shewanella algae* (Konishi et al. 2004) can reduce Au(III) ions in anaerobic environments. In the presence of *S. algae* and hydrogen gas, the Au ions are completely reduced, which results in the formation of 10- to 20-nm gold nanoparticles.

It is already established that silver is highly toxic to most microbial cells. Nonetheless, several bacterial strains are reported as silver resistant (Silver 2003) and may even accumulate silver at the cell wall to as much as 25% of the dry weight biomass, thus suggesting their use for the industrial recovery of silver from ore material. The silver-resistant bacterial strain *Pseudomonas stutzeri* AG259 accumulates silver nanoparticles, along with some silver sulfide, in the cell where particle size ranges from 35 to 46 nm (Slawson et al. 1992). Larger particles are formed when *P. stutzeri* AG259, isolated from a silver mine, is placed in a concentrated aqueous solution of silver nitrate (50 mM; Klaus et al. 1999). Nanoparticles of well-defined size, ranging from a few to 200 nm or more, and distinct morphology are deposited within the periplasmic space of the bacteria. Cell growth and metal incubation conditions may be the reasons for the formation of different particle sizes. The exact reaction mechanisms leading to the formation of silver nanoparticles by this species of silver-resistant bacteria is yet to be elucidated. The ability of microorganisms to grow in the presence of high metal concentrations might result from specific mechanisms of resistance. Such mechanisms include the following: efflux systems, alteration of solubility and toxicity by changes in the redox state of the metal ions, extracellular complexation or precipitation of metals, and the lack of specific metal transport systems (Silver 1996; Beveridge et al. 1997).

Biocomposites of nanocrystalline silver and the bacteria may be thermally treated to yield a carboneous (cermet) nanomaterial with interesting optical properties for potential application in functional thin-film coating (Joerger et al. 2000; Klaus-Joerger et al. 2001). These properties can be modified by varying the silver loading factor. This type of carboneous material is composed primarily of graphite carbon and up to 5% by weight (of the dry biomass) of silver.

Bacteria not normally exposed to large concentrations of metal ions may also be used to grow nanoparticles. The exposure of *Lactobacillus* strains (Nair and Pradeep 2002), which are present in buttermilk, to silver and gold ions resulted to the large-scale production of metal nanoparticles within the bacterial cells. Moreover, the exposure of lactic acid bacteria present in the whey of buttermilk to mixtures of gold and silver ions can be used to grow alloy nanoparticles of gold and silver.

In addition to gold and silver nanoparticles, there is much attention in the development of protocols for the synthesis of semiconductors (the so-called quantum dots) such as CdS, ZnS, and PbS. These luminescent quantum dots are emerging as a new class of materials for biological detection and cell imaging (Chan et al. 2002), based on the conjugation of semiconducting quantum dots and biorecognition molecules. *Clostridium thermoaceticum* (Cunningham and Lundie 1993) precipitates CdS at the cell surface as well as in the medium from CdCl_2 in the presence of cysteine hydrochloride in the growth medium. Most probably, cysteine acts as the source of sulfide. When *Klebsiella aerogenes* is exposed to Cd^{2+} ions in the growth medium, 20- to 200-nm CdS formed on the cell surface. The formation of CdS is confirmed by quantitative energy dispersive x-ray analysis. The buffer composition of the growth medium plays an important role in forming the cadmium sulfide crystallites. Intracellular CdS nanocrystals, composed of a wurtzite crystal phase, are formed when *E. coli* is incubated with cadmium chloride and sodium sulfide (Sweeney et al. 2004). Nanocrystal formation varies dramatically depending on the growth phase of the cells and increases about 20-fold in *E. coli* grown in the stationary phase as compared with that grown in the late logarithmic phase. In a remarkable investigation, Labrenz et al. (2000) has shown that spherical aggregates of 2- to 5-nm-diameter sphalerite (ZnS) particles are formed within natural biofilms dominated by sulfate-reducing bacteria of the family *Desulfobacteriaceae*. A combination of geochemical and microbial processes leads to ZnS biomineralization in a complex natural system. It is appropriate to mention that the concentration of Zn can be significantly reduced to below-acceptable levels for drinking water with the use of this method.

