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Contents

Introduction

The field of nanotechnology is an immensely developing field as a result of its wide-ranging applications in different areas of science and technology. It is gaining importance in areas such as catalysis, optics, biomedical sciences, mechanics, magnetics, and energy science. The word nanoparticle can be defined in nanotechnology as a small object that acts as a whole unit in terms of its transport and properties. The word "nano" is derived from a Greek word meaning dwarf or extremely small (Rai et al. 2008). When used as a prefix, it implies 10^{-9}. A nanometre (nm) is one billionth of a metre, or roughly the length of three atoms side by side. A DNA molecule is 2.5 nm wide, a protein approximately 50 nm, and a flu virus about 100 nm. A human hair is approximately 10,000 nm thick. Nanoparticles are usually 0.1–100 nm in each spatial dimension and are commonly synthesized using two strategies: top-down and bottom-up (Fendler 1998; Thakkar et al. 2010). In top-down approach, the bulk materials are gradually broken down to nanosized materials whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in nanometre range. Bottom-up approach is commonly used for chemical and biological synthesis of nanoparticles (Fendler 1998).

Nanoparticles have characteristic physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical, and biological properties (Schmid 1992). Decreasing the dimension of nanoparticles has pronounced effect on the physical properties that significantly differ from the bulk material. These physical properties are caused by their large surface atom, large surface energy, spatial confinement, and reduced imperfections. Nanoparticles have advantages over bulk materials due to their surface plasmon resonance (SPR), enhanced Rayleigh scattering, and surface-enhanced Raman scattering (SERS) in metal nanoparticles, quantum size effect in semiconductors, and supermagnetism in magnetic materials. Therefore, nanoparticles are considered as building blocks of the next generation of optoelectronics, electronics, and various chemical and biochemical sensors (Wong and Schwaneberg 2003).

An important area of research in nanotechnology is the synthesis of nanoparticles of different chemical compositions, sizes, shapes, and controlled dispersities.

The development of reliable experimental protocols for the synthesis of nanomaterials is one of the challenges in nanotechnology. 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. There are a large number of physical, chemical, biological, and hybrid methods available to synthesize different types of nanoparticles (Mohanpuria et al. 2008; Liu et al. 2011). Although various physical and chemical methods are extensively used to produce monodispersed nanoparticles, the stability and the use of toxic chemicals is the subject of paramount concern. The use of toxic chemicals on the surface of nanoparticles and non-polar solvents in the synthesis procedure limits their applications in biotechnological applications and clinical fields. Therefore, development of clean, biocompatible, non-toxic, and eco-friendly methods for nanoparticles synthesis deserves merit. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for fabrication. The biological systems which have shown potential for the fabrication of different nanoparticles are the plants (Shankar et al. 2014), and various microorganisms like fungi (Sastry et al. 2003), bacteria (Joerger et al. 2001), actinomycetes (Sastry et al. 2003), along with other unicellular and multicellular organisms. 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). As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for fabrication. The inherent, clean, nontoxic, and environment friendly ability of these microorganisms to form the metal nanoparticles is particularly important in the development of nanobiotechnology. The interactions between metals and microbes have also been exploited for various biological applications in the fields of bioremediation, biomineralization, bioleaching, and biocorrosion (Klaus et al. 2009) and the microbial synthesis of nanoparticles has emerged as a promising field of research as nanobiotechnology interconnecting biotechnology and nanotechnology.

Nanoparticles produced by a biogenic enzymatic process are far superior, in several ways, to those particles produced by chemical methods. Although chemical methods are able to produce large quantities of nanoparticles with a defined size and shape in a relatively short time, they are complicated, outdated, costly, and inefficient and produce hazardous toxic wastes that are harmful not only to the environment, but also to human health. With biological methods, the use of expensive chemicals is eliminated; it is not as energy intensive as the chemical method and is

also environment friendly. The biological approach is further supported by the fact that the majority of the microorganisms inhabit ambient conditions of varying temperature, pH, and pressure. The particles using biological approaches have higher catalytic reactivity, greater specific surface area, and improved contact between the enzyme and metal salt due to the microbial carrier matrix (Bhattacharya and Mukherjee 2008). During microbial synthesis of nanoparticles, microorganisms grab the target ions from their environment and then turn the metal ions into the element metal through enzymes generated by cellular activities. The process can be classified into two types: intracellular and extracellular synthesis, which corresponds to the location where nanoparticles is formed (Mann 2001). The intracellular method consists of transporting ions into the microbial cell to form nanoparticles in the presence of enzymes. The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes (Zhang et al. 2011).

Biosynthesis of Nanoparticles by Extremophiles

Extremophiles are the most mysterious category of life on the planet. Although nature offers abundant opportunities to life forms that can consume or produce sufficient energy for their survival, normal survival may not be possible in environments that experience extreme conditions (high or low temperature, salinity, pH, pressure, radiation, chemical extremes, lack of nutrition, osmotic barriers, geological scale/barriers, or polyextremity). Due to extraordinary properties, certain organisms can thrive in such extreme habitats. Thus, they are collectively called, extremophiles. Most known extremophiles are microbes. The domain archaea contains renowned examples, but extremophiles are present in numerous and diverse genetic lineages of bacteria and archaeans, and a few eukaryotes. There are many classes of extremophiles that range all around the globe, each corresponding to the way its environmental niche differs from mesophilic conditions. Extremophiles are defined by the environmental conditions in which they grow optimally. The organisms may be described as acidophilic (optimal growth between pH 1 and pH 5); alkaliphilic (optimal growth above pH 9); halophilic(optimal growth in environments with high concentrations of salt); thermophilic (optimal growth between 60 and 80 °C); hyperthermophilic (optimal growth above 80 °C); psychrophilic (optimal growth at 15 °C or lower, with a maximum tolerant temperature of 20 °C and minimal growth at or below 0 °C); piezophilic or barophilic (optimal growth at high hydrostatic pressure); oligotrophic (growth in nutritionally limited environments); endolithic (growth within rock or within pores of mineral grains); and xerophilic (growth in dry conditions, with low water availability). Some extremophiles are adapted simultaneously to multiple stresses (polyextremophile); common examples include thermoacidophiles and haloalkaliphiles. These classifications are not exclusive. Many

extremophiles fall under multiple categories and are classified as polyextremophiles. For example, organisms living inside hot rocks deep under Earth's surface are thermophilic and barophilic such as *Thermococcus barophilus*. A polyextremophile living at the summit of a mountain in the Atacama Desert might be a radioresistant xerophile, a psychrophile, and an oligotroph. Polyextremophiles are well known for their ability to tolerate both high and low pH levels.

The exploration of extremophiles and their biodiversity in order to make them useful for developing processes and products to serve humanity begins with the categorization of these organisms (MacElroy 1974; Rothschild 2007; Tiquia 2010; Tiquia and Mormile 2010; Tiquia-Arashiro and Mormile 2013; Tiquia-Arashiro 2014a, b). Extremophiles are of biotechnological interest, as they produce extremozymes, defined as enzymes that are functional under extreme conditions. Extremozymes are useful in industrial production procedures and research applications because of their ability to remain active under the severe conditions (e.g. high temperature, pressure, and pH) typically employed in these processes. Studies have not even scratched the surface in identifying extremophiles from natural habitats: less than 1 % of the organisms have been identified and even fewer have been sequenced for their beneficial properties. Table 1 summarizes major known extremophiles and their living conditions. However, the identification of the extremophiles thus far has already provided opportunities for industrial, biotechnological, and medical use, as reviewed by several researchers (Adams and Kelly 2009; Niehaus et al. 1999; Tiquia and Mormile 2010; Oren 2012; Gabani and Singh 2013; Pomaranski and Tiquia-Arashiro 2016).

Table 1 Types of extremophiles, selective species, and their living extremities in the environment (Prasanti et al. 2015)

Types of Extremophile	Selective Species	Living extremities
Thermophiles	*Geobacillus thermodenitrificans*; *Thermus aquaticus* (YT-1)	High temperature above 45 °C and up to 121 °C
Halophiles	*Halobacterium* spp.; *Haloferax* spp.; *Haloarcula* spp.; *Halomonas stenophilia B-100*	High salt concentration such as saltern pond brines and natural salt lakes
Acidophiles	*Ferroplasma acidarmanus*	Extreme pH levels; pH level 0
Alkaliphiles	*Alkaliphilus transvaalensis*	pH level 12.5
Psychrophiles	*Polaromonas vacuolata*	<15 °C
Geophiles	*Geobacillus thermoglucosidasius*; *G. stearothermophilus*; *G. thermodenitrificans*; *G. thermopakistaniensis*	Habitats include rich soils as well as keratin-enriched environments
Barophiles	*Moritella* spp.; *Alphaproteobacterium*	High hydrostatic pressure; 80 Mpa

(continued)

Table 1 (continued)

Types of Extremophile	Selective Species	Living extremities
Radiation-resistant	*Deinococcus depolymerans*; *D. guangriensis*; *D. radiodurans*; *D. wulumuqiensis*; *D. xibeiensis*; *D. gobiensis*; *D. gradis*; *D. misasensis*; *Cellulosimicrobium cellulans* (UVP1); *Bacillus pumilus* (UVP4); *B. stratosphericus* (UVR3); *Enterobacter* sp. (UVP3); *Roultella planticola* (UVR1); *Aeromonas eucrenophila* (UVR4); *Arthrobacter mysorens* (UVR5a); *Micrococcus yunnanensis* (UV20HR); *Stenotrophomonas* sp. (YLP1); *Brevundimonas olei* (BR2)	Resistance to survive under ionizing radiation; UVR resistance >600 J m^{-2}

The traditional and most widely used methods for synthesis of metallic NPs use wet-chemical procedures. A typical procedure involves growing nanoparticles in a liquid medium containing various reactants, in particular reducing agents such as sodium borohydride (Kim et al. 2007), potassium bitartrate (Tan et al. 2003), methoxypolyethylene glycol (Mallick et al. 2004), or hydrazine (Li et al. 1999). To prevent the agglomeration of metallic nanoparticles, a stabilizing agent such as sodium dodecyl benzyl sulphate (Li et al. 1999) or polyvinyl pyrrolidone (Tan et al. 2003) is also added to the reaction mixture. Generally, the chemical methods are low cost for high volume; however, their drawbacks include contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by-products. Therefore, the biological approach for synthesis of nanoparticles becomes important. Nanoparticles produced by a biogenic enzymatic process are far superior, in several ways, to those particles produced by chemical methods. Although chemical methods are able to produce large quantities of nanoparticles with a defined size and shape in a relatively short time, they are complicated, outdated, costly, and inefficient and produce hazardous toxic wastes that are harmful not only to the environment, but also to human health. With biological methods, the use of expensive chemicals is eliminated; it is not as energy intensive as the chemical method and is also environment friendly. The biological approach is further supported by the fact that the majority of the microorganisms inhabit ambient conditions of varying temperature, pH, and pressure. The particles using biological approaches have higher catalytic reactivity, greater specific surface area, and improved contact between the enzyme and metal salt due to the microbial carrier matrix (Bhattacharya and Mukherjee 2008). During microbial synthesis of nanoparticles, microorganisms grab the target ions from their environment and then turn the metal ions into the element metal through enzymes generated by cellular activities. The process can be classified into two types: intracellular and extracellular synthesis, which corresponds to the location where nanoparticles is formed

(Mann 2001). The intracellular method consists of transporting ions into the microbial cell to form nanoparticles in the presence of enzymes. The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes (Zhang et al. 2011).

While microorganisms have unique biocatalysing properties as well as the global tendency to employ eco-friendly processes, the biosynthetic process of NPs has some limitations including (1) the potential toxicity of reactants and products against the microorganisms used, (2) costly mass production of microorganisms or cells in sterile conditions, (3) low productivity, and finally, (4) other economic aspects of bioprocesses such as costly culture media and large reaction volume were used. In order to overcome these shortcomings, extremophilic microorganisms capable of growing in physically or geochemically extreme conditions such as high temperature, extreme pH, and ion concentrations could be considered. Their unique properties pinpoint them as ideal biocatalysts for biosynthesis of NPs while offering very promising solution to the challenges faced. These allow their cultivation under non-sterile conditions by using low-cost media leading to the producing of highly valuable chemicals, bioanalytics, and biomolecules such as novel industrial enzymes.

Extremophilic enzymes hold great promise in industrial biotechnology, as they can often be used under nonconventional harsh conditions, which may result in substrate transformations that are not achievable with normal enzymes (Colombo et al. 1995). Compared with chemical catalysis, enzyme-mediated reactions allow for more specific chemo-, regio-, and stereo-selectivity in organic synthesis (Koeller and Wong 2001). However, long-term stability and recyclability of enzymes have been considered the main limitations to their extensive utilization (Schmid et al. 2011). Recently, several nanoparticles have been employed to improve traditional enzyme immobilization methods in order to enhance loading, activity, and stability of enzymes and to reduce the biocatalyst costs in industrial biotechnology (Abad et al. 2005; Yiu and Keane 2012).

This book provides an extensive overview of the current research worldwide on the use of extremophiles in the biosynthesis of metal nanoparticles and their applications. In view of the tremendous industrial potential of producing nanoparticles from extremophiles, the chapter will shed light in the specifics of fermentation media and recovery of nanoparticles from microbiological process using standard microorganisms. Discussions on limitations and challenges further outline the future of extremophilic-mediated technology. Although a number of approaches can be employed for the synthesis of nanomaterials (i.e. chemical, physical, or biological), there is an avoidable need for the use of stabilizing agent during the synthesis process. Excellent stability and homogeneity of nanomaterials can be achieved through the use of stabilizing agents. There are several physico-chemical procedures available for the stabilization of nanomaterials that operate via one of the four principles: electrostatic, steric, electrosteric stabilization, and stabilization by a ligand or a solvent (Roucoux et al. 2002). Although the physico-chemical procedures for stabilization of nanomaterials are well established, they possess a major disadvantage of being capital intensive and hazardous for the

environment as they involve the use of hazardous chemicals and practices. The nanomaterial synthesizing biocomponents have an added advantage of providing excellent stability to the nanomaterial being synthesized. Despite the fact that the machineries and the underlying mechanisms for the synthesis of nanomaterials have been extensively studied by researchers, the stabilizing entities in the nanomaterial synthesis protocols remain unexplored. This book also provides an introductory overview on the stabilization of nanomaterial by biological entities from extremophiles.

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Chapter 1
Nanoparticles Synthesized by Microorganisms

Abstract Microorganisms capable of synthesizing nanoparticles are prevalent microflora of the terrestrial and marine ecosystems. These microorganisms are involved in biogeochemical cycling of metals in processes such as precipitation (biomineralization), decomposition (bioweathering), and degradation (biocorrosion). The biosynthesis of metal NPs by microbes is a function of heavy metal toxicity resistance mechanisms. Resistance mechanisms range from redox enzymes that convert toxic metal ions to inert forms, structural proteins that bind protein, or through the use of efflux proteins that transport metal ions by proton motive force, chemiosmotic gradients, or ATP hydrolysis, which work together to coordinate synthesis nanoparticle synthesis. This chapter focuses on the biological systems; bacteria, fungi, actinomycetes, and algae for utilization in nanotechnology, especially in the development of a reliable and eco-friendly processes for the synthesis of metallic nanoparticles. The rich microbial diversity points to their innate potential for acting as potential biofactories for nanoparticles synthesis.

1.1 Introduction

Nanoparticles (NPs) fall within the size range of 0.1–100 nm and are capable of exhibiting a range of ideal properties such as near identical strength (e.g. resistance to crushing), active surfaces, which have important catalytic properties; and discrete energy levels that can yield some important tailoring of electronic properties (Daniel and Astruc 2004; Kato 2011). While the chemical compositions of NPs are important, the morphology of the NPs (size and shape) and its surface/colloidal properties are equally essential. For example, smaller size NPs are known to have more antimicrobial activity than larger NPs (Chwalibog et al. 2010). With regard to drug delivery, the smaller the NP, the longer it will remain in the circulatory system and therefore have a greater chance of being distributed among the target sites (Gaumet et al. 2008). NPs in general can be synthetically formed or occur naturally (e.g. by microbial biosynthesis) within the environment. In both instances, variations in the morphology of the resulting NPs are common. NPs can be found as

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nanospheres, nanorods, nanocubes, nanoplates, nanobelts, nanotetrapods, and nanoprisms. These can be loosely grouped into face-centered cubic, cuboctahedron, icosahedrons, regular decahedron, star decahedron, marks decahedron, and round decahedrons (Yacaman et al. 2001). Another extremely important aspect of NPs in addition to their morphology is their composition. For instance NPs containing metal ions have many beneficial properties, which are becoming increasingly more common in new technology and processes. For example, a recent review on silver nanoparticles (AgNP) (Sweet and Singleton 2011) covers the usage of AgNP in a wide range of applications including food storage, photonics, information storage, electronic and optical detection systems, therapeutics, diagnostics, photovoltaics, and catalysts. However, despite the significant advantages of NPs being formed with metals such as silver, the challenge of synthetically controlling the shape of metal NPs has met with variable success, making manipulation aimed at a certain size and/or shape of metal NPs difficult.

Microorganisms capable of synthesizing NPs are especially prevalent microflora of the terrestrial and marine ecosystems. It is well known that microbes are involved in biogeochemical cycles of metals in processes such as precipitation (biomineralization), decomposition (bioweathering), and degradation (biocorrosion). Central to each ecological process is the mobilization, distribution, and chemical modification that govern metal speciation and ultimately toxicity (Gadd 2010). As a consequence of these ecological processes, microbes are often subjected to toxic levels of heavy metals, which unless managed, may induce cell death. For example, silver toxicity can occur through interaction with thiol groups of membrane-bound proteins including enzymes involved in respiration, leading to disruption of the cellular membranes, and subsequent disruption of proton motive force through an inability to maintain a proton gradient. This is thought to promote uncoupling of the respiratory chain from oxidative phosphorylation, due to disruption of electron transport (Holt and Bard 2005). Uncontrolled respiration promotes superoxide and hydroxyl radical formation, leading to induction of SOS response and ultimately cell death. Similar metal toxicity responses are observed with other metals, for example, cadmium (Ahmad et al. 2002), and NP biosynthesis would appear to be a common byproduct of metal resistance. The biosynthesis of metal NPs by microbes is a function of heavy metal toxicity resistance mechanisms, whereby toxic heavy metals are converted to nontoxic species and precipitated as metal clusters of nanoscale dimension and defined shape (Narayanan and Sakthivel 2010). Resistance mechanisms range from redox enzymes that convert toxic metal ions to inert forms, structural proteins that bind protein (Gadd 2010), or through the use of efflux proteins that transport metal ions by proton motive force, chemiosmotic gradients, or ATP hydrolysis (Nies 2003). It is proposed that such mechanisms work to coordinate synthesis. This chapter provides an overview of the different types of NPs and the different microorganisms that synthesize them.

The general scheme of the formation of metallic nanoparticles through biosynthesis is shown in Fig. 1.1. The nanoparticles are produced either intracellularly or

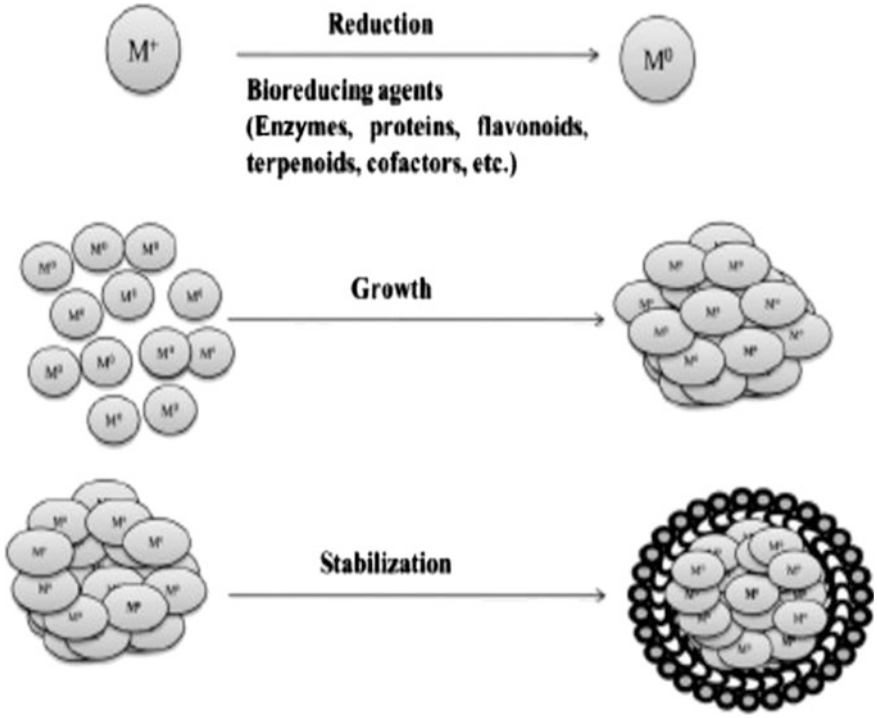

Fig. 1.1 The formation of the metal nanoparticles (Me-NPs) during biosynthesis. *Source* Mittal et al. (2013). Copyright © 2013, Elsevier. Reproduced with permission

extracellularly (Rangarajan et al. 2014). In case of the intracellular synthesis the nanoparticles are produced inside the bacterial cells by the reductive pathways of the cell wall and accumulated in the periplasmic space of the cell. The nanoparticles are produced extracellularly when the cell wall reductive enzymes or soluble secreted enzymes are extracted outside the cell and are involved in the reductive process of metal ions.

One of the enzymes involved in the biosynthesis of metal nanoparticles is the nitrate reductase which reduces the metal ions (Me^{+1}) to the metallic form (Me^{0}). This enzyme is a NADH- and NADPH-dependent enzyme. He et al. (2007) described the hypothetical mechanism for gold nanoparticles biosynthesis carried out by *Rhodopseudomonas capsulate*. These bacteria are known to secrete cofactor NADH- and NADH-dependent enzymes that can be responsible for the biological reduction of Au^{3+} to Au^{0} and the subsequent formation of gold nanoparticles (Fig. 1.2). This reduction is initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier during which the gold ions gain electrons and are therefore reduced to Au^{0}.