Magnetic Fe sulfide nanoparticles are synthesized by using sulfate-reducing bacteria where particles having a size of a few nanometers are formed on the surface (Watson et al. 1999), and the magnetic mineral is separated from the solution by a high-gradient magnetic field of 1 T. Bacterially produced iron sulfide is an adsorbent for a wide range of heavy metals and some anions. High adsorption of radio-

active ions occurs due to high surface area (400–500 m²/g). It may provide a suitable matrix for the long-term safe storage of a number of ions vital to the nuclear industry, particularly the pertechnetate ion (TcO⁴⁻; Watson et al. 2001).

Magnetite is a common product of bacterial iron reduction and could be a potential physical indicator of biological activity in geological settings. Single-domain tiny magnetic particles (<12 nm), which exhibit octahedral shapes, are formed exclusively outside of the bacterial cells by a thermophilic fermentive bacterial strain TOR-39 (Zhang et al. 1998). Transition metals such as Co, Cr, and Ni may be substituted (Roh et al. 2001) for magnetic crystals biosynthesized in the thermophilic iron-reducing bacteria *Thermoanaerobacter ethanolicus* (TOR-39) by way of the electrochemical process. The mineralization processes are highly controlled by the magnetotactic bacteria, leading to the formation of uniform, species-specific magnetic nanoparticles. Sometimes, the particles are assembled into single or multiple chains and anchored inside the cell, enabling the bacteria to passively orient themselves along geomagnetic field lines. Interestingly, the magnetotactic bacteria *Magnetospirillum magnetotacticum* (Philipse and Maas 2002) produce single-domain magnetic crystals (Fe₃O₄) that are subsequently assembled into folded chain and flux-closure ring morphologies. The magnetic crystals with large magnetic moments, when constrained to lie on a two-dimensional surface, are responsible for the head-to-tail assembly. Based on the magnetization measurements and the magnitude of the magnetization field, it is established that biogenic magnetite nanoparticles are not superparamagnetic. Magnetic nanoparticles are also assembled into ordered structures when the motion of the magnetic bacteria *M. magnetotacticum* (MS-1) is controlled by applying a magnetic field (Lee et al. 2004). After assembling the bacteria with microelectromagnets, the cellular membranes of the bacteria are removed by cell lysis to leave the biogenic magnetic nanoparticles at desired locations. Different patterns of magnetic structures (Fig. 1a–c) are observed after removing the cellular membrane of trapped MS-1 bacteria. These types of ordered magnetic structures could serve as a system for studying the interactions between closely spaced magnetic nanoparticles. Thus, a different

approach, the combination of biomineralization and micromanipulation, can be a new procedure for growing and assembling nanoparticles into customized structures.

All magnetotactic bacteria contain magnetosomes, which are intracellular structures comprising magnetic iron mineral crystals enveloped by a membrane vesicle. The magnetosome membrane (MM) is most likely a structural entity that anchors the crystal at particular locations in the cell, as well as the locus of biological control over the nucleation and growth of the magnetosome crystals. Knowledge of biochemical and genetic controls on magnetic production is essential to understanding how the magnetotactic bacteria produce magnetosomes and organize them in chains. The biomineralization of magnetosome particles is achieved by a complex mechanism that involves the uptake and accumulation of iron and the deposition of the mineral particle with a specific size and morphology within a specific section provided by the MM (Schuler 1999). Since the MM is thought to be of paramount importance in magnetosome formation, researchers have focused on the role of MM proteins, which occur in the MM but not in the soluble (periplasmic or cytoplasmic) fraction or in the cytoplasmic or outer membranes, in magnetosome synthesis. The *mam* (MM) genes (Grunberg et al. 2001) appear to be conserved in a large gene cluster within several magnetotactic bacteria (*Magnetospirillum* species and strain MC-1) and may be involved in magnetic biomineralization.

The monodispersity of the silver/gold nanoparticles produced either intra- or extracellularly by the abovementioned methodology is not very high and far inferior to that obtained through conventional chemical methods. More by chance than by design, it is observed that alkalothermophilic (extremophilic) actinomycete, *Thermomonospora* sp., when exposed to gold ions, reduces the metal ions extracellularly, yielding gold nanoparticles with much polydispersity (Ahmad et al. 2003a). A complete reduction of the 10⁻³ M aqueous HAuCl₄ solution at pH 9.0 and 50°C results to spherical and reasonably monodisperse nanoparticles (8 nm; Fig. 2). In contrast, intracellular synthesis of gold nanoparticles occurs in alkalotolerant actinomycete *Rhodococcus* sp. (Ahmad et al. 2003b), where particles are more concentrated on the cytoplasmic membrane than on the cell wall.

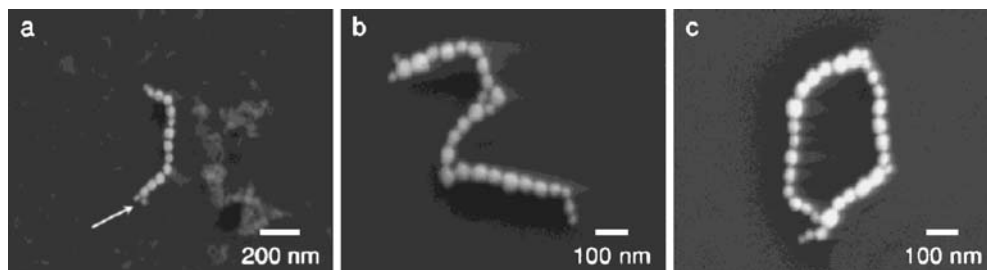
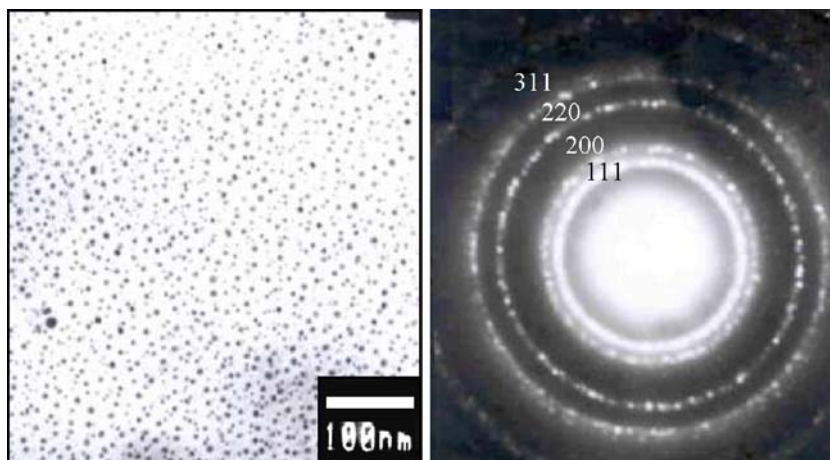


Fig. 1 Scanning electron micrographs of assembled magnetic structures after removing the cellular membrane of trapped bacteria. **a** A single chain of magnetic nanoparticles is shown along with cellular debris. Two small nanoparticles indicated by an *arrow* are still attached to the chain due to the magnetic field from the adjacent

large particle. **b** A long chain from a single bacterium. **c** A ring of magnetic nanoparticles was formed by trapping and lysing two bacteria. (Reproduced with permission from *Nano Lett* 2004, 4, 995–998. Copyright 2004, American Chemical Society)

Fig. 2 TEM micrographs recorded from drop-cast films of the gold nanoparticle solution formed by the reaction of the chloroauric acid solution with *Thermomonospora* sp. biomass for 120 h. Selected area diffraction pattern recorded from the gold nanoparticles (Reproduced with permission from *Langmuir* 2003, 19, 3550–3553. Copyright 2003, American Chemical Society)



There are very few reports regarding alga-mediated synthesis of nanoparticles to date. Suspension of dried cells of alga *Chlorella vulgaris* (Hosea et al. 1986) in the HAuCl_4 solution accumulates elemental gold in the cells. Phytochelation (PC)-coated CdS nanocrystallites formed in

a marine phytoplanktonic alga *Phaeodactylum tricornutum* in response to Cd (Scarano and Morelli 2003; Table 1).

Table 1 Synthesis of nanoparticles by different microorganisms

Microorganisms	Type of nanoparticle
Bacteria	
<i>Bacillus subtilis</i>	Gold
<i>Shewanella algae</i>	Gold
<i>Pseudomonas stutzeri</i>	Silver
<i>Lactobacillus</i>	Gold, silver, Au–Ag alloy
<i>Clostridium thermoaceticum</i>	Cadmium sulfide
<i>Klebsiella aerogenes</i>	Cadmium sulfide
<i>Escherichia coli</i>	Cadmium sulfide
<i>Desulfobacteriaceae</i>	Zinc sulfide
<i>Thermoanaerobacter ethanolicus</i>	Magnetite
<i>Magnetospirillum magnetotacticum</i>	Magnetite
<i>Thermomonospora</i> sp.	Gold
<i>Rhodococcus</i>	Gold
<i>Chlorella vulgaris</i>	Gold
<i>Phaeodactylum tricornutum</i>	Cadmium sulfide
Yeast	
<i>Candida glabrata</i>	Cadmium sulfide
<i>Torulopsis</i> sp.	Lead sulfide
<i>Schizosaccharomyces pombe</i>	Cadmium sulfide
MKY3	Silver
Fungi	
<i>Verticillium</i>	Gold, silver
<i>Fusarium oxysporum</i>	Gold, silver, Au–Ag alloy, cadmium sulfide, zirconia
<i>Colletotrichum</i> sp.	Gold