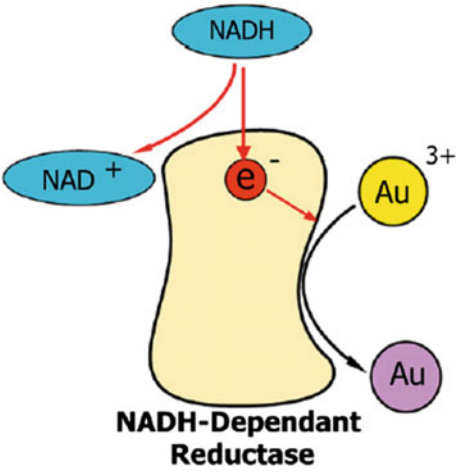

Fig. 1.2 Hypothetical mechanism for silver and gold nanoparticles biosynthesis. *Source* He et al. (2007). Copyright © 2007, Elsevier. Reproduced with permission

1.2 Metallic Nanoparticles

Metallic nanoparticles have fascinated scientist for over a century and are now heavily utilized in biomedical sciences and engineering. They are a focus of interest because of their huge potential in nanotechnology. Metallic nanoparticles have possible applications in diverse areas such as electronics, cosmetics, coatings, packaging, and biotechnology. For example, nanoparticles can be induced to merge into a solid at relatively lower temperatures, often without melting, leading to improved and easy-to-create coatings for electronics applications. Typically, NPs possess a wavelength below the critical wavelength of light. This renders them transparent, a property that makes them very useful for applications in cosmetics, coatings, and packaging. Metallic NPs can be attached to single strands of DNA nondestructively. This opens up avenues for medical diagnostic applications. Nanoparticles can traverse through the vasculature and localize any target organ. This potentially can lead to novel therapeutic, imaging, and biomedical applications. Today these materials can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands, and drugs of interest and thus opening a wide range of potential applications in biotechnology, magnetic separation, and preconcentration of target analytes, targeted drug delivery, and vehicles for gene and drug delivery and more importantly diagnostic imaging (Mody et al. 2010). Some typical metal nanoparticles produced by microorganisms are summarized in Table 1.1.

Table 1.1 Metal nanoparticles synthesized by microorganisms

Microorganism	Type nanoparticle synthesize	Size (nm)	Shape	Reference
Bacteria				
Actinobacter spp.	Magnetite	10–40	Not available	Bharde et al. (2005)
Bacillus lichineformis	Silver	50	Not available	Kalimuthu et al. (2008)
Bacillus cereus	Silver	4–5	Spherical	Babu and Gunasekaran (2009)
Brevibacterium casei	Gold, silver	10–50	Spherical	Kalishwaralal et al. (2010)
Clostridium thermoaceticum	Cadmium sulfide	Not available	Not available	Sweeney et al. (2004)
Corynebacterium glutamicum	Silver	5–50	Irregular	Gurunathan et al. (2009)
Desulfovibrio desulfuricans	Palladium	50	Spherical	Lloyd et al. (1998)
Enterobacter sp.	Mercury	2–5	Spherical	Sinha and Khare (2011)
Escherichia coli	Gold	20–30	Triangles, hexagons	Gericke and Pinches (2006)
Escherichia coli	Cadmium telluride	2-3	Spherical	Bao et al. (2010)
Klebsiella pneumoniae	Gold	5–32	Not available	Malarkodi et al. (2013)
Lactobacillus spp.	Gold, Silver	Not available	Not available	Nair and Pradeep (2002)
Pseudomonas aeruginosa	Gold	15–30	Not available	Husseiny et al. (2007)
Pseudomonas stutzeri	Silver	200 nm	Not available	Klaus et al. (1999)
Pyrobaculum islandicum	Uranium (VI), Technetium (VII), Chromium (VI), Cobalt (III), Manganese (IV)	Not available	Spherical	Kashefi and Lovley (2000)
Rhodococcus sp.	Gold	5–15	Spherical	Ahmad et al. (2003b)
Rhodopseudomonas capsulate	Gold	10–20	Spherical	He et al. (2007)
Shewanella algae	Gold	10–20	Not available	Konishi et al. (2007a)
Shewanella oneidensis	Gold	12–17	Spherical	Suresh et al. (2011)
Shewanella algae	Platinum	5	Not available	Konishi et al. (2007b)

(continued)

Table 1.1 (continued)

Microorganism	Type nanoparticle synthesize	Size (nm)	Shape	Reference
Shewanella sp.	Selenium	181–221	Spherical	Lee et al. (2007)
Thermonospora	Silver	8	Not available	Ahmad et al. (2003a)
Ureibacillus thermosphaericus	Silver	50–70	Not available	Juibari et al. (2011)
Fungi				
Aspergillus flavus	Silver	8–9	Spherical	Vigneshwaran et al. (2007)
Aspergillus fumigatus	Silver	5–25	Spherical	Bhainsa and D'Souza (2006)
Candida utilis	Gold	Not available	Not available	Gericke and Pinches (2006)
Fusarium oxysporum	Silver	5–50	Spherical	Senapati et al. (2004)
Fusarium oxysporum	Silicon	5–10	Spherical	Bansal et al. (2005)
Fusarium oxysporum	Titanium	6–13	Spherical	Bansal et al. (2005)
Neurospora crassa	Gold, silver/gold	32, 20–50	Spherical	Castro-Longoria et al. (2011)
Phaenerochaete chrysosporium	Silver	50–200	Pyramidal	Vigneshwaran et al. (2006)
Trichoderma viride	Silver	5–40	Spherical	Fayaz et al. (2010)
Verticillium luteoalbum	Gold	Not available	Not available	Gericke and Pinches (2006)
Verticillium sp.	Silver	25–32	Spherical	Senapati et al. (2004)
Yarrowia lipolytica	Gold	15	Triangles	Agnihotri et al. (2009)
Algae				
Chlorella vulgaris	Silver	9–20	Not available	Jianping et al. (2007)
Oscillatoria willei	Silver	100–200	Spherical	Mubarak-Ali et al. (2011)
Plectonemaboryanum	Gold	<10–25	Cubic	Lengke et al. (2006a)
Plectonema boryanum UTEX 485	Gold	10–6 μm	Octahedral	Lengke et al. (2006b)
Sargassum wightii	Gold	8–12	Planar	Singaravelu et al. (2007)

(continued)

Table 1.1 (continued)

Microorganism	Type nanoparticle synthesize	Size (nm)	Shape	Reference
Spirulina platenensis	Silver	11.6	Spherical	Mahdieh et al. (2012)
Pterochladia capillacae	Silver	7 (average)	Spherical	El-Rafie et al. (2013)
Jania rubins	Silver	12 (average)	Spherical	El-Rafie et al. (2013)
Ulva faciata	Silver	7 (average)	Spherical	El-Rafie et al. (2013)
Colpmenia sinusa	Silver	20 (average)	Spherical	El-Rafie et al. (2013)

1.2.1 Gold Nanoparticles

Gold nanoparticles (AuNOs) have attracted attention in biotechnology due to their unique optical and electrical properties, high chemical and thermal ability, and good biocompatibility and potential applications in various life sciences related applications including biosensing, bioimaging, drug delivery for cancer diagnosis and therapy (Jiang et al. 2012). Covalently modified gold nanoparticles have attracted a great deal of interest as a drug delivery vehicles. Their predictable and reliable surface modification chemistry, usually through gold-thiol binding, makes the desired functionalization of nanoparticles quite possible and accurate. A variety of therapeutic molecules have been attached in this manner, including various oligonucleotides for gene therapy, bacterial compounds, and anti-cancer drugs. (Jiang et al. 2012). Recently, many advancements were made in biomedical applications with better biocompatibility in disease diagnosis and therapeutics. AuNPs could be prepared and conjugated with many functionalizing agents, such as polymers, surfactants, ligands, dendrimers, drugs, DNA, RNA, proteins, peptides and oligonucleotides. Overall, Au-NPs would be a promising vehicle for drug delivery and therapies. AuNPs can be produced by microorganisms such as bacteria, fungi and algae (Table 1.1).

Bacteria have been used to synthesize AuNPs. For example, microbial synthesis of gold nanoparticles was achieved by Konishi et al. (2007a) using the mesophilic bacterium *Shewanella algae* with H_2 as the electron donor. The authors used varying pH conditions in their study. When the solution pH was 7, gold nanoparticles of 10–20 nm were synthesized in the periplasmic space of *S. algae* cells. Interestingly, when the solution pH was decreased to 1, larger-sized gold nanoparticles (50–500 nm) were precipitated extracellularly (Konishi et al. 2004). In an analogous study, He et al. (2007) showed that the bacteria *Rhodopseudomonas capsulata* produces gold nanoparticles of different sizes and shapes. He et al. incubated *R. capsulata* biomass and aqueous chloroauric acid ($HAuCl_4$) solution at pH values ranging from 7 to 4.16 They found that at pH 7, spherical gold nanoparticles in the

range of 10–20 nm were formed. In contrast, at pH 4, a number of nanoplates were produced (He et al. 2007). In both of these studies, the solution pH was an important factor in controlling the morphology of biogenic gold particles and location of gold deposition. These observations are in line with the findings of Klaus et al. (1999) who observed that variations in incubation conditions lead to variations in particle size. Of note, gold nanoparticles can be used for a variety of applications (e.g., direct electrochemistry of proteins) (Liangwei et al. 2007). The synthesis of gold nanoparticles by two novel strains of *Arthrobacter* sp. 61B and *Arthrobacter globiformis* 151B isolated from basalt rocks in Georgia was studied by Kalabegishvili et al. (2012). Their study has shown that the extracellular formation of nanoparticles took place after 1.5–2 days. They noted that the concentration of gold particles accumulated by increases in bacterial biomass. Gericke and Pinches (2006) reported intracellular gold production by *Pseudomonas stutzeri* NCIMB 13420, *Bacillus subtilis* DSM 10 and *Pseudomonas putida* DSM 291. He et al. (2007) demonstrated that the bacterium *Rhodopseudomonas capsulata* is capable of producing gold particles extracellularly. The gold nanoparticles produced are stable in solution. In the study conducted by Malarkodi et al. (2013), the extracellular biosynthesis of gold nanoparticles is achieved by an easy biological procedure using *Klebsiella pneumoniae* as the reducing agent. After exposing the gold ions to *K. pneumoniae,* rapid reduction of gold ion is observed and leads to the formation of gold nanoparticles in colloidal solution (Figs. 1.3 and 1.4). The method exploits a

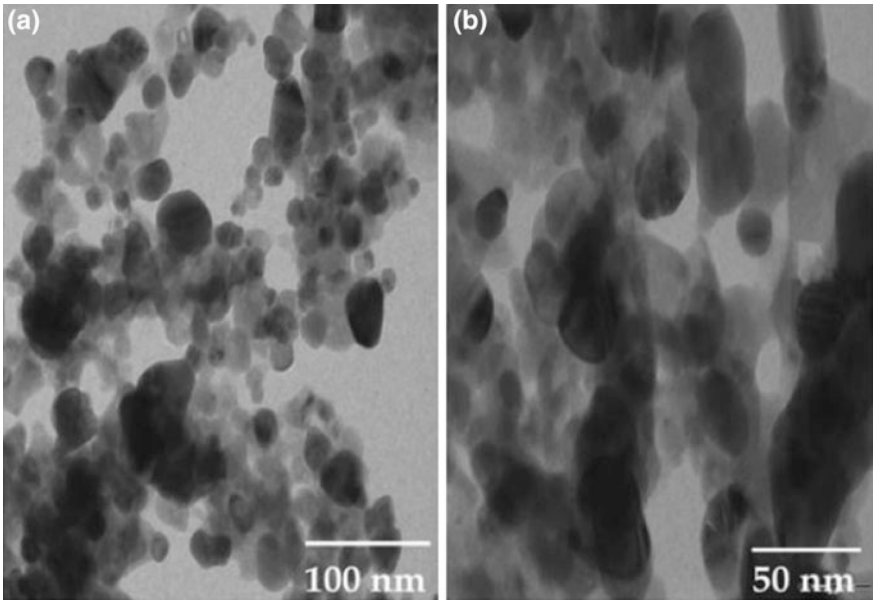

Fig. 1.3 Transmission electron microscopy and photo of gold nanoparticles prepared by using *K. pneumoniae* **a** 100 nm and **b** 50 nm. TEM images of gold nanoparticles using *K. pneumoniae* **a** and **b**. *Source* Malarkodi et al. (2013). Copyright © 2013, Springer. Reproduced with permission

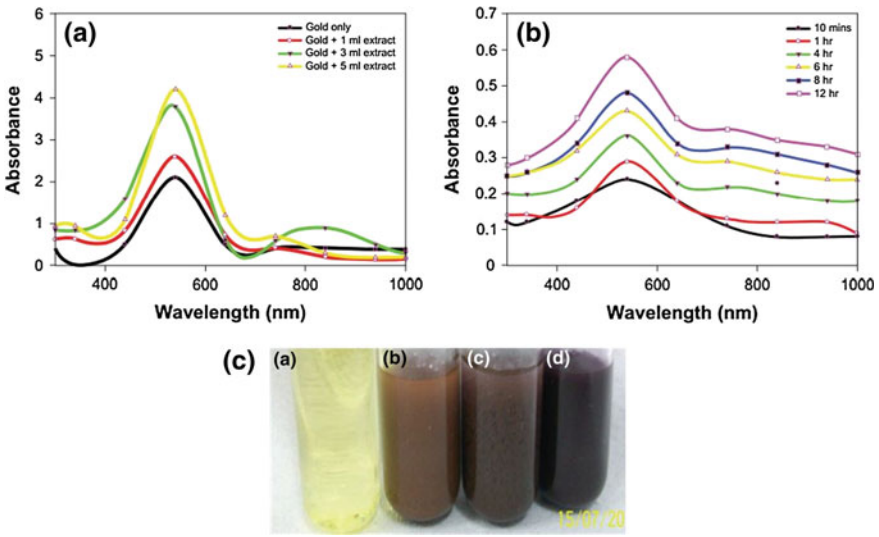

Fig. 1.4 **a** Ultraviolet-visible-near infrared spectra of gold nanoparticles synthesized by exposing various amounts of *Candida albicans* cytosolic extract to a fixed volume (5 mL) of $HAuCl_4$ solution (10–3 M), keeping the final volume (10 mL) of reaction mixture for 24 h. **b** Representative ultraviolet-visible-near infrared spectra depicting kinetics of the reaction of 1 mL of *C. albicans* cytosolic extract with 10 mL of aqueous $HAuCl_4$ solution for specified time periods. The incubation mixture was scanned in the ultraviolet range to analyze characteristic peaks. **c** Color development as a function of surface plasmon resonance in *C. albicans* cytosolic extract-mediated synthesis of gold nanoparticles. (*a*) $HAuCl_4$ aqueous solution, (*b*) Incubation of 5 mL of $HAuCl_4$ aqueous solution with 1 mL of *C. albicans* cytosolic extract keeping the final volume of reaction mixture at 10 mL, (*c*) Incubation of $HAuCl_4$ aqueous solution (5 mL) with 3 mL of *C. albicans* cytosolic extract, making the final volume of reaction mixture 10 mL by adding 2 mL of deionized water, (*d*) 5 mL of *C. albicans* cytosolic extract incubated with 5 mL of aqueous $HAuCl_4$ solution. *Source* Chauhan et al. (2011). Copyright © 2011, Dovepress. Reproduced with permission

cheap and easily available biomaterial for the synthesis of metallic nanoparticles (Malarkodi et al. 2013).

Fungi are taking the center stage of studies on biological generation of metallic nanoparticles because of their tolerance and metal bioaccumulation ability (Sastry et al. 2003). A distinct advantage of using fungi in nanoparticle synthesis is the ease in their scale-up (e.g., using a thin solid substrate fermentation method). Given that fungi are extremely efficient secretors of extracellular enzymes, it is thus possible to easily obtain large-scale production of enzymes. Further advantages of using a fungal-mediated green approach for synthesis of metallic nanoparticles include economic viability and ease in handling biomass. However, a significant drawback of using these bio-entities in nanoparticles synthesis is that the genetic manipulation of eukaryotic organisms as a means of overexpressing specific enzymes (e.g. the ones identified in synthesis of metallic nanoparticles) is relatively much more difficult than that in prokaryotes. Sastry and coworkers have reported the

extracellular synthesis of gold nanoparticles by fungus *Fusarium oxysporum* (Mukherjee et al. 2002). They reported the intracellular synthesis of gold nanoparticles by fungus *Verticillium* sp. as well (Mukherjee et al. 2001a). The extracellular and intracellular biosynthesis of gold nanoparticles by fungus *Trichothecium* sp. was reported by Absar et al. (2005). The gold ions react with the *Trichothecium* sp. fungal biomass under stationary conditions, resulting in the rapid extracellular production of nanoparticles; reaction of the biomass under shaking conditions resulted in intracellular production of the gold nanoparticles. Chauhan et al. (2011) reported biogenic synthesis of gold nanoparticles using cytosolic extract of *Candida albicans*. The study revealed that the shape and the size of nanoparticles formed govern the characteristic features of their spectra (Figs. 1.5 and 1.6). This technique can be extended for rapid, specific, and cost-effective detection of various cancers, hormones, pathogenic microbes, and their toxins if a specific antibody is available.

There are few reports of algae being used as a "biofactory" for synthesis of metallic nanoparticles. Singaravelu et al. (2007) adopted a systematic approach to study the synthesis of metallic nanoparticles by *Sargassum wightii.* This is the first

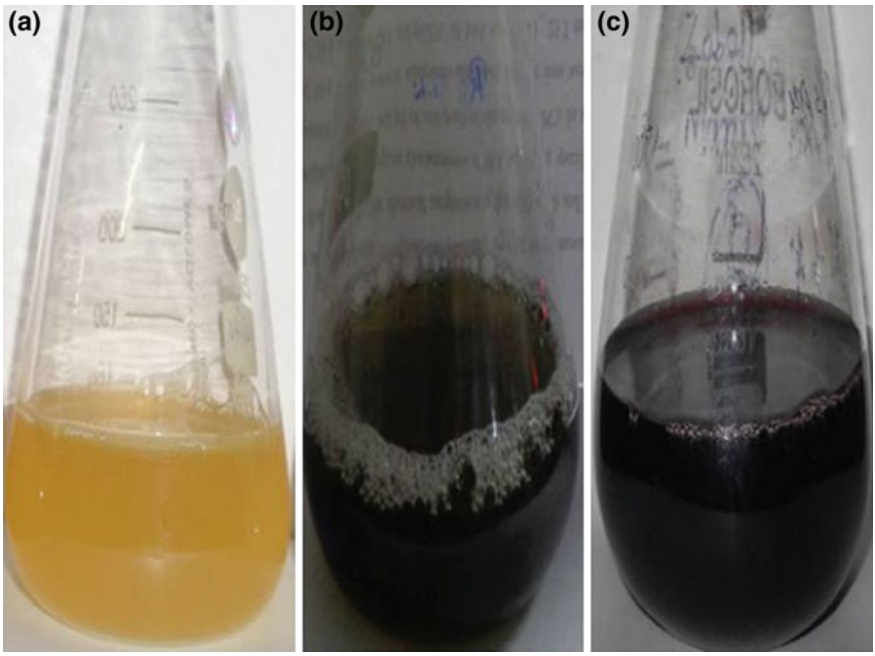

Fig. 1.5 Extracellular synthesis of *K. pneumoniae* biomass and gold chloride mixed with biomass. *K. pneumoniae* biomass (**a**), 1 mM gold chloride mixed with biomass at the beginning of reaction showing a greenish-brown color change (**b**), and after 1 day of reaction showing a dark-purple in color change (**c**). *Source* Malarkodi et al. (2013). Copyright © 2013, Springer. Reproduced with permission

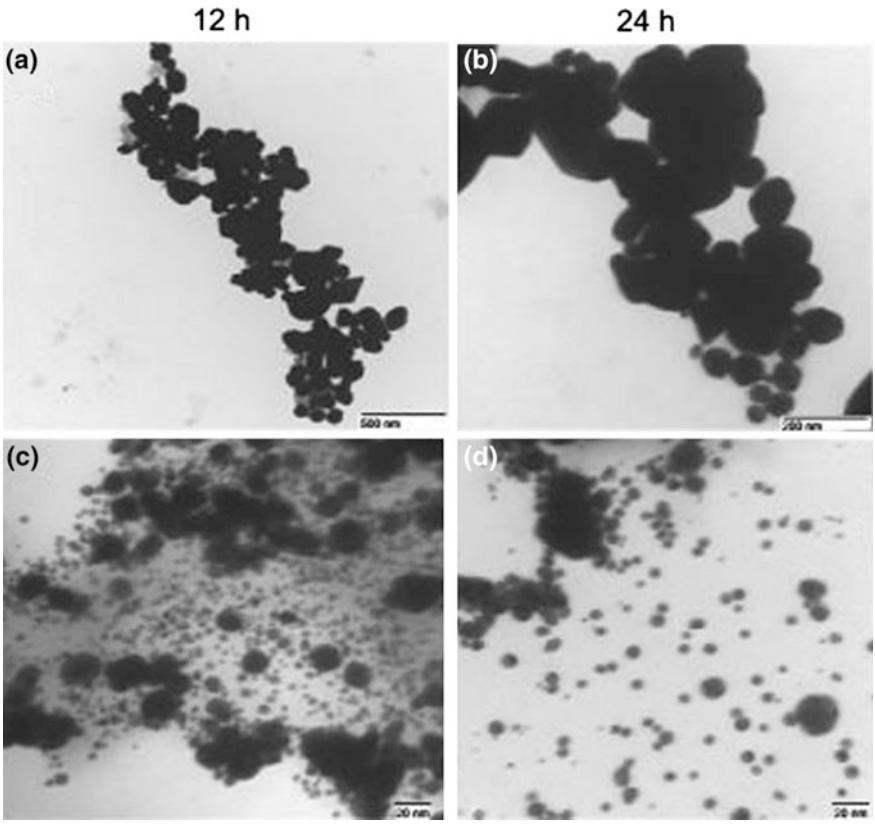

Fig. 1.6 Representative transmission electron micrographs of gold nanoparticles synthesized using various amounts of *Candida albicans* cytosolic extract. Transmission electron micrographs of gold nanoparticles generated upon incubation of 5 mL of $HAuCl_4$ (10−3 M) with 1 mL of *C. albicans* cytosolic extract and making up final volume of reaction mixture (10 mL) using deionized water for **a** 12 h and **b** 24 h. **c** and **d** represent transmission electron microscopic images of nanoparticles obtained as a result of reduction of 5 mL of $HAuCl_4$ solution (10−3 M) by 5 mL of cytosolic extract after 12 h and 24 h, respectively. *Source* Chauhan et al. (2011). Copyright © 2011, Dovepress. Reproduced with permission

report in which a marine alga has been used to synthesize highly stable extracellular gold nanoparticles in a relatively short time period compared with that of other biological procedures. Indeed, 95 % of the bioreduction of $AuCl^{-4}$ ions occurred within 12 h at stirring condition (Singaravelu et al. 2007). The authors extended their report to the formation of palladium and platinum nanoparticles starting with their corresponding metallic chloride—containing salts (Singaravelu et al. 2007). Rajeshkumar et al. (2013, 2014) reported synthesis of gold particles by marine brown algae *Tubinaria conoides*. The nanoparticles showed antibacterial activity against *Streptococcus* sp., *Bacillus subtilis*, *Klebsiella pneumoniae*.