Yeast in nanoparticle synthesis

It has long been recognized that among the eukaryotes, yeasts are explored mostly in the biosynthesis of the semiconductor nanoparticles. Exposure of *Candida glabrata* (Reese and Winge 1988; Dameron et al. 1989) to Cd^{2+} ions leads to the intracellular formation of CdS quantum dots. The synthesis of PC is activated in the presence of Cd. The structure of PC involves a repeating sequence of γ -glutamyl–cysteine pairs to give polypeptides the general formula $(\gamma\text{-Glu-Cys})_n$ Gly, with n values commonly ranging from 2 to 6. They bind Cd ions immediately, forming Cd–PC complexes that are transported into the vacuole. Then, the complex is degraded and the nanoparticles are formed. *Torulopsis* sp., which was found in an extensive screening program (Kowshik et al. 2002a,b), is capable of synthesizing PbS nanocrystals intracellularly when challenged with Pb^{2+} . Crystallites, which are extracted from the biomass by freeze thawing, exhibit a sharp absorption maximum at ~ 330 nm and are 2–5 nm in size.

Intensive research activity is currently in progress to fabricate electronic devices using organic materials due to their flexibility, easy processing, and large quantum efficiency for light emission. To improve their stability, efficiency, and color tunability for diverse applications, composites of organic materials with nanoparticles, porous silicon, etc., have been explored. So far, chemically synthesized nanoparticles have been used for device applications, but biogenic nanoparticles are yet to be explored for the same applications. Addressing this important issue, Kowshik et al. (2002a,b) have shown that CdS quantum dots synthesized intracellularly in *Schizosaccharomyces pombe* yeast cells exhibit ideal diode characteristics. Biogenic CdS nanoparticles in the size range 1–1.5 nm have been used in the fabrication of a heterojunction with poly(*p*-phenylenevinylene). Such a diode exhibits an approximately 75-mA/cm² current in the

forward bias mode at 10 V, while breakdown occurred at ~15 V in the reverse direction.

Though yeast has been used to synthesize intracellular nanoparticles for several years, very recently, silver nanoparticles have been synthesized extracellularly by a silver-tolerant yeast strain, MKY3 (Kowshik et al. 2003). Particles with a 2- to 5-nm size range are formed when challenged with Ag^+ ions in the log phase of growth.

Fungi in nanoparticle synthesis

The use of fungi in the synthesis of nanoparticles is a relatively recent addition to the list of microorganisms. The use of fungi is potentially exciting since they secrete large amounts of enzymes and are simpler to deal with in the laboratory. However, the genetic manipulation of eukaryotic organisms as a means of overexpressing specific enzymes identified in nanomaterial synthesis would be much more difficult than that in prokaryotes.

An extensive screening process resulted to two genera, which, when challenged with aqueous metal ions such as AuCl_4^- and Ag^+ , yielded large quantities of metal nanoparticles either extracellularly (Mukherjee et al. 2002; Ahmad et al. 2003c) or intracellularly (Mukherjee et al. 2001a,b). The appearance of a distinctive purple color in the biomass of *Verticillium* (inset of Fig. 3b) after exposure to the 10^{-4} M HAuCl_4 solution indicates the formation of gold nanoparticles intracellularly (Mukherjee et al. 2001a) and can clearly be seen in the UV-Visible absorption spectrum recorded from the gold-loaded biomass as a resonance at ~550 nm (Fig. 3a, curve 2). This resonance is

clearly missing in the biomass before exposure to gold ions (Fig. 3a, curve 1) and in the filtrate (dotted line, Fig. 3a) after reaction. Further evidence of the intracellular formation is provided by a transmission electron micrograph (TEM) analysis of the thin sections of the cells after the formation of gold nanoparticles (Fig. 3c,d). In the low-magnification TEM image, a number of *Verticillium* cells can be seen; small particles are organized on the walls of the cells and larger particles are observed within the cells. At higher magnification, the 5- to 200-nm-sized nanoparticles with an average size of 20 ± 8 nm are clearly seen populating both the cell wall and the cytoplasmic membrane of the single cell of the fungi. Furthermore, the powder diffraction pattern recorded from the biofilm (Fig. 3b) indicates the crystalline nature of gold nanoparticles. The Bragg reflections are characteristic of face-centered cubic (fcc) gold structure.

The exposure of *Verticillium* sp. to silver ions resulted to a similar intracellular growth of silver nanoparticles (Mukherjee et al. 2001b). The exact mechanism leading to the intracellular formation of gold and silver nanoparticles by *Verticillium* is not fully understood at the moment. Since the nanoparticles are formed on the surface of the mycelia and not in the solution, it is thought that the first step involves the trapping of the metal ions on the surface of the fungal cells possibly via electrostatic interaction between the ions and the negatively charged carboxylate groups in the enzymes present in the cell wall of the mycelia. Thereafter the ions are reduced by enzymes present in the cell wall leading to the formation of the nuclei, which subsequently grow through the further reduction of metal ions and accumulation of these nuclei. The ability of

Fig. 3 a UV-Vis spectra recorded from biofilms of the *Verticillium* fungal cells before (curve 1) and after exposure to 10^{-4} M aqueous HAuCl_4 solution for 72 h (curve 2). The spectrum recorded from the HAuCl_4 solution after immersion of the fungal cells for 72 h is shown for comparison (dashed line). b X-ray diffraction (XRD) pattern recorded from an Au nano-*Verticillium* biofilm formed on a Si(111) wafer. Inset shows pictures of the *Verticillium* fungal cells after removal from the culture medium (flask on top) and after exposure to the 10^{-4} M aqueous solution of HAuCl_4 for 72 h (flask at bottom). c, d TEM images at different magnifications of the thin sections of the stained *Verticillium* cells after reaction with AuCl_4^- ions for 72 h. (Reproduced with permission from *Angew Chem Int Ed* 2001, 40, 3585–3588. Copyright 2001, Wiley-VCH)

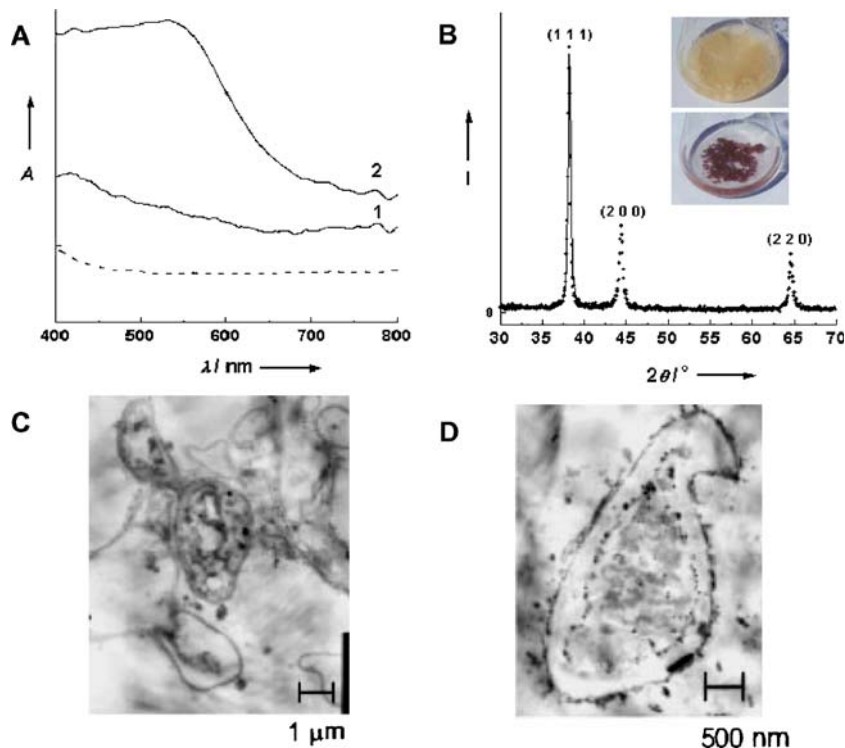
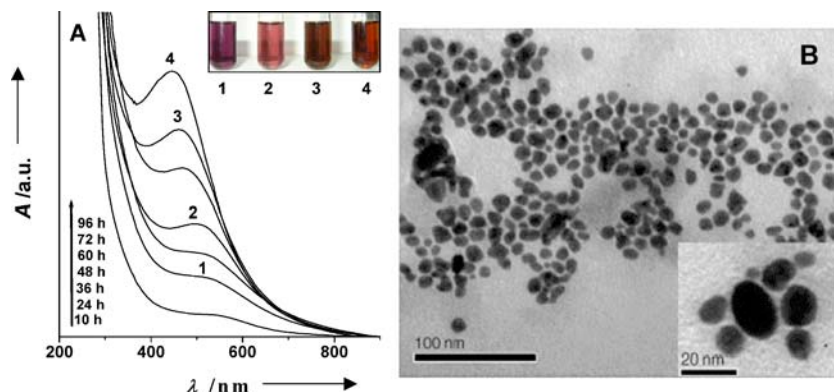