Southam and Beveridge (1996) have demonstrated that gold particles of nanoscale dimensions may readily be precipitated within bacterial cells by incubation of the cells with Au^{3+} ions. Monodisperse gold nanoparticles have been synthesized by using alkalotolerant *Rhodococcus* sp. under extreme biological conditions like alkaline and slightly elevated temperature conditions (Ahmad et al. 2003b). Lengke et al. (2006a) claimed the synthesis of gold nanostructures in different shapes (spherical, cubic, and octahedral) by filamentous cyanobacteria from Au(I)-thiosulfate and Au(III)-chloride complexes and analyzed their formation mechanisms (Lengke et al. 2006a, b). Nair and Pradeep (2002) reported the growth of nanocrystals and nanoalloys using *Lactobacillus*. Some other typical gold nanoparticles produced by microorganisms are summarized in Table 1.1.

1.2.2 Silver Nanoparticles

Silver nanoparticles (AgNps), like their bulk counterpart, show effective antimicrobial activity against Gram-positive and Gram-negative bacteria, including highly multiresistant strains such as methicillin-resistant *Staphylococcus aureus* (Panacek et al. 2006). Silver nanoparticles synthesis by microorganisms is a result of their defense mechanism. The resistance caused by the microorganism for silver ions in the environment is responsible for its nanoparticle synthesis. The silver ions in nature are highly toxic to microorganisms. As a defense mechanism, the microorganism utilizes its cellular machinery to transform the reactive silver ions to stable silver atoms. Parameters such as temperature and pH play an important role in their production. It is now known that more nanoparticles are synthesized under alkaline conditions than under acidic conditions (Saklani and Jain 2012).

The reduction of Ag^+ to colloidal silver by microorganisms in aqueous solutions is a stepwise process. First various complexes of Ag^+ are reduced to metallic silver atoms (Ag^0). This is followed by the agglomeration of Ag^0 into oligomeric clusters (Sharma et al. 2008). It is these clusters that eventually lead to the formation of colloidal AgNPs (Kapoor et al. 1994; Sharma et al. 2008). The low molecular weight peptide, glutathione (GSH) and proteins like metallothioneins and phytochelatins, enzymes such as oxidoreductases, NADH-dependent reductases, nitroreductases, NADH-dependent nitrate reductases (NRs) and cysteine desulfhydrases have been shown to be responsible for nanocrystal formation in yeast, bacteria, and fungi. These microbes are known to reduce the Ag^+ ions to form silver nanoparticles, most of which are found to be spherical particles (Mukherjee et al. 2001b; Ahmad et al. 2003a; Fayaz et al. 2010). In one of the earliest studies in this technology, Slawson et al. (1992) found that a silver-resistant bacterial strain isolated from silver mines, *Pseudomonas stutzeri* AG259, accumulated AgNPs within the periplasmic space. Of note, the particle size ranged from 35 to 46 nm (Slawson et al. 1992). Interestingly, Klaus et al. (1999) observed that when this bacterium was placed in concentrated aqueous solution (50 mM), particles of larger size (200 nm) were formed. Klaus et al. (1999) group attributed the difference in

particle size (in comparison with the report of Slawson et al. 1992) to the differences in cell growth and metal incubation conditions. Klaus et al. (1999) have shown that the bacterium *Pseudomonas stutzeri* AG259, isolated from a silver mine, when placed in a concentrated aqueous solution of silver nitrate, played a major role in the reduction of the Ag^+ ions and the formation of silver nanoparticles (AgNPs) of well-defined size and distinct topography within the periplasmic space of the bacteria (Klaus et al. 1999). An important application of such a bacterium would be in industrial Ag recovery. Intriguingly, the exact mechanism(s) of AgNPs synthesis by this bacterium is still unclear. However, the molecular basis of biochemical synthesis of AgNPs from *Morganella* sp. RP-42, an insect midgut isolate (Parikh et al. 2008). Parikh et al. (2008) observed that *Morganella* sp. RP-42 when exposed to silver nitrate ($AgNO_3$) produced extracellular crystalline AgNPs of size 20 ± 5 nm. Three gene homologues (*sil*E, *sil*P, and *sil*S) were 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, which encodes a periplasmic silver-binding protein (Parikh et al. 2008). This is the only report that elucidates the molecular evidence of silver resistance in bacteria, which could be linked to synthesis mechanism. In another study, Nair and Pradeep (2002), exposed common *Lactobacillus* strains present in buttermilk to large concentrations of metal ions to produce microscopic gold, silver, and gold-silver alloy crystals of well-defined morphology. The bacteria produced these intracellularly and, remarkably, the cells preserved their viability even after crystal growth (Nair and Pradeep 2002) Notably, even cyanobacteria have been observed to produce AgNPs. For example, the biosynthesis of AgNPs has been successfully conducted using *Plectonema boryanum* UTEX 485, a filamentous cyanobacterium (Lengke et al. 2007). The authors posit that the mechanisms of AgNPs production via cyanobacteria could involve metabolic processes from the use of nitrate at 25 °C and/or organics released from the dead cyanobacteria at 25° (Fig. 1.7) to 100 °C (Fig. 1.8).

AgNPs were synthesized in the form of a film or produced in solution or accumulated on the cell surface of fungi, *Verticillium*, *Fusarium oxysporum*, or *Aspergillus flavus*, were employed (Mukherjee et al. 2001b; Senapati et al. 2004; Bhainsa and D'Souza 2006; Vigneshwaran et al. 2007; Jain et al. 2011). Mukherjee et al. (2001b) studied the synthesis of intracellular AgNPs using the fungus *Verticillium.* The authors observed that exposure of the fungal biomass to aqueous Ag^+ ions results in the intracellular reduction of the metal ions and formation of 25 ± 12 nm AgNPs. A shortcoming of the study by Mukherjee et al. (2001b) was that the metallic nanoparticles were synthesized intracellularly. Indeed, when the site of nanoparticle synthesis is intracellular, downstream processing becomes difficult and often defeats the purpose of developing a simple and cheap process. In this regard, Bhainsa and D'Souza (2006) have reported extracellular biosynthesis of AgNPs using the filamentous fungus *Aspergillus fumigatus* and the synthesis process was rapid. AgNPs were formed within minutes of silver ion coming in contact with the cell filtrate. The ultraviolet-visible (UV-Vis) spectrum of the aqueous medium containing Ag^+ ion showed a peak at 420 nm corresponding with

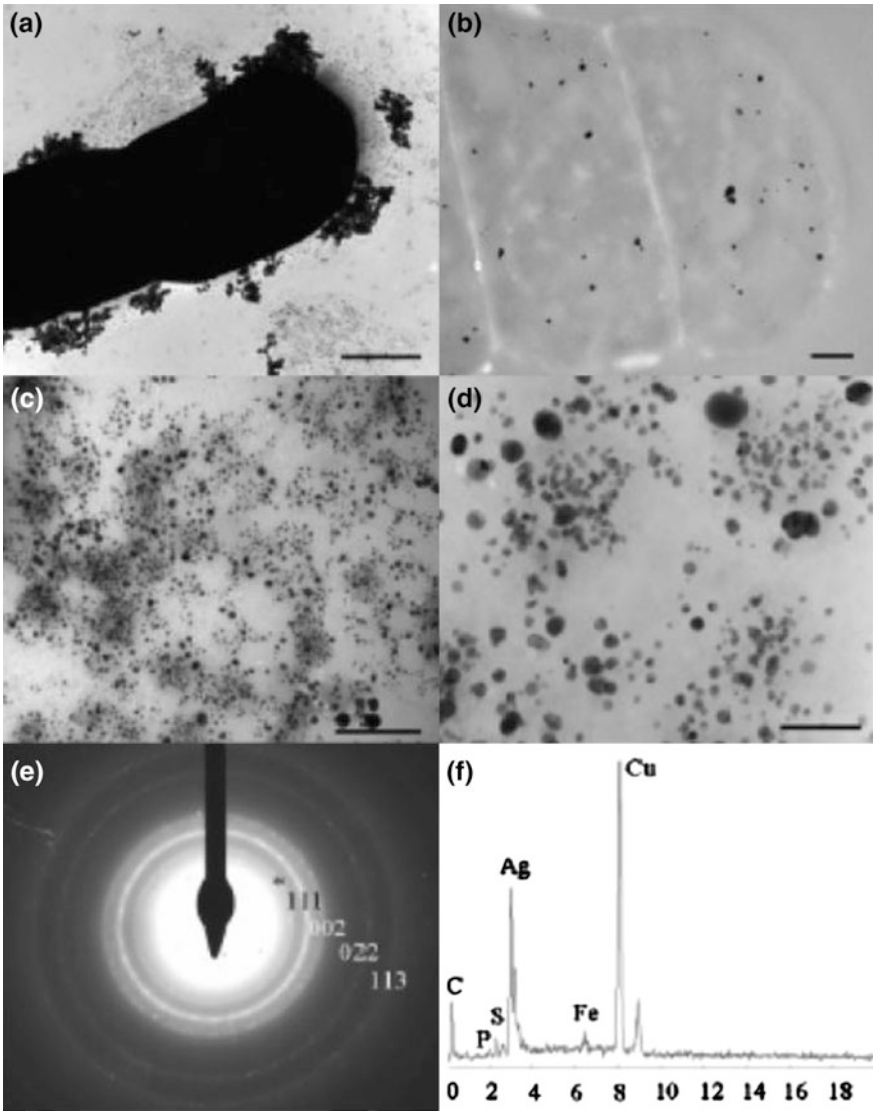

Fig. 1.7 TEM micrographs of whole mounts of cyanobacterial cells cultured in the presence of $AgNO_3$ at 25 °C and 28 days. **a** Precipitation of silver nanoparticles on the cyanobacterial surface. **b** TEM micrograph of a thin section of cyanobacteria cells with nanoparticles of silver deposited inside the cell. **c**, **d** Spherical nanoparticles of silver precipitated in solution. **e** TEM-SAED diffraction powder-ring pattern consistent with crystalline nanoparticles of Ag with a possible trace of silver sulfide (*). *d* spacings of 0.235, 0.204, 0.144, and 0.123 nm corresponding to reflections 111, 200, 220, and 311, respectively. **f** TEM-EDS for area D. Scale bars in A, B, C, and D are 0.5 and 0.2 nm and 25 and 50 nm, respectively. *Source* Lengke et al. (2007). Copyright © 2007, American Chemical Society. Reproduced with permission

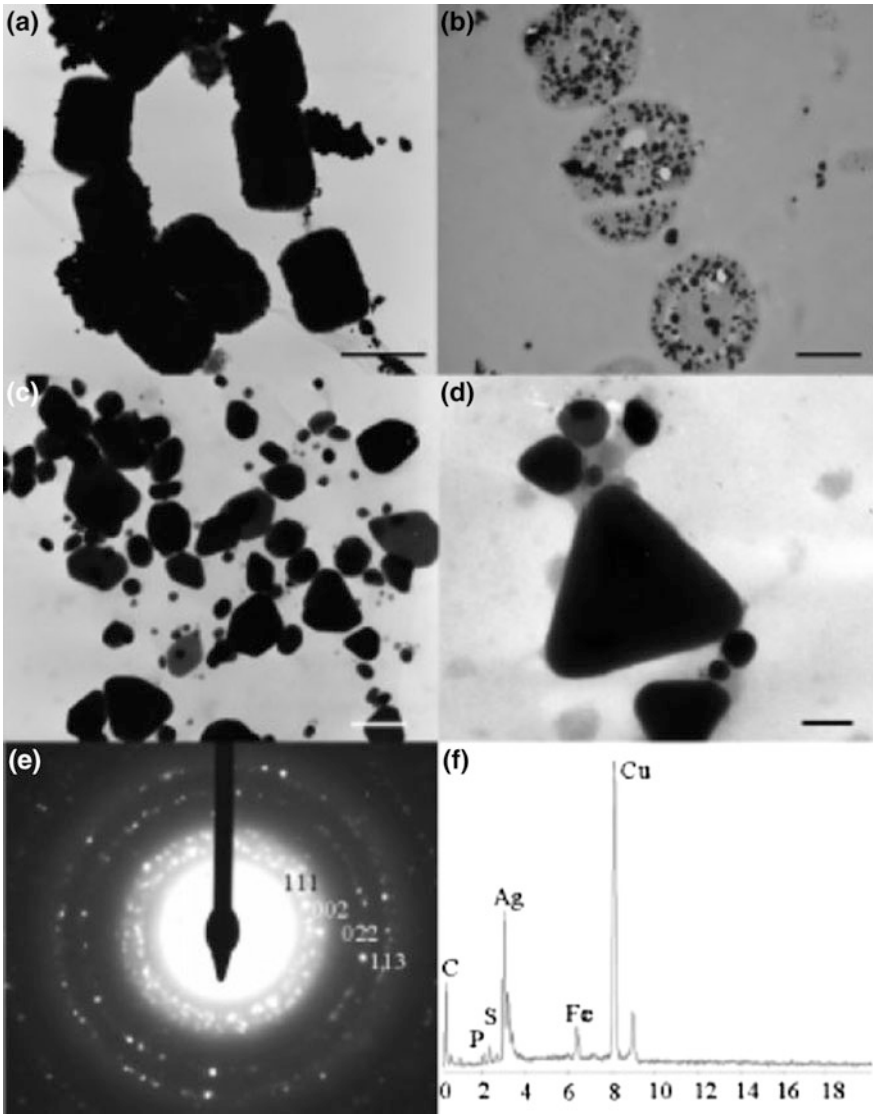

Fig. 1.8 TEM micrographs of whole mounts of cyanobacterial cells cultured in the presence of $AgNO_3$ at 100 °C and 28 days. **a** Silver nanoparticles encrusted on cyanobacterial cells. **b** TEM micrograph of a thin section of cyanobacteria cells with nanoparticles of silver deposited inside the cell. **c** Octahedral, spherical, and anhedral nanoparticles of silver precipitated in solution. **d** Octahedral silver platelets. **e** TEM-SAED diffraction powder-ring pattern consistent with crystalline nanoparticles of Ag; *d* spacings of 0.235, 0.204, 0.144, and 0.123 nm correspond to reflections 111, 200, 220, and 311, respectively. **f** TEM-EDS for area B. Scale bars in A, B, C, and D are 1, 0.05, and 0.1 and 50 nm, respectively. *Source* Lengke et al. (2007). Copyright © 2007, American Chemical Society. Reproduced with permission

the plasmon absorbance of AgNPs. The crystalline AgNPs were well dispersed in the range of 5–25 nm. Remarkably, the nanoparticles present in the aqueous medium were quite stable, even up to 4 months of incubation at 25 °C. Mukherjee et al. (2008) demonstrated green synthesis of highly stabilized nanocrystalline silver particles by a nonpathogenic and agriculturally important fungus, *Trichoderma asperellum*. An interesting aspect of this study is the mechanism of formation of AgNPs. The process of growing nanoparticles comprises two key steps: bioreduction of $AgNO_3$ to produce AgNPs followed by stabilization and/or encapsulation of the same by a suitable capping agent. The size of the silver particles using TEM and XRD studies is found to be in the range 13–18 nm. These nanoparticles are found to be highly stable and even after prolonged storage for over 6 months they do not show significant aggregation (Fig. 1.9).

A mechanism behind the formation of silver nanoparticles and their stabilization via capping has been investigated using FTIR (Fig. 1.8) and surface-enhanced resonance Raman spectroscopy (Fig. 1.9). Both the spectra exhibit a broad intense band at $\sim$3400 cm^{-1} with overlapping shoulders on either side assigned to the N–H stretching frequency arising from the peptide linkages present in the proteins

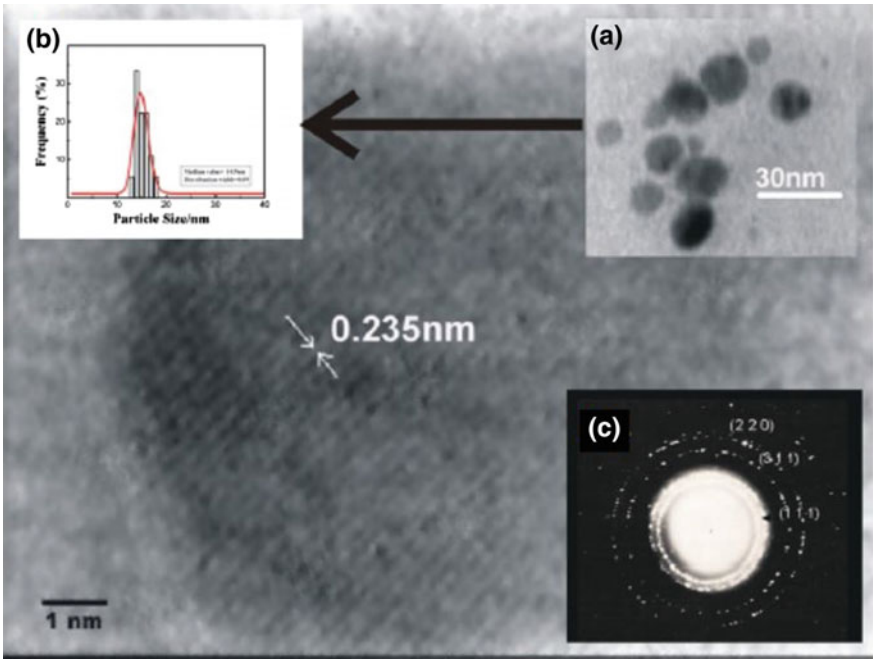

Fig. 1.9 High-resolution transmission electron micrograph of drop-cast silver nanoparticles preserved for over 6 months. Inset: **a** low-resolution micrographs showing size of the particulates, **b** histogram of particle size distribution as obtained from TEM and **c** SAED pattern recorded on the same sample. *Source* Mukherjee et al. (2008). Copyright © 2008, IOP Science. Reproduced with permission

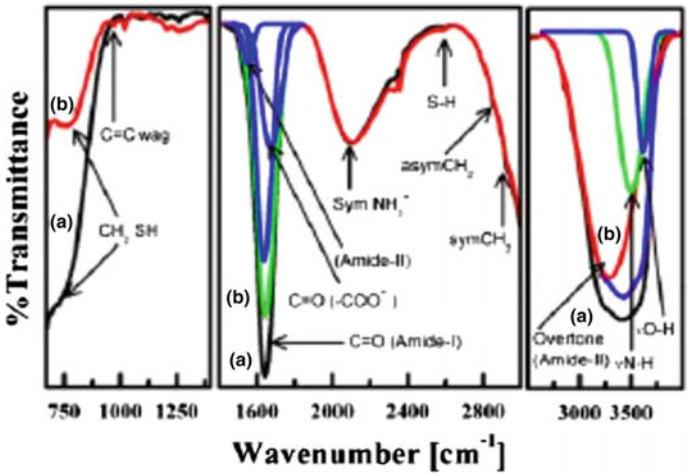

Fig. 1.10 FTIR spectra of the cell-free extract **a** before addition of $AgNO_3$ and **b** after removal of silver nanoparticles by centrifugation. *Source* Mukherjee et al. (2008). Copyright © 2008, IOP Science. Reproduced with permission

of the extract (Fig. 1.10). Upon deconvolution, the side bands were respectively identified to be the overtone of the amide-II band ($\sim$3270 cm^{-1}) and the stretching frequency of the O–H band ($\sim$3600 cm^{-1}) possibly arising from the carbohydrates and/or proteins present in the sample. However, it can arise from the aqueous solvent as well. It may be observed that the intensity of the first two bands diminishes significantly in (b), indicating a decrease in the concentration of the peptide linkages in the solution. The spectra also exhibit an intense band at $\sim$1640 cm^{-1} and a broad asymmetric band at $\sim$2100 cm^{-1}, the latter assigned to the N–H stretching band in the free amino groups of biomacromolecules and a low intensity peak at $\sim$2600 cm^{-1} due to S–H stretching vibrations. It may be noted that the intensity of the band at $\sim$2100 cm^{-1} remains almost unchanged in the two spectra while that due to S–H stretching shifts towards lower wavenumbers in (b). Upon deconvolution, the band at $\sim$1640 cm^{-1} is found to be a superposition of three different bands centred at $\sim$1550, 1640 and 1670 cm^{-1}, respectively assigned to the amide-II band, carbonyl and carboxylic C=O stretching bands of the peptide linkages (Mukherjee et al. 2008).

Figure 1.11 shows the aforesaid Raman spectrum which clearly exhibits an intense band at $\sim$240 cm^{-1} identified to be due to stretching vibrations of Ag–N bonds and two broad bands at $\sim$1350 and 1565 cm^{-1} attributed, respectively, to symmetric and asymmetric C=O stretching vibrations of the CO_2 ions apart from a few weak features at $\sim$692, 940, 970 and 1050 cm^{-1} assigned, respectively, to the stretching vibrations of C–S, C–CO_2, C–C and Cα–N bonds. Selective enhancement of these Raman bands clearly indicates that C=O bonds of the carboxylate

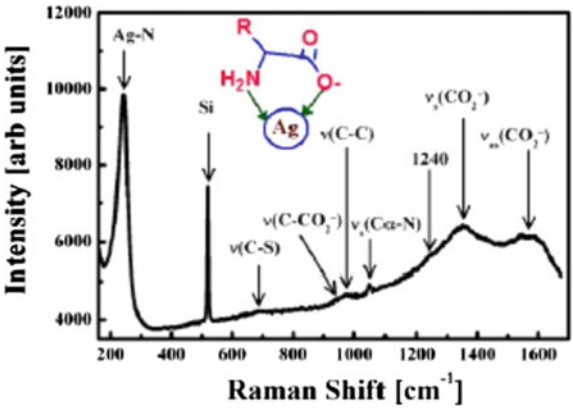

Fig. 1.11 Macro-Raman spectrum of silver nanoparticles drop-cast on Si (100) single crystals. *Source* Mukherjee et al. (2008). Copyright © 2008, IOP Science. Reproduced with permission

ions and Ag–N bonds from the free amine groups are lying perpendicular to the nano silver surface and are directly associated with the capping of the same. This is further supported from the fact that both the symmetric and asymmetric stretching bands of CO_2 are significantly broadened due to distortion in the respective bond angles and bond lengths that have resulted from the strain induced following encapsulation of silver nanoparticles (Mukherjee et al. 2008). The band at 240 cm^{-1} is direct evidence of the formation of a chemical bond between silver nanoparticles and the nitrogen of the amine group present in the amino acids. The probing technique itself manifests the feasibility of using these nanoparticles as potential templates for surface-enhanced resonance Raman spectroscopy (SERS) (Mukherjee et al. 2008). Some other silver nanoparticles produced by microorganisms are listed in Table 1.1.

1.2.3 Cadmium Nanoparticles

The health risks posed by cadmium toxicity have been investigated for over 50 years. Yet knowledge in this area is still expanding, as evidenced by the excellent reviews appearing in this volume. At the level of the organism, cadmium toxicity is associated with liver and kidney injury, osteomalacia, osteoporosis, skeletal deformations, neurological, and other deficits. Cadmium is classified as a category 1 carcinogen, but is not directly genotoxic or mutagenic in bacteria. It is known to affect genome stability via inhibition of DNA repair and generation of free radical-induced DNA damage. At the cellular level, cadmium induces oxidative stress by depletion of endogenous antioxidants such as glutathione and is associated with mitochondrial damage, induction of apoptosis, and disruption of intracellular calcium signaling. Despite the extensive studies on cadmium toxicity, there

continues to be much territory left to cover regarding its mechanism of action, intracellular damage, and environmental exposure. At present, the primary cadmium nanoparticles are those of CdSe or CdTe, encapsulated in various coatings in the form of semiconductor quantum dots (Bao et al. 2010). Semiconductor nano-crystals, which have unique optical, electronic, and optoelectronic properties have potential application in the field of nano-electronics.

Kumar et al. (2007) synthesized CdSe nanoparticles (9–15 nm) using a fungus, *Fusarium oxysporum*, in a mixture of $CdCl_2$ and $SeCl_4$. Cui et al. (2009) synthesized CdSe using a yeast strain: *Saccharomyces cerevisiae*. Pearce et al. (2008) also synthesized CdSe nanoparticles by adding $CdCl_2O_8$ to selenide (Se[II]) produced from selenite (Se[IV]) by an anaerobic bacterium: *Veillonella atypica*. In the latter two studies (Pearce et al. 2008; Cui et al. 2009), cadmium was added after microbial formation of selenide for CdSe synthesis, probably because of its toxicity to the microbes. Consequently, they synthesized CdSe in two-vessel processes consisting of reduction of selenite to selenide and subsequent synthesis of CdSe from selenide and cadmium ion. In contrast, only Kumar et al. (2007) reported a one-vessel process in which the fungus generates CdSe in the co-presence of selenite and cadmium ion, which might improve economic efficiency through its simple operation of fewer reaction vessels. Synthesis of CdSe was observed in *Pseudomonas* sp. RB. by Ayano et al. (2014). Transmission electron microscopy and EDS revealed that this strain accumulated nanoparticles (10–20 nm) consisting of selenium and cadmium inside and on the cells when cultivated in the same medium for the enrichment culture. This report is the first report describing isolation of a selenite-reducing and cadmium-resistant bacterium (Ayano et al. 2014).

Cadmium telluride (CdTe), an important group II–VI semiconductor material with large exciton Bohr radius (7.3 nm) and narrow bulk band gap of 1.5 eV has shown significant potential for LED (energy), FRET (electronics), and biomedical applications (Yang et al. 2009) due to their size dependent properties. These nanoparticles provide excellent photostability, narrow emission and high quantum yield in comparison with organic dyes and therefore explored in live cell bio-imaging (Pan and Feng 2009). Syed and Ahmad (2013) uses the fungus *Fusarium oxysporum* to synthesize highly fluorescent extracellular CdTe (quantum dot) nanoparticles. The process utilizes Cd and Te precursors in a very dilute form and allows bottom-up, one-step preparation of nanoparticles. Different techniques were employed for their characterization such as SAED and XRD which confirmed the crystalline nature of biosynthesized nanoparticles. These biosynthesized nanoparticles are capped by proteins secreted by the fungus in the reaction mixture, which makes them water dispersible and provides stability in solution by preventing their agglomeration. These nanoparticles also showed antibacterial activity against Gram-positive and Gram-negative bacteria. This study demonstrates that fungus based approach provides a novel, rational and environment friendly synthesis protocol for nanomaterials synthesis. This is the first demonstration of a fungal-mediated approach for the synthesis of CdTe quantom dots (Syed and Ahmad 2013).

1.3 Alloy Nanoparticles

Alloy nanoparticles or bimetallic nanoparticles exhibit unique electronic, optical, and catalytic properties that are different from those of the corresponding individual metal particles (Harada et al. 1993). For instance gold nanoparticles supported on metal oxide or gold-containing bimetallic nanoparticles are found to exhibit enhanced catalytic activity (Bond 2002).

1.3.1 Gold—Silver (Au–Ag) Nanoparticles

In many functional properties, the performances of Au–Ag alloy nanoparticles are superior to the corresponding monometallic ones, such as in surface enhanced Raman spectroscopy (SERS), sensors, and catalysis. Therefore, many synthesis approaches of bimetallic Au–Ag nanoparticles have been developed, such as digestive ripening, laser synthesis method, seed growth method, ligand binding method, and galvanic reaction. Since the seminal discovery of catalytic activity by gold nanoparticles, supported Au–Ag alloy nanoparticles have been receiving increasing attentions for possible enhancement in catalytic activity (Liu et al. 2013).

In 2005, Liu et al. reported that Au and Ag showed obvious synergetic effect in CO oxidation reaction over an alloy nanocatalyst Au–Ag@MCM-41 catalyst. Since then, the Au–Ag alloy system has been applied to various reactions including oxidation of alcohols, acetylene hydrogenation, and photocatalytic reaction. Sandoval et al. (2011) investigated the Au–Ag alloy nanoparticles supported on TiO_2 by a sequential precipitation-deposition method, where they deposited Ag first and Au at the second step, and found that Au–Ag alloy nanoparticles are also very stable under high temperature pretreatment. However, the effect of the deposition sequence on the formation of Au–Ag bimetallic nanoparticles supported on SiO_2 and their catalytic performances have not been investigated yet. Since Sun and Xia (2002) found the replacement reaction method could synthesize Au–Ag bimetallic systems, various Au–Ag nanostructures have been developed including hollow cubes, porous surfaces and alloy nanoparticles.

Senapati et al. (2005) reported the synthesis of bimetallic Au–Ag alloy by *Fusarium oxysporum* and argued that the secreted cofactor NADH plays an important role in determining the composition of Au–Ag alloy nanoparticles. Using TEM, well-separated nanoparticles with occasional aggregation in the size range 8–14 nm are clearly observed (Fig. 1.12). The amount of cofactor NADH plays an important role in determining the nanoalloy composition (Senapati et al. 2005).

Zheng et al. (2010) studied Au–Ag alloy nanoparticles biosynthesized by yeast cells (Fig. 1.13). Fluorescence microscopic and transmission electron microscopic characterizations indicated that the Au–Ag alloy nanoparticles were mainly synthesized via an extracellular approach and generally existed in the form of irregular

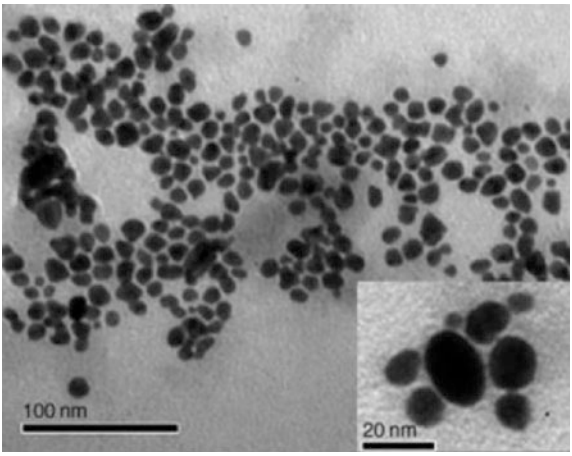

Fig. 1.12 TEM images of Au–Ag nanoparticles formed by reaction of a mixture of 1 mm $HAuCl_4$ and 1 mm $AgNO_3$ with 60 g *Fusarium oxysporum* wet biomass for 96 h. *Source* Senapati et al. (2005). Copyright © 2013, Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission

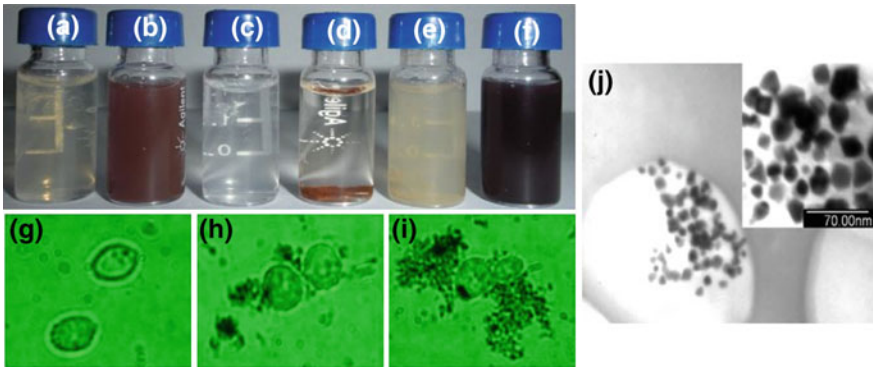

Fig. 1.13 Yeast cell solutions containing $HAuCl_4$ (**a** and **b**), $AgNO_3$ (**c** and **d**) and the mixture of $HAuCl_4$ and $AgNO_3$ (**e** and **f**) before (**a**, **c** and **e**) and after (**b**, **d** and **f**) standing for 24 h, fluorescence microscopy bright field images of the surface of yeast cells' film after reacting with $AgNO_3$ (**g**), $HAuCl_4$ (**h**) and the mixture of $AgNO_3$ and $HAuCl_4$ (**i**), and TEM images of Au–Ag alloy nanoparticles (**j**). *Source* Zheng et al. (2010). Copyright © 2010, Elsevier. Reproduced with permission

polygonal nanoparticles. Electrochemical investigations revealed that the vanillin sensor based on Au–Ag alloy nanoparticles modified glassy carbon electrode was able to enhance the electrochemical response of vanillin for at least five times.

Sawle et al. (2008) demonstrated the synthesis of core-shell Au–Ag alloy nanoparticles from fungal strains *Fusarium semitectum* and showed that the

nanoparticle suspensions are quite stable for many weeks. As *Fusarium. oxysporum* is known to synthesize technologically important metal sulfide quantum dots extracellularly, this procedure can further be extended for the synthesis of other composite nanoparticles such as Au–CdS, Ag–CdS, and CdS–PbS.

1.4 Oxide Nanoparticles

The industrial use of metallic oxide nanoparticles in a wide variety of applications has been rapidly expanding in the last decade. Such applications include the use of silicon, titanium, iron, and other metallic oxide nanoparticles, thereby increasing the occupational and other environmental exposure of these nanoparticles to humans and other species (Lai et al. 2007a). Nevertheless, the health effects of exposure of humans and other species to metallic oxide nanoparticles have not been systematically investigated as their impact on the environment has not been under the scrutiny of regulatory control (Lai et al. 2007b). Oxide nanoparticle is an important type of compound nanoparticle synthesized by microbes. In this section, Li et al. (2011) reviewed the biosynthesis of oxide nanoparticles. Most of the examples of the magnetotactic bacteria used for the production of magnetic oxide nanoparticles and biological systems for the formation of nonmagnetic oxide nanoparticles (Table 1.2).

1.4.1 Cerium Oxide Nanoparticles

Cerium, which is the first element in the lanthanide group with 4*f* electrons, has attracted much attention from researchers in physics, chemistry, biology and materials science. It is the most abundant of rare-earth metals found in the Earth's crust. Several Ce-carbonate, -phosphate, -silicate, and -(hydr)oxide minerals have been historically mined and processed for pharmaceutical uses and industrial applications. Of all Ce minerals, cerium dioxide has received much attention in the global nanotechnology market due to their useful applications for catalysts, fuel cells, and fuel additives. When combined with oxygen in a nanoparticle formulation, cerium oxide adopts a fluorite crystalline structure that emerges as a fascinating material. This cerium oxide nanoparticle (CeONP) has been used prolifically in various engineering and biological applications, such as solid-oxide fuel cells, high-temperature oxidation protection materials, catalytic materials,, solar cells and potential pharmacological agents (Xu and Qu 2014). Although useful because of its various properties and applications, the main application of CeONPs is in the field of catalysis, and stems from their unique structure and atomic properties compared with other materials. Cerium nanoparticles have found numerous applications in the biomedical industry due to their strong antioxidant properties. Industrial applications include its use as a polishing agent, ultraviolet absorbing compound in

Table 1.2 Oxide nanoparticles synthesized by microorganisms

Microorganism	Type nanoparticle produce	Size (nm)	Shape	Reference
Curvularia lunata	CeO_2	5	Spherical	Munusamy et al. (2016)
Fusarium oxysporum	TiO_2	6–13	Spherical	Bansal et al. (2005)
Fusarium oxysporum	$BaTiO_3$	4–5	Spherical	Bansal et al. (2006)
Fusarium oxysporum	ZrO_2	3–11	Spherical	Bansal et al. (2004)
Lactobacillus sp.	$BaTiO_3$	20–80	Tetragonal	Jha and Prasad (2010)
Lactobacillus sp.	$T1O_2$	8–35	Spherical	Jha et al. (2009a)
Saccharomyces cerevisiae	Sb_2O_3	2–10	Spherical	Jha et al. (2009b)
Shewanella oneidensis	Fe_3O_4	40–50	Rectangular, rhombic, hexagonal	Perez-Gonzalez et al. (2010)
Shewanella oneidensis MR-1	Fe_3O_3	30–43	Pseudohexagonal/irregular or rhombohedral	Bose et al. (2009)
Yeast cells	Fe_3O_4	Not available	Wormhole- like	Zhou et al. (2009)
Penicillium sp.	CuO	Not available	Spherical	Honary et al. (2012)
Fusarium oxysporum	CuO	2–5	Spherical	Hosseini et al. (2012)
Stereum hirsutum	CuO	5–20	Spherical	Cuevas et al. (2015)

sunscreen, solid electrolytes in solid oxide fuel cells, as a fuel additive to promote combustion, and in automotive exhaust catalysts (Sindhu et al. 2015). CeONPs have also been used in fighting inflammation and cancer, and in radiation protection of cells (Shah et al. 2012).

A recent mass flow modeling study predicted that a major source of CeO_2 nanoparticles from industrial processing plants (e.g., electronics and optics manufactures) is likely to reach the terrestrial environment such as landfills and soils. The environmental fate of CeO_2 nanoparticles is highly dependent on its physicochemical properties in low temperature geochemical environment. Though there are needs in improving the analytical method in detecting/quantifying CeO_2 nanoparticles in different environmental media, it is clear that aquatic and terrestrial organisms have been exposed to CeO_2 NPs, potentially yielding in negative impact on human and ecosystem health. Interestingly, there has been contradicting reports about the toxicological effects of CeO_2 nanoparticles, acting as either an antioxidant

or reactive oxygen species production-inducing agent) (Dahle and Arai 2015). This poses a challenge in future regulations for the CeO_2 nanoparticle application and the risk assessment in the environment.

Arumugam et al. (2015) successfully synthesize CeO_2 nanoparticles using *Gloriosa superba* L. leaf extract. The synthesized nanoparticles retained the cubic structure, which was confirmed by X-ray diffraction studies. The oxidation states of the elements (C [1s], O [1s] and Ce [3d]) were confirmed by XPS studies. TEM images showed that the CeO_2 nanoparticles possessed spherical shape and particle size of 5 nm. The Ce–O stretching bands were observed at 451 cm^{-1} and 457 cm^{-1} from the FT-IR and Raman spectra respectively. The band gap of the CeO_2 NPs was estimated as 3.78 eV from the UV–visible spectrum. From the photoluminescence measurements, the broad emission composed of eight different bands were found. The antibacterial studies performed against a set of bacterial strains showed that Gram-positive bacteria were relatively more susceptible to the NPs than Gram-negative bacteria. The toxicological behavior of CeO_2 NPs was found due to the synthesized NPs with uneven ridges and oxygen defects in CeO_2 NPs.

CeO_2 NPs have been successfully synthesized using the fungus *Curvularia lunata* (Munusamy et al. 2016). The XRD patterns, Micro Raman spectra and SAED pattern studies suggest the formation CeO_2 NPs cubic fluorite structure. The TEM images showed spherical morphology with the average size of 5 nm. Synthesized CeO_2 NPs were investigated by antibacterial activity. The perusal results observed at 100 µg CeO_2 NPs had most significant effect of antibacterial activity due to the strong electrostatic forces to binding the bacterial cell membrane to inhibit the bacterial growth.

1.4.2 Silica Dioxide Nanoparticles

Silica, or silicon dioxide, is the same material used to make glass. In nature, silica makes up quartz and the sand you walk on at the beach. Unlike metals such as gold and iron, silica is a poor conductor of both electrons and heat. Despite these limitations, silica (silicon oxide) nanoparticles form the framework of silica aerogels. *Silica aerogels* are composed of silica nanoparticles interspersed with nanopores filled with air. As a result, this substance is mostly made up of air. Because air has very low thermal conductivity and silica has low thermal conductivity, they are great materials to use in insulators. These properties make nano aerogels one of the best thermal insulators known to man. Silica nanoparticles can also be functionalized by bonding molecules to a nanoparticle that also is able to bond to another surface, such as a cotton fiber. The functionalized silica nanoparticles attach to the cotton fiber and form a rough surface that is hydrophobic (water repellent), giving an effect similar to the water repellency of lotus leaves. Another type of silica nanoparticle is riddled with nanoscale pores. Researchers are developing drug delivery methods where therapeutic molecules stored inside the pores are slowly released in a diseased region of the body, such as near a cancer tumor (Argyo et al. 2014).

Application of silica nanoparticles as fillers in the preparation of nanocomposite of polymers has drawn much attention, due to the increased demand for new materials with improved thermal, mechanical, physical, and chemical properties. The chemical synthesis of silica-based materials are relatively expensive and exo-hazardous, often requiring extreme temperature, pressure and pH. Singh et al. (2008) demonstrated the synthesis of silicon/silica nanoparticle composites by *Acinetobacter* sp. The formation of silicon/silica nanocomposite is shown to occur when the bacterium is exposed to K_2SiF_6 precursor under ambient conditions. This bacterium has been shown to synthesize iron oxide and iron sulfide nanoparticles. It is hypothesized that this bacterium secretes reductases and oxidizing enzymes which lead to Si/SiO_2 nanocomposite synthesis. The synthesis of silica nanoparticles by bacteria demonstrates the versatility of the organism, and the formation of elemental silicon by this environmentally friendly process expands further the scope of microorganism-based nanomaterial synthesis.

1.4.3 Titatium Oxide Nanoparticles

Titanium dioxide (TiO_2) has become part of our everyday lives. It is found in various consumer goods and products of daily use such as cosmetics, paints, dyes and varnishes, textiles, paper and plastics, food and drugs, and even paving stones. 4.68 million tons of titanium dioxide were produced worldwide in 2009. Titanium dioxide (TiO_2) is a material of great significance in many fields, e.g., photocatalysis, solar cell devices, gas sensors, and biomaterials (Gong and Selloni 2005). The non-toxic and biocompatible properties of Titania find its applications in biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries (Jha et al. 2009a). TiO_2 catalysts have been confirmed to be excellent and efficient photocatalysts for the degradation and inhibition of numerous toxic environmental contaminants. Various applications of titanium dioxide include air and water cleaning and surface cleaning (Pan et al. 2010). Titanium is recommended for desalinization plants because of its strong resistance to corrosion from seawater. In medical applications the titanium pins are due to because of their non-reactive nature when contacting bone and flesh (Prasad et al. 2007). The TiO_2 nanoparticles are synthesized using various methods such as sol gel, hydrothermal, flame combustion, solvothermal, fungal mediated biosynthesis

Titanium oxide nanoparticles can be synthesized from *Bacillus subtilis* (Kirthi et al. 2011). The TiO_2 nanoparticles have shown spherical clusters of the nanoparticles. Nanoparticles were spherical, oval in shape, individual as well as a few aggregates having the size of 66–77 nm. The particle size distribution reveals the morphological homogeneity with the grain size falling mostly in submicron range. The energy yielding material—glucose, which controls the value of oxidation—reduction potential (rH_2), the ionic status of the medium pH and overall rH_2, which is partially controlled by the bicarbonate, negotiate the synthesis of TiO_2 nanoparticles in the presence of *B. subtilis*. The synthesis of n-TiO_2 might have

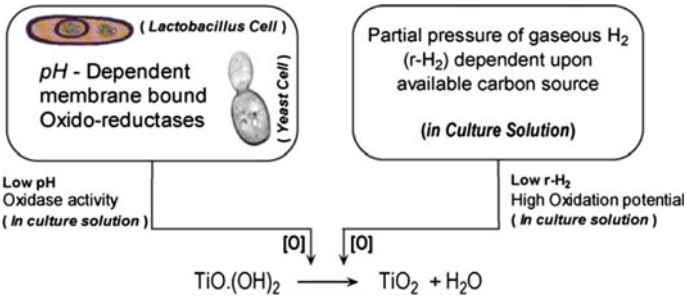

Fig. 1.14 Schematics for the biosynthesis of *n*-TiO_2. *Source* Jha et al. (2009a). Copyright © 2009, Elsevier. Reproduced with permission

resulted due to pH-sensitive membrane bound oxidoreductases and carbon source dependent rH_2 in the culture solution (Kirthi et al. 2011).