Fig. 4 **a** UV–Vis spectra of Au–Ag alloy nanoparticles, exhibiting increasing Ag mole fraction with time, after the reaction of a mixture of a solution containing 1 mM H₂AuCl₄ and 1 mM AgNO₃ with 60 g *F. oxysporum* wet biomass for 96 h. *Inset* shows test tubes (1–4) containing these diluted colloidal solution. **b** TEM images of Au–Ag nanoparticles. (Reproduced with permission from *Small* 2005, 1, 517–520. Copyright 2005, Wiley-VCH)

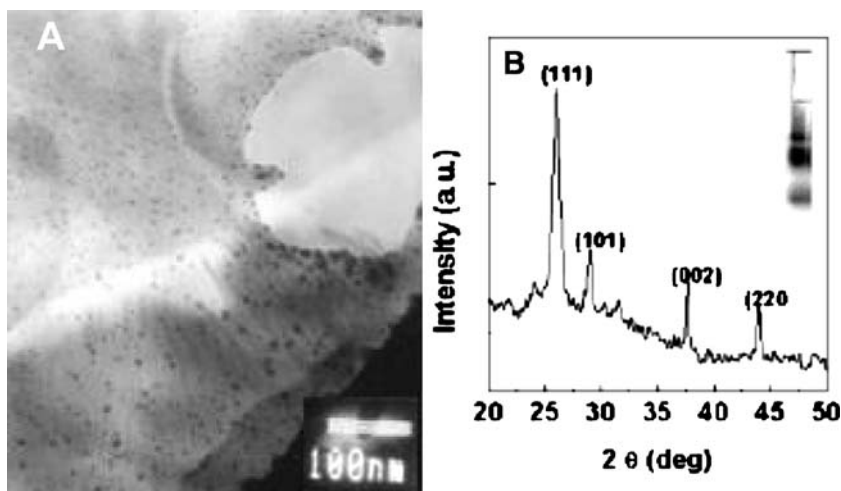


Verticillium cells to multiply after exposing to metal ions proves the capability of using microorganisms in the synthesis of nanomaterials (Mukherjee et al. 2001b).

From the application point of view, it would be imperative to harvest the metal nanoparticles formed within the fungal biomass. It is possible to release the intracellular silver and gold nanoparticles via ultrasound treatment of the biomass–nanoparticles composite or via reaction with suitable detergents. Nonetheless, it would be far more practical if the metal ions exposed to the fungus could be reduced outside the fungal biomass, leading to the formation of metal nanoparticle in the solution. Quite surprisingly, the plant pathogenic fungal strain *Fusarium oxysporum* behaved considerably differently; the reduction of the metal ions occurred extracellularly, resulting in the rapid formation of highly stable gold (Mukherjee et al. 2002) and silver (Ahmad et al. 2003c) nanoparticles of 2- to 50-nm dimensions. Moreover, the aqueous extract of the fungal biomass can reduce gold and silver ions to the corresponding nanoparticles. Most probably, the reduction of the AuCl₄⁻ and Ag⁺ ions occurs due to reductases released by the fungus into the solution, thus opening up a novel fungal/enzyme-based in vitro approach to nanomaterials. The long-term stability of the nanoparticles in the solution may be due to the stabilization of proteins, like cysteine (Gole et al. 2001). Apart from individual metal nanoparticles, the bimetallic Au–Ag alloy can be synthe-