A low-cost green and reproducible microbes (*Lactobacillus* sp. and *Saccharomyces cerevisiae*) mediated biosynthesis of TiO_2 nanoparticles was carried out by Jha et al. (2009a). The synthesis was performed akin to room temperature in the laboratory ambience. X-ray and transmission electron microscopy analyses were performed to ascertain the formation of TiO_2 nanoparticles. Individual nanoparticles as well as a few aggregate having the size of 8–35 nm are found. Concentric Scherrer rings in the selected area electron diffraction pattern indicated that the nanoparticles are having all possible orientations. A possible involved mechanism for the biosynthesis of nano-TiO_2 has also been proposed in which pH as well as partial pressure of gaseous hydrogen (rH_2) or redox potential of the culture solution seems to play an important role in the process (Fig. 1.14).

1.4.4 Iron Oxide Nanoparticles

Eight iron oxides are known (Cornell and Schwertmann 2003), among these iron oxides, hematite (α-Fe_2O_3), magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are very promising and popular candidates due to their polymorphism involving temperature-induced phase transition. Each of these three iron oxides has unique biochemical, magnetic, catalytic, and other properties which provide suitability for specific technical and biomedical applications. As the most stable iron oxide and n-type semiconductor under ambient conditions, hematite (α-Fe_2O_3) is widely used in catalysts, pigments and gas sensors due to its low cost and high resistance to corrosion. It can also be used as a starting material for the synthesis of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), which have been intensively pursued for both fundamental scientific interests and technological applications in the last few decades (Wu et al. 2010a). As shown in Fig. 1.15b, Fe_3O_4 has the face centered cubic spinel structure, based on 32 O_2^- ions and close-packed along the direction. Fe_3O_4 differs from most

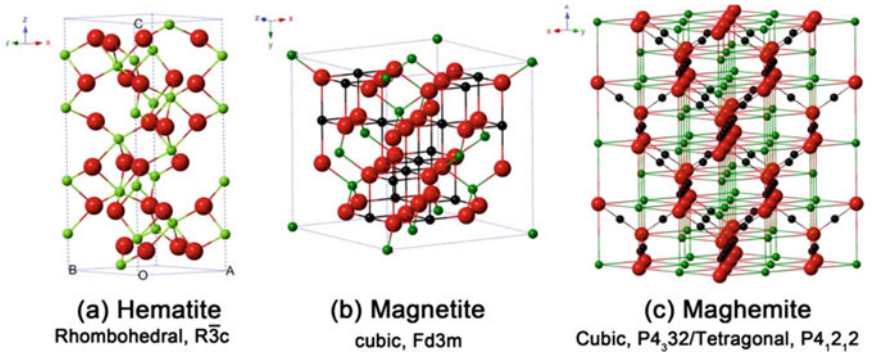

Fig. 1.15 Crystal structure and crystallographic data of the hematite, magnetite and maghemite (the *black ball* is Fe^{2+}, the *green ball* is Fe^{3+} and the *red ball* is O_2. *Source* Wu et al. (2015). Copyright © 2015, Taylor and Francis. Reproduced with permission

other iron oxides in that it contains both divalent and trivalent iron. Fe_3O_4 has a cubic inverse spinel structure that consists of a cubic close packed array of oxide ions, where all of the Fe^{2+} ions occupy half of the octahedral sites and the Fe^{3+} are split evenly across the remaining octahedral sites and the tetrahedral sites. Fe_3O_4 has the lowest resistivity among iron oxides due to its small bandgap (0.1 eV). As shown in Fig. 1.15c, the structure of γ-Fe_2O_3 is cubic; each unit of maghemite contains 32 O_2^- ions, 211/3 Fe^{3+} ions and 21/3 vacancies. Oxygen anions give rise to a cubic close-packed array while ferric ions are distributed over tetrahedral sites (eight Fe ions per unit cell) and octahedral sites (the remaining Fe ions and vacancies). Therefore, the maghemite can be considered as fully oxidized magnetite, and it is an n-type semiconductor with a bandgap of 2.0 eV.

The bacterium *Actinobacter* sp. has been shown to be capable of extracellularly synthesizing iron based magnetic nanoparticles, namely maghemite (γ-Fe_2O_3) and greigite (Fe_3S_4) under ambient conditions depending on the nature of precursors used (Bharde et al. 2008). More precisely, the bacterium synthesized maghemite when reacted with ferric chloride and iron sulfide when exposed to the aqueous solution of ferric chloride-ferrous sulfate. Challenging the bacterium with different metal ions resulted in induction of different proteins, which bring about the specific biochemical transformations in each case leading to the observed products. Maghemite and iron sulfide nanoparticles show superparamagnetic characteristics as expected. Compared to the earlier reports of magnetite and greigite synthesis by magnetotactic bacteria and iron reducing bacteria, which take place strictly under anaerobic conditions, the present procedure offers significant advancement since the reaction occurs under aerobic condition. Moreover, reaction end products can be tuned by the choice of precursors used. The process of magnetic nanoparticles mineralization can be divided into four steps (Faramazi and Sadighi 2013): (1) vesicle formation and iron transport from outside of the bacterial membrane into the cell; (2) magnetosomes alignment in chain; (3) initiation of crystallization; and (4) crystal maturation (Fig. 1.16).

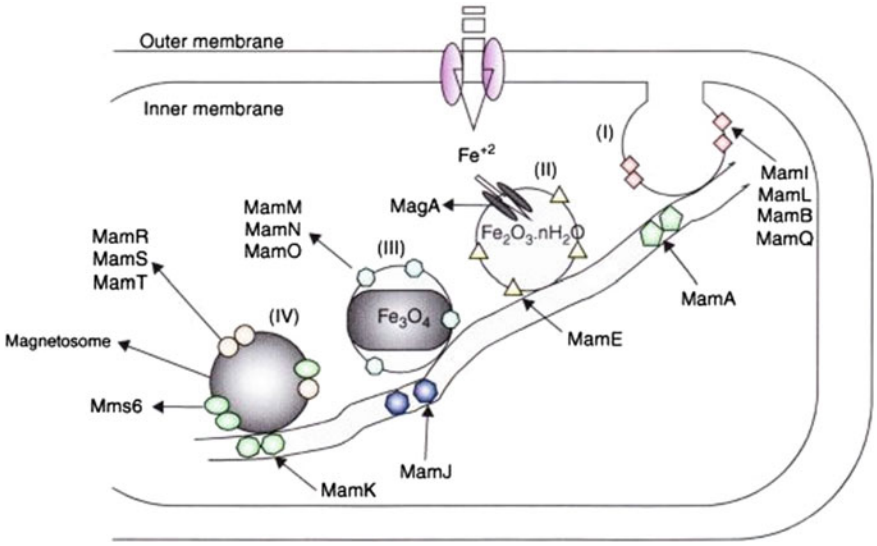

Fig. 1.16 Magnetosome biomineralization in magnetotactic bacteria (MTB). (*I*) MamI, MamL, MamB, and MamQ proteins initiate the membrane invagination and form a vesicular membrane around the magnetosome structure. (*II*) The protease-independent function of MamE recruits other proteins such as MamK, MamJ and MamA to align magnetosomes in a chaib. (*III*) Iron uptake occurs via MagA, a transmembrane protein, and initiation of magnetic crystal biomineralization occurs through MamM, MamNm and MamO proteins. (*IV*) Finally, MamR, MamS, MamT, MamP, MamC, MamD, MamF, MamG, the protease-dependent of MamE and Mms6, a membrane tightly bounded GTPase, regulate crystal growth and determine morphology of the produced magnetic nanoparticles. *Source* Faramazi and Sadighi (2013). Copyright © 2013, Elsevier. Reproduced with permission

1.4.5 Zirconium Dioxide (ZrO_2) Nanoparticles

Zirconium is a strong transition metal that resembles titanium. Because of its strong resistance to corrosion, it is used as an alloying agent in materials that are exposed to corrosive agents such as surgical appliances, explosive primers, vacuum tube getters and filaments. Since it has a very negative reduction potential (−1.55 V), it is never found as the native metal. It is obtained mainly from the mineral zircon, which can be purified with chlorine (Eshed et al. 2011). Because of its intrinsic physicochemical properties such as hardness, shock wear, strong acid and alkali resistance, low frictional resistance, and high melting temperature, zirconia can be used as an abrasive, as a hard, resistant coating for cutting tools, and in high temperature engine components. For these reasons, it is often called ceramic steel. Zirconia nanoparticles are of great interest due to their improved optical and electronic properties with application as a piezoelectric, electro-optic and dielectric material. Zirconia is also emerging as an important class of catalyst. The synthesis of zirconia has been realized by physico-chemical methods such as sol–gel synthesis, aqueous precipitation,

thermal decomposition and hydrothermal synthesis (Bansal et al. 2004). However, all these methods require extremes of temperature (in the case of thermal synthesis) and pressure (hydrothermal synthesis).

Bansal et al. (2004) have shown that the fungus *Fusarium oxysporum* secretes proteins capable of hydrolyzing aqueous ZrF_6^{2-} ions extracellularly to form zirconia at room temperature. Particularly interesting is the fact that the fungus is capable of hydrolyzing tough metal halide precursors under acidic conditions. While the hydrolytic proteins secreted by *Fusarium oxysporum* are yet to be sequenced and studied for their role in the fungus metabolic pathways, our studies indicate that they are cationic proteins of molecular weight centered around 24 and 28 kDa and thus, similar to silicatein. The regenerative capability of biological systems coupled with the fact that that fungi such as *Fusarium oxysporu*m are capable of hydrolyzing metal complexes that they never encounter during their growth cycle shows enormous promise for development, particularly in large-scale synthesis of metal oxides.

1.4.6 Antimony Trioxide (Sb_2O_3) Nanoparticles

Antimony trioxide (Sb_2O_3) is a good semiconducting material and an excellent catalyst for the production of PET plastic used in the packaging of mineral water and soft drinks, which has been confirmed by the World Health Organization and the European Food Safety Authority. In addition, Sb_2O_3/Sb_2O_5 is a potential compound for the synthesis of antimony gluconate, which is considered to be an effective medicine against Kala azar (visceral leishmaniasis). Common salts of antimony are irritants, thus an oral administration produces nausea, vomiting and diarrhea; they are therefore administered parenterally. It is also a cumulative drug. Antimony compounds are avoided in cases of pulmonary tuberculosis, jaundice, nephrites and dysentery (Acharya 1972). Nanoscale antimony compounds could prove to be less toxic to the body because they can cross the renal barrier. Further, Sb_2O_3 greatly increases flame retardant effectiveness when used as a synergist in combination with halogenated flame retardants in plastics, paints, adhesives, sealants, rubber and textile back coatings (Ye et al. 2006). Sb_2O_3 also has several applications, such as a fining agent or as a degasser (to remove bubbles) in glass manufacturing, an opacifier in porcelain and enameling services, and a white pigment in paints. Sb_2O_3 nanoparticles have been synthesized using different techniques by different groups of researchers (Guo et al. 2000; Friedrichs et al. 2001; Ye et al. 2002). A microbe (*Lactobacillus* sp.)-mediated biosynthesis of Sb_2O_3 nanoparticles was reported by Jha et al. (2009b). The synthesis was performed at around room temperature. X-ray and transmission electron microscopy analyses were performed to ascertain the formation of Sb_2O_3 nanoparticles. X-ray analysis indicated that Sb_2O_3 nanoparticles had a face-centered cubic unit cell structure. Individual nanoparticles as well as a few aggregate of 3–12 nm were found.

1.4.7 Copper Oxide (CuO) Nanoparticles

Copper as a metal or copper oxides exhibit broad-spectrum biocidal activity, and several studies during the last two years found that copper demonstrates remarkable antibacterial activity at the nanoscale (Cuevas et al. 2015). In contrast to silver nanoparticles, which have been studied extensively for antibacterial application, copper is an essential element for living organisms and may be suitable for biomedical applications (Rubilar et al. 2013). It is important to note that copper is approximately 10-fold cheaper than silver in the market, and therefore, a method utilizing copper would prove to be quite cost-effective. On the other hand, it has been reported that copper nanoparticles are less toxic than silver nanoparticles (Bondarenko et al. 2013). Microorganisms such as *Fusarium oxysporum* are able to leach copper from integrated circuits present on electronic boards under ambient conditions (Cuevas et al. 2015). The analysis of the biogenic synthesis of copper oxides from $CuSO_4$ has been observed in by *Penicillium aurantiogriseum*, *P. citrinum*, *P. waksmanii*, and *F. oxysporum* showed no large polydispersity in the pH range of 5–9 (Honary et al. 2012; Hosseini et al. 2012). A limited number of studies have been published, and these evaluated different fungal strains for the biosynthesis of copper nanoparticles. Fungi, such as *Penicillium* sp. and *F. oxysporum* strains, have been reported to biosynthesize copper oxide and Cu_2S nanoparticles (Honary et al. 2012; Hosseini et al. 2012).

Cuevas et al. (2015) evaluated the ability to synthesize copper and copper oxide nanoparticles using a mycelium-free extract produced by *Stereum hirsutum*, a white-rot fungus, in the presence of three different copper salts and to characterize and assess the involvement of proteins in the formation of the nanoparticles. The nanoparticles biosynthesis in presence of all copper salts demonstrated higher formation with 5 mM $CuCl_2$ under alkaline conditions. TEM analysis confirmed that the nanoparticles were mainly spherical (5–20 nm). The presence of amine groups attached to nanoparticles was confirmed by FTIR, which suggests that extracellular protein of fungus is responsible for the formation of the nanoparticles.

1.4.8 Zinc Oxide (ZnO)

Zinc oxide (ZnO) NPs have unique optical and electrical properties, and as a wide band gap semiconductor, they have found more uses in biosensors, nanoelectronics, and solar cells. These NPs are being used in the cosmetic and sunscreen industry due to their transparency and ability to reflect, scatter, and absorb UV radiation and as food additives. Furthermore, zinc oxide NPs are also being considered for use in next-generation biological applications including antimicrobial agents, drug delivery, and bioimaging probes (Jayaseelan et al. 2012). A low-cost and simple procedure for synthesis of zinc oxide NPs using reproducible bacterium, *Aeromonas hydrophila*, was reported. X-ray diffraction (XRD) confirmed the crystalline nature

of the NPs, and atomic force microscopy (AFM) showed the morphology of the nanoparticle to be spherical, oval with an average size of 57.72 nm. The antibacterial and antifungal activity was ended with corresponding well diffusion and minimum inhibitory concentration. The maximum zone of inhibition was observed in the ZnO NPs (25 μg/mL) against *Pseudomonas aeruginosa* ($\sim 22 \pm 1.8$ mm) and *Aspergillus flavus* ($\sim 19 \pm 1.0$ mm) (Jayaseelan et al. 2012).

1.5 Sulfide Nanoparticles

In addition to oxide nanoparticles, sulfide nanoparticles have also attracted great attention in both fundamental research and technical applications as quantum-dot fluorescent biomarkers and cell labeling agents because of their interesting and novel electronic and optical properties (Yang et al. 2005). Examples of sulfide-producing nanoparticles are listed in Table 1.3.

Table 1.3 Sulfide nanoparticles synthesized by microorganisms

Microorganism	Type nanoparticle produce	Size (nm)	Shape	Reference
Coriolus versicolor	CdS	100–200	Spherical	Sanghi and Verma (2009)
Desulfobacteraceae	CdS	2–5	Hexagonal lattice	Labrenz et al. (2000)
Escherichia coli	CdS	2–5	Wurtzite crystal	Sweeney et al. (2004)
Fusarium oxysporum	CdS	5–20	Spherical	Ahmad et al. (2002)
Lactobacillus	CdS	4.7–5.1	Spherical	Prasad and Jha (2010)
Rhodopseudomonas palustris	CdS	8	Cubic	Bai et al. (2009)
Schizosaccharomyces pombe	CdS	1–1.5	Hexagonal lattice	Kowshik et al. (2002)
Yeast	CdS	3.4–3.8	Spherical	Prasad and Jha (2010)
Rhodopseudomonas sphaeroides	PbS	10.35–10.65	Spherical	Bai and Zhang (2009)
Sulfate-reducing bacteria	FeS	2	Spherical	Watson et al. (1999)
Fusarium oxysporum	CuS	10–40	Not available	Schaffie and Hosseini (2014)
Rhodopseudomonas sphaeroides	ZnS	8	Hexagonal lattice	Bai et al. (2006)

1.5.1 Cadmium Sulfide (CdS) Nanoparticles

CdS nanocrystal is one typical type of sulfide nanoparticle and has been synthesized by microorganisms (Prasad and Jha 2010; Kowshik et al. 2002). Cunningham and Lundie found that *Clostridium thermoaceticum* could precipitate CdS on the cell surface as well as in the medium from $CdCl_2$ in the presence of cysteine hydrochloride in the growth medium where cysteine most probably acts as the source of sulfide (Cunningham and Lundie 1993). Dameron et al. (1989) have used *Schizosaccharomyces pombe* and *Candida glabrata* (yeasts) to produce intracellular CdS nanoparticles with cadmium salt solution. ZnS and PbS nanoparticles were successfully synthesized by biological systems. *Rhodobacter sphaeroides* and *Desulfobacteraceae* have been used to obtain ZnS nanoparticles intracellularly with 8 nm and 2–5 nm in average diameter, respectively (Bai et al. 2006; Labrenz et al. 2000).

Kang et al. (2008) reported phytochelatin-mediated intracellular synthesis of CdS nanocrystals in engineered *E. coli*. By controlling the population of the phytochelatin (PCs), *E. coli* cells were engineered as an eco-friendly biofactory to produce uniformly sized PC-coated CdS nano-crystals. This is the first systematic approach toward tunable synthesis of semiconductor nano-crystals by genetically engineered bacteria. The first report on the production of semiconductor nano-crystal synthesis in bacteria was published by Sweeney et al. (2004). The study revealed that *E. coli* has the endogenous ability to direct the growth of nano-crystals. Parameters such as growth phase and strain type are essential for initiating nano-crystal growth. El-Shanshoury et al. (2012) reported a rapid and low-cost biosynthesis of CdS using culture supernatants of *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Lactobacillus acidophilus* DSMZ 20079T. The CdS nanoparticles synthesis were performed at room temperature and were formed within 24 h. The process of extracellular and fast biosynthesis may help in the development of an easy and eco-friendly route for synthesis of CdS nanoparticles. Mousavi et al. (2012) reported the synthesis of CdS nanoparticles using *Escherichia coli* PTTC 1533 and *Klebsiella pneumoniae* PTTC 1053. The synthesis of 5-200 nm nanoparticles occurred after 96 h of incubation at 30 °C and pH 9.

A plausible mechanism has been made to understand the synthesis of CdS nanoparticles (Fig. 1.17). At the initial phase of the reaction, $CdCl_2$ dissociates into Cd^{2+} and accumulates around the bacterial membrane because of the negative potential of the bacterial membrane (Triphati et al. 2014). Cd^{2+} being a heavy metal ion creates stress conditions for bacteria, thus leading bacterial biomass to initiate a defense mechanism. This leads bacteria to secrete certain enzymes/proteins in order to detoxify the metal ions that created the metal stress condition. The secreted protein by bacterial biomass binds up with Cd^{2+}. Subsequently, Na_2S being added dissociates into S^{2-} in the solution and it also binds with the protein. The CdS nuclei then grow following the process of Ostwald ripening leading to the formation of CdS nanoparticles. Thus, protein secreted in this process becomes incorporated as it serves a capping layer for synthesis of CdS nanoparticles (Triphati et al. 2014).

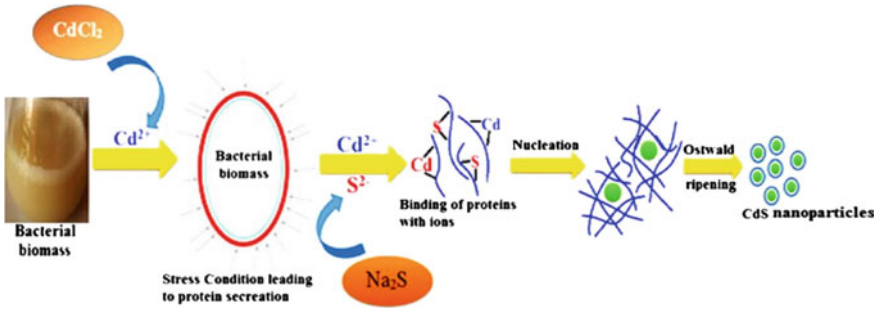

Fig. 1.17 Biosynthesis mechanism of CdS nanoparticles. *Source* Tripathi et al. (2014). Copyright © 2014, IOP Science

1.5.2 Lead Sulfide (PbS) Nanoparticles

Semiconductor PbS nanoparticles have attracted great attention in recent decades as a result of their interesting optical and electronic properties, and some of them are used for the fabrication of devices size (Bai and Zhang 2009). Some new chemical methods for the preparation of lead sulfide nanoparticles require special container, high temperature or long time for initiating the reaction. Biological synthesis method is one of the promising methods due to requiring a relatively milder condition, one step synthetic procedure, clean, and controllable size distribution. An earlier study found that *Torulopsis* sp. is capable of synthesizing PbS nanocrystals intracellularly when challenged with Pb^{2+}. PbS nanoparticles were also synthesized by using *Rhodobacter sphaeroides*, whose diameters were controlled by the culture time (Bai and Zhang 2009).