sized by *F. oxysporum*. Very recently, it has been shown that when the biomass of *F. oxysporum* is exposed to equimolar solutions of H₂AuCl₄ and AgNO₃, highly stable Au–Ag alloy nanoparticles of varying mole fractions can be achieved (Senapati et al. 2005). Variation in the amount of biomass reveals that secreted cofactor NADH plays an important role in determining the composition of Au–Ag alloy nanoparticles. The presence of only one plasmon resonance, shifting gradually from nanogold to nanosilver plasmon bands (Fig. 4a), clearly suggests the formation of gold–silver alloy nanoparticles, rather than either a segregated metal or core/shell-type structure. TEM shows well-separated nanoparticles (Fig. 4b) with occasional aggregation in the size range 8–14 nm. An almost-uniform contrast for each particle in the TEM analysis suggests that the electron density is homogeneous within the volume of the particle, which is unlikely in the case of the core/shell structure. Even more exciting is the finding that the exposure of *F. oxysporum* to the aqueous CdSO₄ solution yields CdS quantum dots extracellularly (Fig 5a; Ahmad et al. 2002). The particles are reasonably monodisperse and range in size from 5 to 20 nm. X-ray diffraction analysis of a film of the particles formed on a Si(111) wafer clearly shows that the particles are nanocrystalline with Bragg reflection, which is characteristic of hexagonal CdS (Fig. 5b). Reaction of the fungal biomass with the aqueous CdNO₃ solution for an extended period of time

Fig. 5 **a** Bright-field TEM picture of CdS nanoparticles formed by the reaction of CdSO₄ with the fungal biomass for 12 days. **b** XRD pattern recorded from the CdS nanoparticle film deposited on a Si (111) wafer. *Inset* shows the native gel electrophoresis of the aqueous protein extract obtained from *F. oxysporum* mycelia; 10% (w/v) polyacrylamide slab gel, pH 4.3 (Reproduced with permission from *J Am Chem Soc* 2002, 124, 12108–12109. Copyright 2002, American Chemical Society)



does not yield CdS nanoparticles, indicating the possibility of the release of a sulfate reductase enzyme into the solution. Polyacrylamide gel electrophoresis indicates the presence of at least four protein bands in the aqueous extract of the fungal biomass. Reaction of the protein extract after dialysis (using a dialysis bag with a 3-kDa molecular weight cutoff) with the CdSO₄ solution does not yield CdS nanoparticles. However, the addition of ATP and NADH to the dialysate restores the CdS formation capability of the protein extract. It is believed that the same proteins are also responsible for the reduction of gold and silver ions. Another significant application of this fungus is in the synthesis of zirconia nanoparticles (Bansal et al. 2004), which are a technologically promising oxide. The challenging of the aqueous solution of K₂ZrF₆ to the fungus *F. oxysporum* results to protein-mediated extracellular hydrolysis of the zirconium hexafluoride anions and room temperature formation of crystalline zirconia nanoparticles. An endophytic fungus (*Colletotrichum* sp.) growing in the geranium leaves, when exposed to aqueous chloroaurate ions, produces gold nanoparticles (Shankar et al. 2003) of rodlike and prismatic morphology.

Summary and outlook

In summary, a brief overview of the use of microorganisms such as bacteria, yeasts, algae, fungi, and actinomycetes in the biosynthesis of metal nanoparticles has been depicted. This is an interdisciplinary field “bionanotechnology” that requires the collaboration between physicists, chemists, biologists, and engineers. A case for the serious investigation of microorganisms has been made as a possible alternative to the more popular physical and chemical methods that are currently prevalent. A number of issues need to be addressed from the nanotechnology and microbiology points of view before such biosynthetic procedures can compete with the traditional protocols. The elucidation of biochemical pathways leading to metal ion reduction in the different classes of microbes is necessary to develop a rational microbial nanoparticle synthesis procedure. The surface chemistry of biogenic nanoparticles should be properly recognized. Genetic engineering techniques can potentially be used to improve the particle properties and to control their composition. The shift from bacteria to fungi as a means of developing natural “nanofactories” has the added advantage that downstream processing and handling of the biomass would be much simpler. At present, microbial methods in the synthesis of nanomaterials of varying composition are extreme limited and confined to metal, some metal sulfide, and very few oxides. An extension of the procedures to enable reliable synthesis of nanocrystals of other oxides (TiO₂, ZrO₂, etc.), nitrides, carbides, etc., could make microbial synthesis a commercially feasible proposition.

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