1.5.3 Iron Sulfide Nanoparticles

Ahmad et al. (2002) have found eukaryotic organisms such as fungi to be a good candidate for the synthesis of metal sulfide nanoparticles extracellularly. Another kind of sulfide nanoparticle was magnetic Fe_3S_4 or FeS nanoparticle. Bazylinski et al. (1995) reported the formation of Fe_3S_4 by uncultured magnetotactic bacteria. They examined a sediment sample that contained approximately 1×10^5 magnetotactic bacteria per cm^3, and approximately 10^5 cells were obtained after purification by the racetrack method. Magnetosomes in the uncultured cells exhibited elongated rectangular shape. The average magnetosome number per cell was approximately 40, and they were mainly located as a large cluster within the cell. Aligned magnetosomes forming a chainlike structure were also observed beside the large cluster. Sulfate-reducing bacteria were capable of producing magnetic FeS nanoparticles (Watson et al. 1999).

1.5.4 Copper Sulfide Nanoparticles

Copper sulfide (CuS) nanoparticles have attracted increasing attention from biomedical researchers across the globe, because of their intriguing properties which have been mainly explored for energy- and catalysis-related applications to date. Recently, CuS nanoparticles are gradually emerging as a promising platform for sensing, molecular imaging, photothermal therapy, drug delivery, as well as multifunctional agents that can integrate both imaging and therapy (Goel et al. 2014). Although copper sulfide nanoparticles have been previously synthesized by several chemical, electrochemical, organic and enzymatic methods, the first report on a biosynthesis approach was published in 2012 by Hosseini et al. in which the copper sulfide nanoparticles were synthesized from a pure copper sulfate solution by *F. oxysporum*. Industrially, the heavy metals in wastewaters are precipitated in the sulfide forms via dissimilatory reduction of sulfate that is performed by anaerobes. However, the performance of these sulfate-reducing bacteria is limited to anaerobic environments. Metal sulfides are also formed from sulfate by assimilatory sulfate reduction (Tiquia 2008; Tiquia et al. 2006) performed via aerobic pathways by overproducing two unique enzymes called serine acetyltransferase (SAT) and cysteine desulfhydrase. The precursor of cysteine biosynthesis (O-acetylserine) is produced by the acetylation of serine that is catalyzed by SAT, but a single amino acid change renders the SAT insensitive to feedback inhibition by cysteine that results in cysteine overproduction by the microorganism. Then the excess cysteine is converted into pyruvate, ammonia and sulfide ions by cysteine desulfhydrase. Finally, the secreted sulfide precipitates metal ions like copper and removes it from the solution as copper sulfide nanoparticles. Schaffie and Hosseini (2014) demonstrated in their study that is feasible to produce copper sulfide nanoparticles from acid mine drainage (AMD) through a biological approach. The properties of the produced nanoparticles were the same as the nanoparticles synthesized from the pure copper sulfate solution. These nanoparticles have the same composition like covelite and an average size of 10–40 nm.

1.5.5 Silver Sulfide Nanoparticles

Silver sulfide nanoparticles possess unique semi-conducting, optical, and electrical properties and are highly stable. Owing to these features, they are broadly used in solar cell batteries (Tubtimtae et al. 2010), thermoelectric sensors, etc. (Yan et al. 2011). The recently obtained Ag_2S/graphene nanocomposite is promising for the development of super capacitors (Mo et al. 2012). The great potential of practical applications of Ag_2S nanoparticles brought into existence numerous protocols for their preparation. The thermolysis of silver xanthates with long aliphatic chains at 200 °C brings about egg shaped particles with a narrow range of sizes (Zhang et al. 2012). Rod shaped Ag_2S nanocrystals have been obtained from silver nitrate and

thioacetamide (Zhao et al. 2007). Lea fshaped Ag_2S nanolayers can be produced by autoclaving an ethanol solution of silver nitrate and carbon disulfide at 160 °C (Chen et al. 2008). Bacteria of the genus *Shewanella* are commonly used in the preparation of nanoparticles of metals, oxides, and sulfides (Perez-Gonzalez et al. 2010). These bacteria can reduce many substances, including metal oxides, nitrates, sulfates, etc. They have been employed in the synthesis of gold nanoparticles (Suresh et al. 2011), arsenic sulfide nanotubes (Jiang et al. 2009), and uranium dioxide nanoparticles (Burgos et al. 2008). Debabova et al. (2013) synthesize Ag_2S nanoparticles using the metal-reducing bacterium *Shewanella oneidensis* MR1 an aqueous solution of $AgNO_3$ and $Na_2S_2O_3$ at an ordinary temperature and pressure. The nanoparticles vary in size within 2–16 nm, and the fraction 6–12 nm in size constitutes about 70 %. The maximum yield of nanoparticles in silver equivalent is 53 %. Being visualized by transmission electron microscopy, the particles look like spheres with average diameters varying from 7 ± 2 to 9 ± 2 nm. The elemental composition of synthesized nanoparticles has been analyzed by energy dispersive X-ray spectroscopy, and the estimated silver to sulfur atomic ratio is 2:1. The presence of living bacterial cells is mandatory for the formation of Ag_2S nanoparticles in the aqueous salt solution. Changes in the reaction conditions (reagent concentrations, temperature, and cell incubation time in the reaction mixture) influence the yield of nanoparticles dramatically, but have little influence on their size.

1.5.6 Zinc Sulfide (ZnS) Nanoparticles

Zinc sulfide (ZnS), are the most attractive materials for applications in areas such as IR optical devices and fast optical switching devices. Zinc sulfide nanoparticles can be prepared by different methods, such as colloidal aqueous and micellar solution synthesis method (Khiewa et al. 2005), using ultrasonic waves (Behboudnia et al. 2005), microwaves (Ni et al. 2004), and gamma-irradiation (Qiao et al. 2000). In most cases, particles prepared by these methods have some problems including poor reproducibility, control of particle size, distribution and shape. Some reactions require high temperature, and/or high pressure for initiating the reaction, and/or inert atmosphere protection, and/or using toxic matters such as H_2S, toxic template and stabilizer, and metallic precursors. When zinc sulfide nanoparticles are used as biological probes in clinic examinations, the synthesis of zinc sulfide is expected to be clean (Dubertret et al. 2002). Consequently, researchers in nanoparticles synthesis have turned to biological systems for inspiration. Spherical aggregates of 2–5 nm sphalerite ZnS particles were formed by sulfate-reducing bacteria under anaerobic conditions (Mandal et al. 2006).

A novel, clean biological transformation reaction by immobilized *Rhodobacter sphaeroides* has been developed for the synthesis of zinc sulfide (ZnS) nanoparticles was developed by Bai et al. (2006). *Rhodobacter sphaeroides* is a purple, non-sulphur, photosynthetic bacterium. It can grow not only aerobically

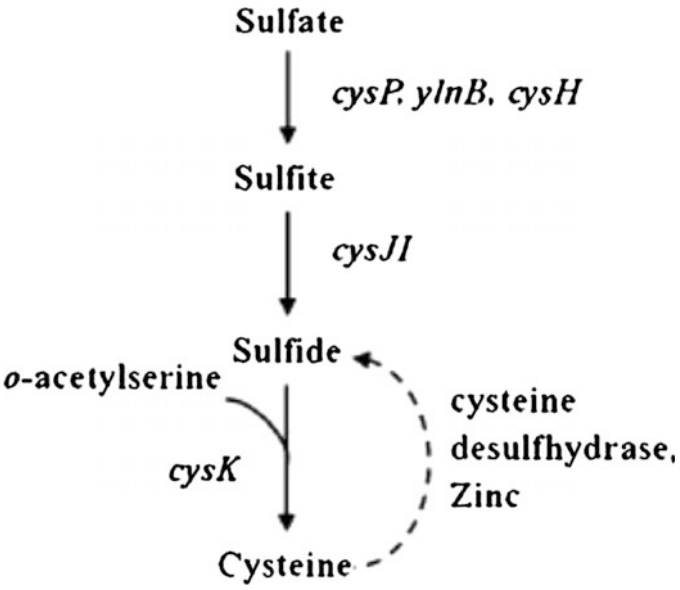

Fig. 1.18 Sulfate assimilation action of *Rhodobacter sphaeroides*. The enzymes present in *Rhodobacter sphaeroides* are indicated by the corresponding genes: *cys*P, sulfate permease; *yln*B, ATP-sulfurylase; *cys*H, phosphoadenosine phosphosulfate reductase; *cys*JI, sulfite reductase; *cys*K, o-acetylserine synthase. *Source* Bai et al. (2006). Copyright © 2013, Springer. Reproduced with permission

in the dark but also anaerobically in the light and has tolerance to heavy metals (Giotta et al. 2006). In the biological synthetic process for ZnS nanoparticles by *Rhodobacter sphaeroides*, soluble sulfate acts as the source of sulfur. The formation mechanism of ZnS nanoparticles by biological transformation reaction of Rhodobacter sphaeroides can be explained in (Fig. 1.18). First, the soluble sulfate enters into immobilized beads via diffusion, and later is carried to the interior membrane of *Rhodobacter sphaeroides* cell facilitated by sulfate permease Then, the sulfate is reduced to sulfite by ATP sulfurylase and phosphoadenosine phosphosulfate reductase, and next sulfite is reduced to sulfide by sulfite reductase. The sulfide reacts with O-acetylserine to synthesize cysteine via O-acetylserine thiolyase (Holmes et al. 1997; Auger et al. 2005), and then cysteine produces S^{2-} by a cysteine desulfhydrase in presence of zinc. After this process, S^{2-} reacts with the soluble zinc salt and the ZnS nanoparticles are synthesized. Finally, ZnS nanoparticles are discharged from immobilized cells to the solution. In the synthetic process, the particle size is controlled by the culture time of the *Rhodobacter sphaeroides*, and simultaneously the immobilized beads act on separating the ZnS nanoparticles from the *Rhodobacter sphaeroides*. Although the detailed mechanism study of this process is in progress, it suggests that many other high grade binary metal sulfides can also be produced using this method (Bai et al. 2006).

1.5.7 Antimony Sulphide (Sb_2S_3) Nanoparticles

Sb_2S_3 exhibits important applications in photovoltaic, photosensors, optical nanodevices, and photoelectronics. It has been are used in electronics as poor conductors of heat and electricity. Very pure antimony is used to make certain types of semiconductor devices, such as diodes and infrared detectors (Grigorescu and Stradling 2001; Wei et al. 2006). Antimony, as a metallic form, is not soluble in body fluids and, as reported in old literature, cannot produce any effect on human system (Filella et al. 2002a, b). In contrast, organic or inorganic salts of antimony can be decomposed by the fluids and have been used for therapeutic purposes (Filella et al. 2002a, b; Johnson et al. 2005). Antimony salts are currently chosen for the treatment of leishmaniasis, a disease that affects 12 million people annually around the world (Berman 1997; Haldar et al. 2011). Moreover, trivalent antimony compounds have been used in treating bilharzia, trypanosomiasis and kala-azar for more than a century (Berman 1997; Isago et al. 2008).

Bahrami et al. (2012) explored the biological synthesis of antimony sulfide using *Serratia marcescens*. The bacterium was isolated from the Caspian Sea in northern Iran and was used for intracellular biosynthesis of antimony sulfide nanoparticles. This isolate was identified as nonpigmented *Serratia marcescens* using conventional identification assays and the 16S rDNA fragment amplification method, and was used to prepare inorganic antimony nanoparticles. Antimony-supplemented nutrient agar (NA) plates ($SbCl_3$, 1 % w/v) were inoculated with the bacterial isolate. These inoculated NA media were incubated aerobically at 30 °C. After 72 h, bacterial cells were harvested from the surface of culture media. The biogenic nanoparticles were released by liquid nitrogen and extracted using two sequential solvent extraction systems. The energy-dispersive x-ray demonstrated that the extracted nanoparticles consisted of antimony and sulfur atoms. The transmission electron micrograph showed the small and regular non-aggregated nanoparticles ranging in size less than 35 nm. Although the chemical synthesis of antimony sulfide nanoparticles has been reported in the literature, the biological synthesis of antimony sulfide nanoparticles has not previously been published. This is the first report to demonstrate a biological method for synthesizing inorganic nanoparticles composed of antimony (Bahrami et al. 2012).

1.6 Palladium and Platinum Nanoparticles

Palladium is an excellent hydrogenation and dehydrogenation catalyst available in organo-metallic forms. Palladium nanoparticles have found to be effective catalysts in a number of chemical reactions due to their increased surface area over the bulk metal. The sulfate-reducing bacterium, *Desulfovibrio desulfuricans* and metal iron-reducing bacterium, *Shewanella oneidensis*, were capable of reducing soluble

palladium (II) into insoluble palladium (0) with formate, lactate, pyruvate, or H_2 as the electron donor (Lloyd et al. 1998; Yong et al. 2002a; de Windt et al. 2005).

Coker et al. (2010) demonstrated a novel biotechnological route for the synthesis of heterogeneous catalyst, consisting of reactive palladium nanoparticles arrayed on a nanoscale biomagnetite support. The magnetic support was synthesized at ambient temperature by the Fe (III)-reducing bacterium *Geobacter sulfurreducens*. The palladium nanoparticles were deposited on the nanomagnetite using simple one-step method to an organic coating priming the surface for Pd adsorption, which was produced by the bacterial culture during the formation of the nanoparticles. A recent biological method used to produce palladium nanoparticles is the precipitation of Pd on a bacterium. These palladium nanoparticles can be applied as catalyst in dehalogenation reactions. Large amounts of hydrogen are required as electron donors in these reactions, resulting in considerable cost. A study carried out by Hennebel et al. (2011) demonstrates how bacteria is cultivated under anaerobic conditions and can be used to reductively precipitate the palladium catalysts and generate the hydrogen (electron donor). This avoids the cost coupled to hydrogen supply. Batch reactors with nanoparticles formed by *Citrobacter braakii* showed the highest diatrizoate dehalogenation (Hennebel et al. 2011).

Konishi et al. (2007b) demonstrated that resting cells of *Shewanella algae* were able to deposit platinum NPs by reducing $PtCl_6^{2-}$ ions within 60 min at pH 7 and 25 °C. Biogenic platinum NPs of about 5 nm were located in the periplasmic space. In this case, the cell suspension changed the color from pale yellow to black in 10 min. The black appearance provided a convenient visible signature for the microbial formation of metallic platinum NPs. The observed decrease in aqueous platinum concentration was presumably caused by the rapid reduction of $PtCl_6^{2-}$ ions into insoluble platinum. In the absence of lactate, however, *S. algae* cells were not able to reduce the $PtCl_6^{2-}$ ions. They reported that the $PtCl_6^{2-}$ ions were not chemically reduced by lactate. Yong et al. (2002b) also reported that the sulfate-reducing bacterium *Desulfovibrio desulfuricans* was able to adsorb only 12 % of platinum (IV) ions on the bacterial cells from 2 mM platinum chloride solution. In another study, Gram-negative cyanobacterium, *Pediastrum boryanum* UTEX 485, extracellularly produced Pt (II)-organics and metallic platinum NPs at 25–100 °C for up to 28 days and 180 °C for 1 day with different morphologies of spherical, bead-like chains and dendritic in the size range of 30 nm–0.3 μm (Lengke et al. 2006c).

1.7 Selenium Tellurium Nanoparticles

Selenium is of considerable environmental importance as it is essential at low concentrations but toxic at high concentrations for animals and humans, with a relatively small difference between these values (Fordyce 2005). Selenium occurs in different oxidation states as reduced form (selenide, Se^{2-}), least mobile elemental selenium (Se^0) and water soluble selenite (SeO_3^2)/selenate (SeO_4^2) oxyanions.

Selenium possesses several applications in medicine, chemistry and electronics. Selenium (Se), as a functional material, is an important semiconductor and photoelectric element due to its special physical properties (Zhang et al. 2011). Therefore, Se is used in many applications ranging from photocells, photographic exposure meters and solar cells to semiconductor rectifiers. Recently, there has been increasing interest in the synthesis of nanoparticles using microorganisms, leading to the development of various biomimetic approaches (Mohampuria et al. 2008). However, most methods used to synthesize SeNPs are characterized by elevated temperatures and high pressures and are hazardous to the environment (Zhang et al. 2011).

Stenotrophomonas maltophilia SELTE02 showed promising transformation of selenite (SeO_3^{-2}) to elemental selenium (Se^0) accumulating selenium granules either in the cell cytoplasm or in the extracellular space. In addition, *Enterobacter cloacae* SLD1a-1, *Rhodospirillum rubrum*, and *Desulfovibrio desulfuricans* have also been found to bioreduce selenite to selenium both inside and outside the cell with various morphologies like spherical, fibrillar, and granular structure or with small atomic aggregates. *E. coli* also deposited elemental selenium both in periplasmic space and cytoplasm, and *P. stutzeri* also aerobically reduced selenite to elemental selenium (Narayanan and Sakthivel 2010). Under aerobic conditions, Hunter and Manter (2008) reported that *Tetrathiobacter kashmirensis* bioreduced selenite to elemental red selenium. A 90-kDa protein present in the cell-free extract was believed to be responsible for this bioreduction. Moreover, Yadav et al. (2008) showed that *P. aeruginosa* SNT1 biosynthesized nanostructured selenium by biotransforming selenium oxyanions to spherical amorphous allotropic elemental red selenium both intracellularly and extracellularly.

Oremland et al. (2004) reported the biogenesis of SeNPs under anaerobic conditions. Se^0 nanoparticles (SeNPs) formed by the Se-respiring bacteria, such as *Sulfurospirillum barnesii*, *Bacillus selenitireducens*, and *Selenihalanaerobacter shriftii*, are structurally unique when compared to Se^0 formed by chemical synthesis. However, anaerobic conditions have limitations, such as culture conditions and isolate characteristics that make biosynthesis processes tedious and challenging (Prakash et al. 2009). The generation of SeNPs by soil bacteria *Pseudomonas aeruginosa* and *Bacillus* sp. under aerobic conditions has been reported; however, these studies only include the partial characterization of selenium nanospheres (Yadav et al. 2008; Prakash et al. 2009). The characterization of the nanospheres in relation to size is of great importance, both in industrial and biologic activities. Recent reports describe that Se^0 nanoparticles with a size under 100 nm have a greater bioavailability (Thakkar et al. 2009; Dhanjal and Cameotra 2010). In addition, other studies mention that a smaller size increases the ability to trap free radicals with greater antioxidant effect (Huang et al. 2003). Peng et al. (2007) mentioned that the size of Se^0 nanoparticles plays an important role in their biologic activity and, as expected, 5–200 nm nano-Se can directly scavenge free radicals in vitro in a size dependent fashion. The bio-reduction of selenite (Se [IV]) by *Pantoea agglomerans* generates nanoparticles with sizes ranging between 30 and 300 nm was reported by Torres et al. (2012). Their study demonstrated that,

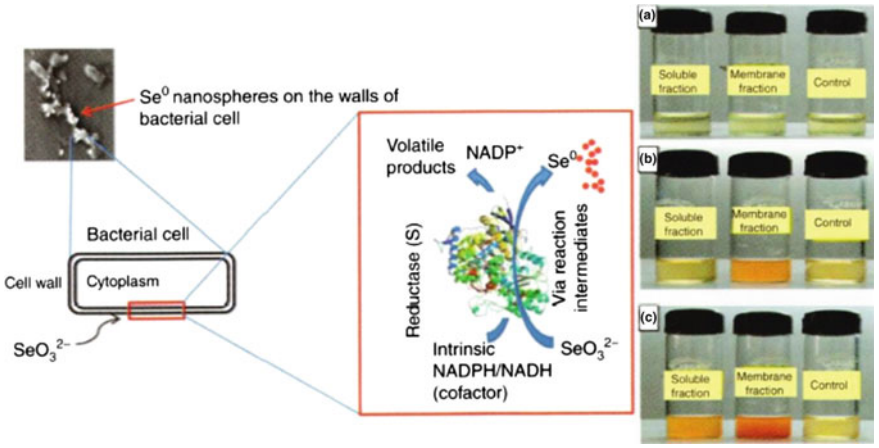

Fig. 1.19 Proposed mechanism for biogenesis of selenium nanospheres at different time intervals: **a** selenite reduction at 0 h; **b** formation of red elemental selenium in membrane fraction after 3–4 h of incubation; and **c** insoluble fraction after 12 h of incubation. *Source* Dhanjal and Cameotra (2010). Copyright © 2010, BioMed Central. Reproduced with permission

Pantoea agglomerans strain UC-32 produce amorphous SeNPs under aerobic conditions with a size optimal for biotechnological use (such as trapping free radicals in the induction of selenoenzymes) after at least 20 h of incubation. These results are of great importance due to the low culture requirements of UC-32 strain with the subsequent low cost of biologically active SeNPs production.

Synthesis of S^0 under aerobic conditions by *Bacillus cereus* was investigated by Dhanjal and Cameota (2010). The aerobic reduction of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) to Se^0 is depicted in Fig. 1.19. The electron transfer was initiated from NADPH/NADH by NADPH/NADH-dependent electron carrier. The results show: (1) selenite reduction in 0 h; (2) formation of red elemental selenium in membrane fraction after 3–4 h of incubation; and (3) prolonged incubation for 12 h resulted in formation of red elemental selenium in soluble fraction.

Tellurium (Te) has been reduced from tellurite to elemental tellurium by two anaerobic bacteria, *Bacillus selenitireducens* and *Sulfurospirillum barnesii*. *B. selenitireducens* initially formed nanorods of 10 nm in diameter and 200 nm in length were clustered together to form larger rosettes of $\sim$1000 nm but with *S. barnesii* small irregularly shaped extracellular nanospheres of diameter <50 nm were formed (Baesman et al. 2007). In another study, tellurium-transforming *Bacillus* sp. BZ isolated from the Caspian Sea in northern Iran was used for the intracellular biosynthesis of elemental tellurium NPs. The biogenic NPs were released by liquid nitrogen and purified by an n-octyl alcohol water extraction system. TEM analysis showed rod-shaped NPs with dimensions of about 20 × 180 nm. The produced NPs had a hexagonal crystal structure (Zare et al. 2012).

1.8 Bismuth Nanoparticles

The bismuth nanoparticles (BiNPs) has attracted a great deal of interest because of its potential applications in X-ray radiation therapy, catalysts, thermoelectricity, and optical uses (Hossain and Su 2012; Wang and Buhro 2010; Carotenuto et al. 2009; Lin et al. 2011). BiNPs electrodes have been applied in the detection of heavy metal ions as a substitute for bismuth film (Sahoo et al. 2013) In addition, bismuth compounds, such as $BiPO_4$ (Pan et al. 2010), $BiVO_4$ (Qu et al. 2013), Bi_2O_3, (Zhou et al. 2009) and Bi_2S_3 nanoparticles (Wu et al. 2010b) were also reported over the past decades as novel catalysts for photodegradation of environmental pollutants. Several approaches have been employed to fabricate BiNPs including thermal plasma (Wang et al. 2007) an electrochemical method (Reim et al. 2013) a gas condensation method (Lee et al. 2007) and solution phase chemical methods. The latter is the most popular method, which often involves the reduction of relevant metal salts with various reductants in the presence of morphology-controlling surfactants.

The biological synthesis of BiNPs was explored using *Serratia marcescens* by Nazari et al. (2012). The biogenic bismuth NPs were released by liquid nitrogen and purified using an n-octanol water two-phase extraction system. The energy-dispersive X-ray and X-ray diffraction (XRD) patterns demonstrated that the purified NPs consisted of only bismuth and are amorphous. In addition, the transmission electron micrograph showed that the small NPs formed larger aggregated NPs around 150 nm.

1.9 Conclusions and Future Perspectives

Biological systems; bacteria, fungi, actinomycetes, and algae have many opportunities for utilization in nanotechnology, especially in the development of a reliable and eco-friendly processes for the synthesis of metallic nanoparticles. The rich microbial diversity points to their innate potential for acting as potential biofactories for nanoparticles synthesis. Despite some related reports, many aspects of nanoparticles biosynthesis remain unclear especially with regards to why and how the size and shapes of the synthesized nanoparticles are influenced by the biological systems. The biochemical and molecular mechanisms of biosynthesis of metallic nanoparticles need to be better understood to improve the rate of synthesis and monodispersity of the product. The properties of NPs can be controlled by optimization of important parameters which control the growth condition of organisms, cellular activities, and enzymatic processes (optimization of growth and reaction conditions). The large-scale synthesis of NPs using microorganisms is interesting because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes. It seems that by optimizing the reaction conditions and selecting the best microbes, these natural nanofactories can be used in the synthesis of stable NPs with well-defined sizes, morphologies, and compositions.

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Chapter 2
Halophiles in Nanotechnology

Abstract Halophiles are salt loving organisms that flourish in saline environments such as marine and estuarine environments, solar salterns, salt lakes, brines and saline soils. They offer potential applications in various fields of biotechnology. They can be used as a source of metabolites, compatible solutes and other compounds of industrial value. The biodegradation of organic pollutants in hypersaline environments and treatment of saline effluents contaminated with organic by halophiles have been investigated. Some halophiles are a potential source of extracellular hydrolases like proteases with a wide array of industrial applications. These enzymes exhibit stability over a range of saline conditions and harsh conditions of pH or/and ionic strength. Recently, they are being explored as potential sources of metal tolerant microorganisms with the ability to synthesize metallic nanoparticles. This chapter covers the various halophilic organisms and their by-products that have been exploited for nanomaterial synthesis, the mechanisms that may be involved in the nanomaterial fabrication and the possible applications of the fabricated nanoparticles.

2.1 Introduction

Halophiles are salt loving organisms that flourish in saline environments. They include mainly prokaryotic and eukaryotic microorganisms with the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salts. Currently, over 182 species distributed in 48 validly described genera in *Halobacteriaceae* cover aerobic halophiles and a few anaerobic and halophilic methanogens are known in *Archaea* domain. The number of halophiles in *Bacteria* domain is higher and they are distributed in many groups (phyla). Representatives of halophilic bacteria are included in Phylum *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria*, *Firmicutes* and *Sulphur-Green* bacteria. Furthermore, halophilic Fungi, Plants, Ciliates and Flagellates have also been known in *Eucarya* domain. These organisms cope with harsh environmental conditions by two strategies, namely salt-in and compatible solutes (Oren 1999a, b, 2002a, b). The salts are

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accumulated inside of cells to osmolarity equivalent with external environment in first strategy, and synthesis of organic molecules known as compatible solutes occurs or they are accumulated from environments if they are present in the second strategy (Oren 1999a, b). Special adaptations are requested for proteins and enzymes in case of fi rst strategy, and the most well-known fact is the increasing acidic amino acids residues on their surface (Lanyi 1974; Graziano and Merlino 2014). Among halophilic microorganisms are a variety of heterotrophic and methanogenic archaea; photosynthetic, lithotrophic, and heterotrophic bacteria; and photosynthetic and heterotrophic eukaryotes (DasSarma 2009; DasSarma and DasSarma 2012). Halophiles can be loosely classified as slightly, moderately or extremely halophilic, depending on their requirement for NaCl. The extremely halophilic archaea, in particular, are well adapted to saturating NaCl concentrations and have a number of novel molecular characteristics, such as enzymes that function in saturated salts, purple membrane that allows phototrophic growth, sensory rhodopsins that mediate the phototactic response, and gas vesicles that promote cell flotation.

The saline environments that halophiles inhabit include the marine and estuarine environments, solar salterns, salt lakes, brines and saline soils (Oren 1999a, b, 2002a, b; Tiquia et al. 2007; DasSarma and DasSarma 2012). In the latter, the matrix potential of the soil adds to the water stress caused by high salt concentrations. High saline waters originate either by seawater condensation (thalassohaline) or by evaporation of inland surface water (athalassohaline). The salt composition of thalassohaline waters resembles that of seawater with NaCl as the main constituent. Athalassohaline lakes can differ in their ion composition from seawater derived lakes. Some athalassohaline waters have a very high concentration of divalent cations (for example, the Dead Sea with the main cation Mg^{2+} instead of Na^{+}), while others are free of magnesium and calcium due to the presence of high levels of carbonate. Increased carbonate concentrations lead to the formation of soda lakes, which have pH-values well above 10 (for example, the Wadi Natrun in Egypt). Microflora have been found in all of the above types of saline waters, indicating that halophilic microorganisms tolerate high salinity and can adapt to different stressors like high pH or extreme temperatures (Kunte et al. 2002). The salinity of saline environments is generally understood by biologists in terms of sodium chloride content. This compound represents over 90 % of the total salt content in many cases and the presence of other compounds in terms of influence to physical or chemical parameters of the saline area could be considered as low. On the other hand, these compounds could have a signifi cant infl uence on the diversity of halophilic microorganisms if we considered the high concentrations of magnesium in Dead Sea or carbonates in soda lakes such as Magadi, in Kenya, Wadi Natrum in Egypt, Sambhar, India (Enache et al. 2015). The predominant microorganisms in the saline environments are represented by both halophilic archaea and bacteria (DasSarma and DasSarma 2012).

Halophilic bacteria grow over an extended range of salt concentrations (3–15 % NaCl, w/v and above), unlike the truly halophilic archaea whose growth is restricted to high saline environments (Litchfield 2002). According to Kushner (1978), many

marine organisms are slight halophiles (with 3 % w/v NaCl in seawater). Moderate halophiles optimally grow at 3–15 % w/v NaCl; extreme halophiles at 25 % w/v NaCl (halobacteria and halococci) and borderline extreme halophiles require at least 12 % w/v salt. Halophilic microorganisms usually adopt either of the two strategies of survival in saline environments: 'compatible solute' strategy and 'salt-in' strategy (Ventosa et al. 1998). Compatible solute strategy is employed by the majority of moderately halophilic and halotolerant bacteria, some yeasts, algae and fungi. In this strategy cells maintain low concentrations of salt in their cytoplasm by balancing osmotic potential through the synthesis or uptake of organic compatible solutes. Hence these microorganisms are able to adapt to a wide range of salt concentrations. The compatible solutes include polyols such as glycerol, sugars and their derivatives, amino acids and their derivatives, and quaternary amines such as glycine betaine and ectoines. The salt-in strategy is employed by true halophiles, including halophilic archaea and extremely halophilic bacteria. These microorganisms are adapted to high salt concentrations and cannot survive when the salinity of the medium is lowered. They generally do not synthesize organic solutes to maintain the osmotic equilibrium. This adaptation involves the selective influx of K^+ ions into the cytoplasm. All enzymes and structural cell components must be adapted to high salt concentrations for proper cell function (Shivanand and Mugeraya 2011).

Halophilic bacteria offer potential applications in various fields of biotechnology (Tiquia and Mormile 2010; Margesin and Schinner 2001). These microorganisms can be used as a source of metabolites, compatible solutes and other compounds of industrial value. Novel halophilic biomolecules may also be used for specialized applications, e.g. bacteriorhodopsin for biocomputing, pigments for food colouring and compatible solutes as stress protectants (DasSarma and DasSarma 2012). Biodegradation of organic pollutants by halophilic bacteria and archaea has been reviewed (Borgne et al. 2008; Tiquia 2010). These microorganisms are good candidates for the bioremediation of hypersaline environments and treatment of saline effluents. Understanding the degradation process would also shed light on the enzymes involved and on the regulation of metabolism. Halophilic bacteria are a potential source of extracellular hydrolases like proteases with a wide array of industrial applications. These enzymes exhibit stability over a range of saline conditions (Shivanand and Jayaraman 2009).

Halophiles organisms represent a valuable resource of enzymes (extremozymes) with stability in harsh conditions of pH or/and ionic strength. Thus, their investigations as biocatalysts in the presence of novel nanomaterials are attractive. Several biomolecules produced by these halophilic organisms, i.e. enzymes, halocins (halobacterial proteins with antibiotic activities), exopolysaccharides etc. show biological activity in harsh conditions. Combining of these bio-molecules with various nanomaterials like thin-layers, nanotubes, nanospheres results in novel compounds harboring both biological properties of biomolecules and physico-chemical characteristics of nanomaterials. Recently, they are being explored as potential sources of metal tolerant microorganisms with the ability to synthesize metallic nanoparticles (Agnihotri et al. 2009; Kathiresan et al. 2009; Venkatpurwar

and Pokharkar 2011; Ali et al. 2011). This chapter covers the various halophilic organisms and their by-products that have been exploited for nanomaterial synthesis, the mechanisms that may be involved in the nanomaterial fabrication and the possible applications of the fabricated nanoparticles.

2.2 Nanoparticle Synthesis by Halophiles

Marine environments could be good source of metal tolerant microbes as most of these organisms exist at the bottom of the sea, and contribute towards biogeochemical cycling of inorganic elements. Besides, the marine econiche is continuously exposed to metallic pollution due to volcanic eruptions, natural weathering of the rocks, anthropogenic activities such as mining, combustion of fuels and industrial and urban sewage. Estuaries and solar salterns may also contain high concentrations of metals as they serve as effective traps for river borne metals (Chapman and Wang 2001). Thus, halophiles are continuously exposed to metals and could be exploited for nanoparticle synthesis. Nanoparticles synthesis by halophiles has been reported in few organisms like bacteria, archaea, fungi, and algae (Table 2.1).

2.2.1 Nanoparticle Synthesis by Halophilic Bacteria

Reports on nanoparticles synthesis by halophilic bacteria and their metabolites are mostly confined to metallic nanoparticles. These halophilic bacteria include, *Halomonas salina*, *H. maura*, *Pseudomonas* sp., and *Idiomarina* sp. PR-58-8 (Table 2.1). A highly silver tolerant halophilic marine bacterium *Idiomarina* sp. PR 58-8 synthesizes intracellular crystalline silver nanoparticles (SNPs) with an average particle size of 26 nm. Non-protein thiols that are known to be expressed in response to metal stress are involved in metal tolerance (Seshadri et al. 2012). This bacterium synthesizes SNPs when silver is added at the time of inoculation unlike the terrestrial bacteria such as *Lactobacillus* sp. and *Escherichia coli*, wherein silver is added in the mid-log phase of growth. This is attributed to the high silver-tolerance of *Idiomarina* sp. PR58-8 and eliminates the requirement of growth phase monitoring during synthesis of SNPs. UV-visible absorbance scan of the 48 h culture from 300–800 nm revealed a broad peak at 450 nm, a characteristic of SNPs. XRD of lyophilized cell pellets obtained from 48 h cultures corresponded to silver (3C-syn) in the ICDD. TEM showed presence of SNPs in the 26 nm size range which upon purification could be applied in bio-labelling, antimicrobial coatings etc. The bacterium was found to respond to silver stress by inducing the expression of NP–SHs at extremely high levels (261 % on average) over the control, peaking at 42 h. Thus *Idiomarina* sp. PR58-8 is a promising microorganism for metal accumulation and metal nanoparticle synthesis.

Table 2.1 Halophiles in biosynthesis of nanoparticles

Halophile	NPs	Reference
Bacteria		
Idiomarina sp. PR 58-8	Ag	Seshadri et al. (2012)
Pseudomonas sp. *591786*	Ag	Muthukannan and Karuppiah (2011)
Halomonas salina	Au	Shah et al. (2012)
Bacillus megaterium BSB6	Se	Mishra et al. (2011)
Bacillus megaterium BSB12	Se	Mishra et al. (2011)
Archaea		
Halococcus salifodinae BK3	Ag	Srivastava et al. (2013)
Halococcus salifodinae BK18	Ag	Srivastava et al. (2014)
Fungi		
Penicillium fellutatum	Ag	Kathiresan et al. (2009)
Aspergillus niger	Ag	Kathiresan et al. (2010)
Pichia capsulata	Ag	Manivannan et al. (2010)
Yarrowia lipolytica	Ag	Bankar et al. (2009)
Yarrowia lipolytica	CdO	Pawar et al. (2012)
Yarrowia lipolytica	CdS	Pawar et al. (2012)
Rhodosporidium diobovatum	PbS	Seshadri et al. (2012)
Thraustochytrium sp	Ag	Gomathi (2009)
Algae		
Sargassum wightii	Ag	Singaravelu et al. (2007)
Sargassum wightii	Au	Oza et al. (2012)
Sargassum ongifolium	Au	Rajeshkumar et al. (2014)
Pterocladia capillacae	Ag	El-Rafie et al. (2013)
Jania rubins	Ag	El-Rafie et al. (2013)
Ulva faciata	Ag	El-Rafie et al. (2013)
Colpmenia sinusa	Ag	El-Rafie et al. (2013)
Navicula atomus	Au	Schrofel et al. (2011)
Diadesmis gallica	Au	Schrofel et al. (2011)

Similarly, a novel halophilic strain of *Pseudomonas* sp. 591786 can also synthesize polydisperse intracellular silver nanoparticles (Muthukannan and Karuppiah 2011). The silver nanoparticles formed were polydisperse, predominantly spherical with some nanotriangles in the range of 20–100 nm. Some small particles in the regime of 10–20 nm are also present. On treatment of aqueous solution of 1 mM silver nitrate with bacterial strain, SNPs can be rapidly fabricated at intracellular level. The formation of intracellular SNPs occurred only after 6 h of incubation.

The halophilic proteobacteria *Halomomas salina* can synthesize anisotropic gold nanoparticles under acidic conditions and spherical nanoparticles under alkaline conditions. The nanoparticle synthesis is extracellular and the NADH-dependent nitrate reductase is involved in the silver reduction and nanoparticle synthesis (Shah et al. 2012). *Halomonas salina* is a halophilic *Proteobacteria*, rich in reductases.

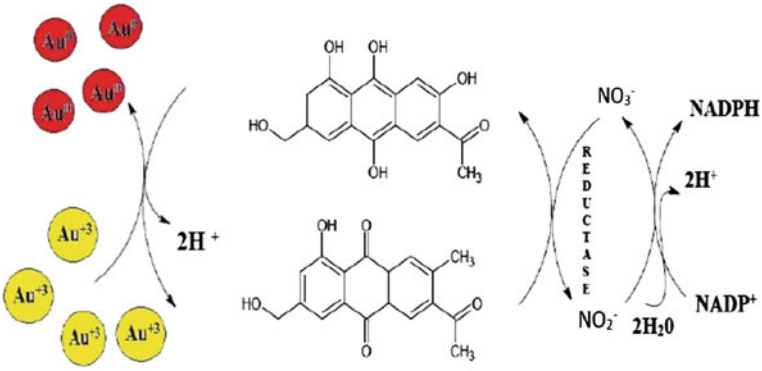

Fig. 2.1 Possible mechanism of reduction of gold salt and formation of gold nanoparticles. *Source* Shah et al. (2012). Copyright © 2012, Scholar Research Library

Studies have indicated that NADH and NADH-dependent nitrate reductase enzyme are important factors in the biosynthesis of metal nanoparticles. *Halomonas salina* is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, which may be acting as a scaffold or nucleating agent and might be responsible for the bioreduction of Au^{+3} to Au^0 and the subsequent formation of gold nanoparticles (Fig. 2.1). The same enzyme later then acts as a capping agent, thus ensuring complete formation of thermodynamically stable nanostructures.

Similarly, two halophilic strains of *Bacillus megaterium* BSB6 and *Bacillus megaterium* BSB12 isolated from Bhitarkanika mangrove soils, synthesize spherical selenium nanoparticles (SeNPs) both intra- and extra-cellularly with an average size of 200 nm). The mechanism involved for the reduction of selenite to selenium, however remains unexplored (Mishra et al. 2011). Both the strains were found capable of reducing Se(IV) to elemental selenium even in the presence of high salt concentrations. Under optimized set of conditions (at 37 C, initial pH 7.5) almost complete reduction of Se(IV) up to 0.25 mM was achieved within 40 h incubation. Microbial reduction of selenium oxyanions generates red elemental selenium particles with either crystalline or amorphous structure. Often Se^0 formed by bacterial reduction is structurally unique compared to elemental selenium formed by chemical synthesis (Oremland et al. 2004). The formation of selenium nanoparticles was also evident from the UV–Vis spectra (Fig. 2.2) of the centrifuged and washed culture with selenium particles. The absorption bands with maxima at 226 and 285 nm, located between 200 and 300 nm, was due to the formation of selenium nanoparticles from Se(IV).

The intracellular selenite reduction is usually driven by reduced thiols, e.g., glutathione, in microorganisms (Kessi and Hanselmann 2004; Kessi 2006). Selenite reacts with glutathione to form selenodiglutathione (GS-Se-SG), which can be further reduced by NADPH to unstable selenopersulfide (GS-Se–) in the presence of glutathione reductase. Then, dismutation of GS-Se– will produce GSH and Se^0. In addition to the thiol groups, terminal reductases for anaerobic respiration in some

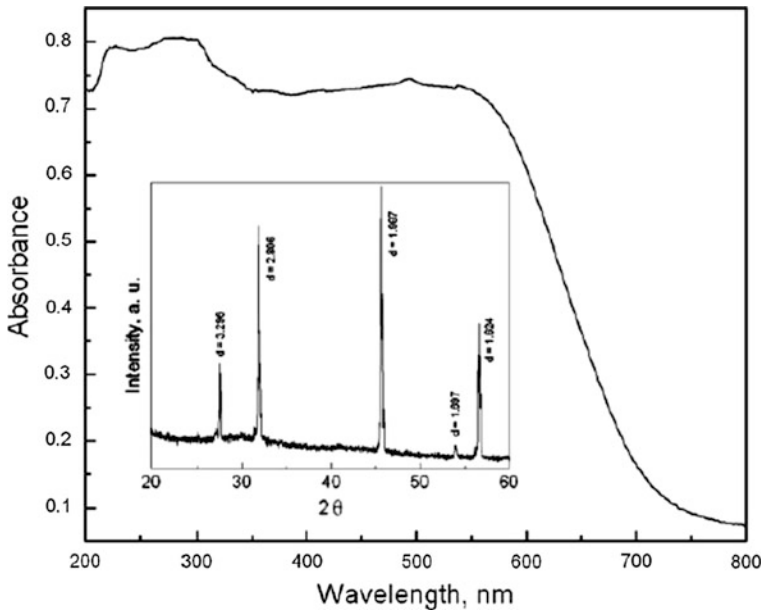

Fig. 2.2 UV–Visible diffuse reflectance spectra of Se^0 particles associated with *B. megaterium* (BSB12). (*Inset*) X-ray diffraction patterns of Se^0 particles associated with *B. megaterium* (BSB12). *Source* Mishra et al. (2011). Copyright © 2011, Elsevier. Reproduced with permission

microorganisms may also reduce selenite as they are redox-reactive in cells. It is reported that two nitrite reductases and an inducible sulfite reductase are able to conduct selenite reduction in cells (DeMoll-Decker and Macy 1993; Basaglia et al. 2007; Harrison et al. 1984). However, the possible involvement of other various respiration reductases in selenite reduction, as well as the physiological and ecological influence of this process to cells (Fig. 2.3). For instance in *Shewanella oneidensis* MR-1, selenite reduction is dependent on central respiration c-type cytochrome CymA. In contrast, nitrate reductase, nitrite reductase, and the Mtr electron transfer pathway do not work as selenite reductases (Li et al. 2014).

2.2.2 *Nanoparticle Synthesis by Halophilic Archaea*

Haloarchaea are the predominant population of thalassohaline and athalassohaline environments where salinity reaches up to 300 g/L (Zafrilla et al. 2010) and contribute to the red coloration of solar salt crystallizer. These organisms maintain osmotic balance with hypersaline surroundings by building up potassium ion concentration within their cells (Oren 2008). Haloarchaea are also known to encounter metals in their environment, but their metal tolerance has not been well documented (Srivastava and Kowshik 2013). Metal resistance genes have been

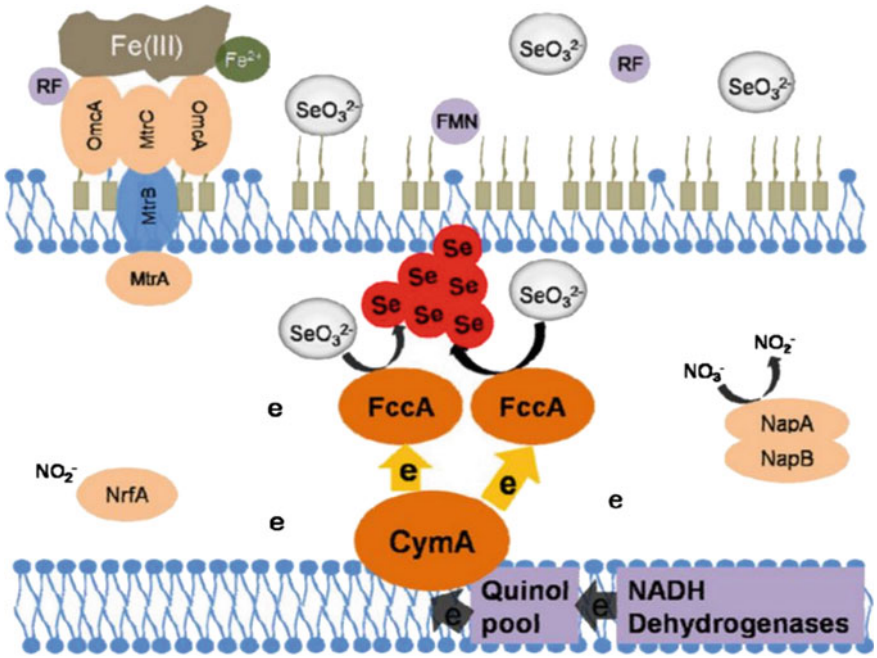

Fig. 2.3 Schematic diagram of the proposed pathways of selenite reduction and anaerobic respiration in *Shewanella oneidensis* MR-1. The oxidation of lactate provides electrons in the form of NADH, which further deliver the electrons to CymA through NADH dehydrogenases and the quinol pool. Shunt of electrons from CymA to various reductases enables execution of usual anaerobic respiration and selenite reduction. *Source* Li et al. (2014). Copyright © Scientific Reports (Springer Nature). Reproduced with permission

annotated in model organism *Halobacterium* sp. strain NRC-1 (Ng et al. 2000), but only arsenic resistance has been demonstrated experimentally (Wang et al. 2004). A system-level analysis of this organism has shown various strategies such as enhanced efflux and reduced influx that result in metal tolerance (Kaur et al. 2006). Haloarchaea are known to encounter metals in their natural habitat, yet reports on metal tolerance and nanoparticles synthesis by haloarchaea are few.

With the exception of two organisms, *Halococcus salifodinae* BK3 and H. *salifodinae* BK6, there are no other reports on metallic nanoparticles synthesis by haloarchaea. The intracellular synthesis of silver nanoparticles by *H. salifodinae* BK3 and BK6 involves the enzyme NADH-dependent nitrate reductase that helps in silver ion reduction. These organisms adapt to the metal stress and thus their growth kinetics parameters in presence of silver nitrate are similar to that of organism grown without silver nitrate. The reduction of silver ions to its metallic form in bacterial and fungal systems has also been shown to involve enzymatic detoxification by enzymes like nitrate reductase, where toxic metal form is converted to non-toxic nanoparticulate form (Srivastava et al. 2013, 2014).

Nitrate reductase is normally involved in the reduction of nitrate to nitrite using NADH as the electron donor, however, in the presence of silver the electron is shuttled from NADH to reduction of silver ions. The haloarchaeal isolate *H. salifodinae* BK3 exhibited nitrate reductase activity as indicated by the pink color development on addition of Griess Ilosvays reagent (Srivastava et al. 2013). This mechanism has been excellently described in the organism *B. licheniformis* (Kalimuthu et al. 2008). *B. licheniformis* is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, that might be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of silver nanoparticles. Figure 2.4 shows that the nitrate reductase present in the bacteria may aid the synthesis of silver nanoparticles (Kalimuthu et al. 2008). The silver nanoparticles synthesized by *H. salifodinae* BK3 and BK6 exhibit good antibacterial activity against both Gram-positive and Gram-negative bacteria (Srivastava et al. 2013, 2014). The following are the effects by which silver ions exhibit their antimicrobial functions. (1) binding of silver ions to the negatively charged DNA thereby making the DNA to lose its structure and also inhibiting the replication of DNA; (2) binding of silver ions with the thiol-containing proteins,

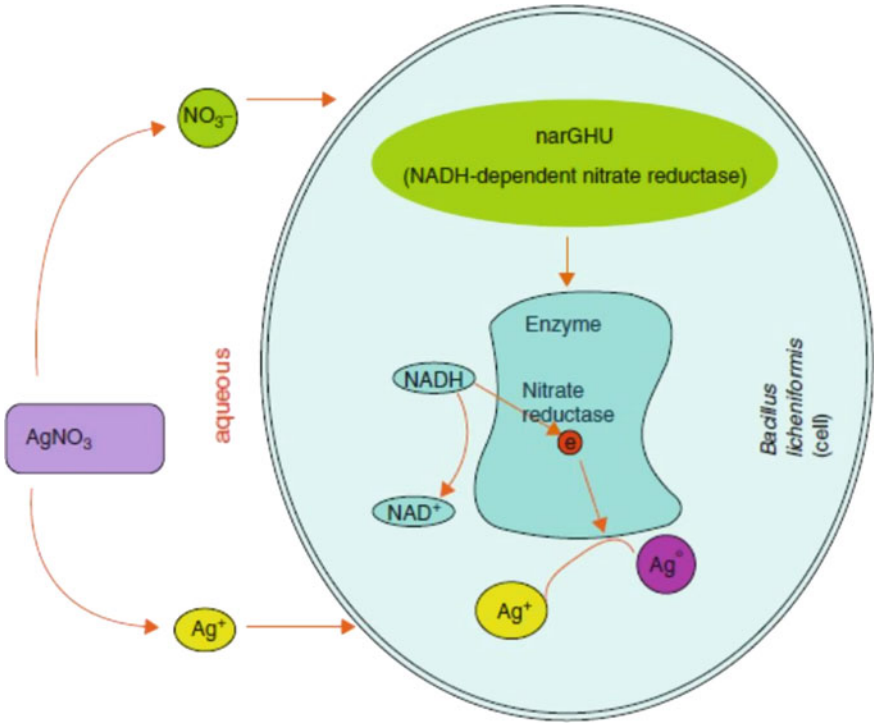

Fig. 2.4 Reduction of silver ions to silver atom by the enzyme nitrate reductase. *Source* Kalimuthu et al. (2008). Copyright © 2008, Elsevier. Reproduced with permission

thereby inhibiting the function of proteins; and (3) induction of reactive oxygen species synthesis leading to the formation of highly reactive radicals that destroy the cells (Deepak et al. 2011).

2.2.3 Nanoparticle Synthesis by Halophilic Fungi

A few halophilic yeasts and fungi are known to synthesize nanomaterials. *Pichia capsulata*, a mangrove derived halophilic yeast, is capable of synthesizing silver nanoparticles extracellularly (Manivannan et al. 2010). Yeasts are unicellular fungi that predominantly reproduce by budding. A rapid method for the synthesis of silver nanoparticles from *P. capsulata* was demonstrated. The optimum conditions were pH 6:0, 0:3 % NaCl concentration, and a temperature of 5 °C. The nanoparticles were mostly spherical with a size of 525 nm. An NADH-dependent (NADH: nicotinamide adenine dinucleotide) protein similar to nitrate reductase was partially purified and was suggested to mediate the reduction process.

Yarrowia lipolytica is biotechnologically important in the bioremediation of hydrophobic substrate contaminated environments, in the treatment or up-gradation of wastes, biotransformation of organic compounds, production of novel enzymes, and in the cloning and expression of heterologous proteins. This yeast has been isolated from polluted areas containing toxic and hazardous metals. Metal tolerance in this yeast is attributed to the presence of superoxide dismutase (a copper tolerating protein), reductases, CRF1, metallothioneins, efflux mechanisms, and melanin (Bankar et al. 2009). A tropical marine isolate of *Y. lipolytica* (NCIM 3589), obtained from oil-polluted seawater near Mumbai, India, mediated the synthesis of gold nanoparticles. The synthesis took place at 30 °C within 72 h. The cell wall-associated synthesis of nanoparticles was confirmed by TEM analysis. The yeast, as well as the mycelial forms of this dimorphic fungus, synthesized the gold nanostructures. The size of the nanoparticles was found to be pH dependent. At pH 2:0, gold nucleation was observed within 15 min. Over a period of time, these developed into large triangular and hexagonal plates. The size of the nanostructures at pH 7:0 and at 9:0 was 15 nm (Agnihotri et al. 2009). In a further study, this system was used in the custom designing of gold nanoparticles with specific sizes. With increasing cell numbers and the same concentration of gold salt, the particle size was found to decrease. On the other hand, with increasing concentration of the gold salt and the same cell numbers, there was an increase in the size of the particles. The cell-associated gold nanoparticles could be released into the medium by incubation at 20 °C (Pimprikar et al. 2009). Melanin, a dark-colored pigment from this yeast was found to be one of the factors responsible for nanoparticle synthesis. Cell-extracted and induced melanin (obtained by incubating resting cells with L-3,4-dihydroxyphenylalanine (L-DOPA) mediated the synthesis of gold nanostructures. Another cold-adapted marine strain of *Y. lipolytica* (NCIM 3590) also synthesized gold nanostructures (Fig. 2.5a). The synthetic ability in this case was also associated with the dark-colored pigment, melanin. These nanoparticles

displayed antibiofilm activity against pathogenic bacteria. Since the inherent content of melanin in this organism was low, the yeast was induced to overproduce melanin by incubation with a precursor, L-DOPA. This melanin also mediated the rapid formation of silver and gold nanoparticles. The former displayed effective antifungal properties against a wall-disfigurement causing fungus (Apte et al. 2013). In addition, the tropical marine strain (*Y. lipolytica* NCIM 3589) described above was able to synthesize CdO and CdS nanostructures in a cell-associated and extracellular manner. The SEM images of cell-associated nanostructures in this yeast are depicted in Fig. 2.5b. Certain functional groups on the cell surface were thought to play a role in the reductive and stabilization processes (Pawar et al. 2012).

Another fungus, *Penicilium fellutanum* associated with the rhizosphere of an Indian endemic mangrove plant (*Rhizophora annamala*) was isolated and evaluated

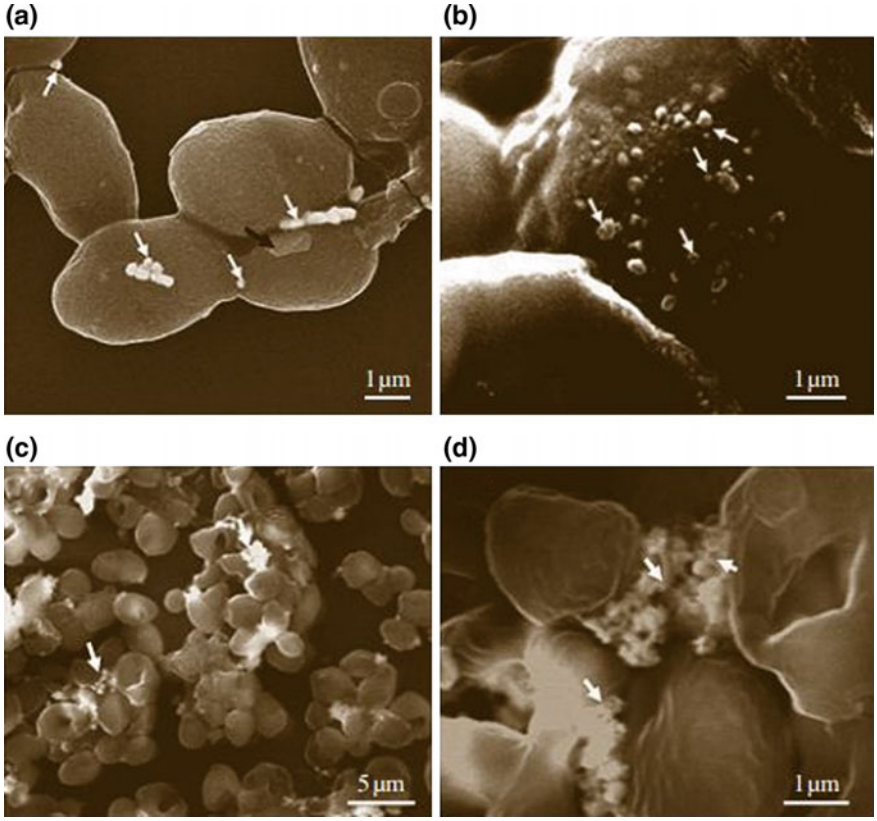

Fig. 2.5 Nanostructures associated with marine yeasts. **a** Gold nanoparticles and microplates synthesized by Yarrowia lipolytica NCIM 3590; **b** CdO nanoparticles mediated by *Y. lipolytica* NCIM 3589. *White arrows* point to nanostructures and the *black arrow* to a microplate. *Source* Pawar et al. (2012). Copyright © 2012, American Scientific Publishers

for the synthesis of silver nanoparticles. The biosynthesis of nanoparticles was the maximum when the culture filtrate was treated with 1.0 mM $AgNO_3$, maintained at 0.3 % NaCl and pH 6.0, incubated at 5 °C for 24 h. The extracellularly synthesized nanoparticles are spherical in shape with size ranging from 5–25 nm. A protein of about 70 kDa from the cell-free supernatant was proposed to be responsible for converting the metal ions to their zero valence state (Kathiresan et al. 2009). Presence of silver nanoparticles in the culture filtrate was confirmed by absorption peak at 430 nm, as well under transmission electron microscope.

Silver nanoparticles are also synthesized by the halophilic fungi *Aspergillus niger*. *Aspergillus species* are found in almost all climatic conditions worldwide. This fungus is often associated with environments that are rich in starchy material. Since mangrove ecosystems offer such conditions (due to the accumulation of debris from leaves, inflorescence, and stems), *Aspergillus* sp. have been isolated from these habitats (Seelan et al. 2009). A strain of *A. niger* (AUCAS 237) isolated from a mangrove sediment (Vellar estuary, India) synthesized silver nanoparticles. The nanoparticles were 5–35 nm in diameter and spherical in shape. They displayed effective antimicrobial activity towards clinical pathogens (especially Gram negative bacteria and some fungi). The activity was enhanced when the nanoparticles were stabilized with polyvinyl alcohol. FTIR analysis revealed the possibility of proteins as possible capping and stabilizing agents (Kathiresan et al. 2010). Silver nanoparticles were also synthesized by using another strain of *A. niger* isolated from the Gulf of Cambay, India. The nanoparticles were spherical, 5–26 nm in size, and displayed laser optical speckles, which could have several applications in the future (Vala et al. 2012). Microorganisms are known to be symbiotically associated with different marine forms such as sponges. A strain of *A. terreus* (MP1) was isolated from a marine sponge. The mycelial extract of this fungus synthesized silver nanoparticles. SEM and TEM analysis showed the presence of spherical nanoparticles 1520 nm in size. These particles effectively inhibited the growth of pathogenic bacterial strains of *Staphylococcus aureus, Klebsiella pneumoniae*, and *Salmonella typhi* (Meenupriya et al. 2011).

Rhodospiridium diobovatum, a marine yeast, synthesizes lead sulphide (PbS) nanoparticles intracellulary with the help of non-protein thiols (Seshadri et al. 2011). The PbS nanoparticles were characterized by UV-visible absorption spectroscopy, X-ray diffraction (XRD) and energy dispersive atomic spectroscopy (EDAX). UV-visible absorption scan revealed a peak at 320 nm, a characteristic of the nanosize range. XRD confirmed the presence of PbS nanoparticles of cubic structure. Crystallite size as determined from transmission electron microscopy was found to be in the range of 2–5 nm. Elemental analysis by EDAX revealed the presence of particles composed of lead and sulfur in a 1:2 ratio indicating that PbS nanoparticles were capped by a sulfur-rich peptide. A quantitative study of lead uptake through atomic absorption spectrometry revealed that 55 % of lead in the medium was accumulated in the exponential phase, whereas a further 35 % was accumulated in the stationary phase; thus, the overall recovery of PbS nanoparticles was 90 %. The lead-exposed yeast displayed a marked increase (280 % over the control) in nonprotein thiols in the stationary phase. A sulfur-rich peptide was

suggested to be the capping agent. In the presence of lead, this yeast produced increasing contents of non-protein thiols during the stationary phase. These were possibly involved in forming the nanoparticles (Seshadri et al. 2011).

2.2.4 Nanoparticle Synthesis by Halophilic Algae

The reports on nanoparticles synthesis by halophilic algae are few and mostly recent. All studies so far are on extracellular synthesis of inorganic (metallic) nanoparticles. Algae inhabit natural as well as metal-contaminated freshwater and marine environments. Various species of algae are also known to interact with heavy metal ions. Some of these species are involved in the detoxification and bioremediation of metal wastes from water (Scarano and Morelli 2003; Mehta and Gaur 2005). The first alga reported to synthesize gold nanoparticles was the brown alga Sargassum wightii (Singaravelu et al. 2007). The marine brown algae *Sargassum wightii* synthesizes stable gold (30–100 nm) and silver nanoparticles (8–12 nm) when its extract is exposed to gold chloride and silver nitrate, respectively (Singaravelu et al. 2007; Oza et al. 2012). The first report on the synthesis of highly stable gold nanoparticles using marine alga, *S. wightii* was carried out by Singaravelu et al. (2007). The reduction of the metal ions resulted in the formation of high density, extremely stable gold nanoparticles in the size ranging from 8 to 12 nm with an average size ca. 11 nm. Figure 2.6a shows the powder of marine alga with gold ions at the beginning of the reaction and Fig. 2.6b shows the color change of the medium to ruby red after 15 h of incubation. The change in color of the medium was noted by visual observation. An important potential benefit of the described method of synthesis of nanoparticles using marine algae is that they are quite stable in solution and this is a very important advantage over other biological methods currently in use (Singaravelu et al. 2007).

Similarly, extracts of *S. longifolium* can reduce silver nitrate to spherical silver nanoparticles that exhibit excellent antifungal activity. The extracts contain various active molecules rich in hydroxyl groups or carboxyl groups that are responsible for the reduction of the metallic ion (Rajeshkumar et al. 2014). The synthesized silver nanoparticles exhibited excellent antibacterial activity against pathogenic fungi such as *Aspergillus fumigatus*, *Candida albicans*, and *Fusarium* sp. (Fig. 2.7). The antifungal activity of *S. longifolium* mediated synthesized silver nanoparticles against harmful pathogenic fungi at different concentrations (50, 100, and 150 μL) was carried out. As the concentration of silver nanoparticles increased, the zone of inhibition increased.

Diatoms are unicellular photosynthesizing microorganisms belonging into the group of brown algae (division *Chromophyta*, class *Bacillariophyceae*) encased in siliceous cell walls—frustules (Fig. 2.8). The biosynthesis of gold nanoparticles has been successfully conducted using two strains of diatoms (*Navicula atomus* and *Diadesmis gallica*) mixed with aqueous $HAuCl_4$ ($\sim$500 mg/L Au) at laboratory conditions. The interaction of diatoms with aqueous salt promoted the precipitation

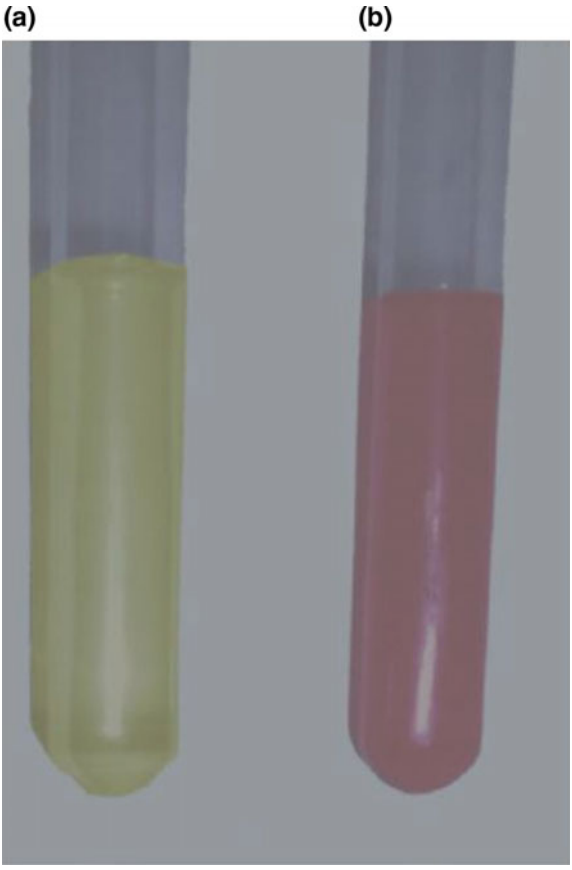

Fig. 2.6 *Yellow color* due to aqueous auric chloride (**a**) and *ruby red color* indicating the formation of gold nanoparticles (**b**). *Source* Singaravelu et al. (2007). Copyright © 2007, Elsevier. Reproduced with permission

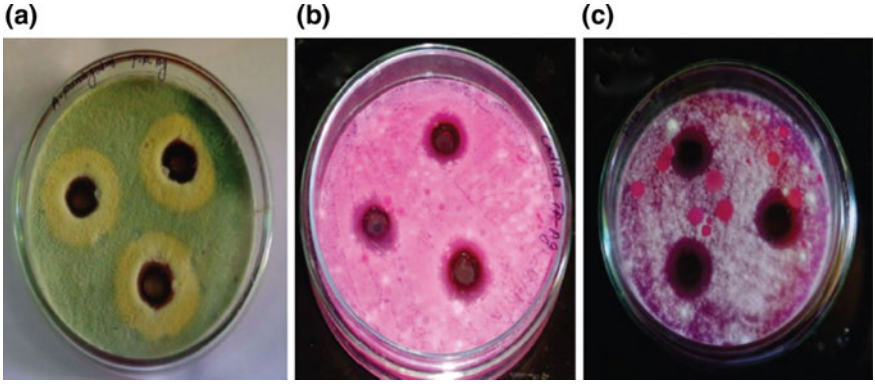

Fig. 2.7 Antifungal activity of AgNPs synthesized by using marine algae *S. longifolium*. *Source* Rajeshkumar et al. (2014). Copyright © 2014, Hindawi Publishing Corporation. Reproduced with permission

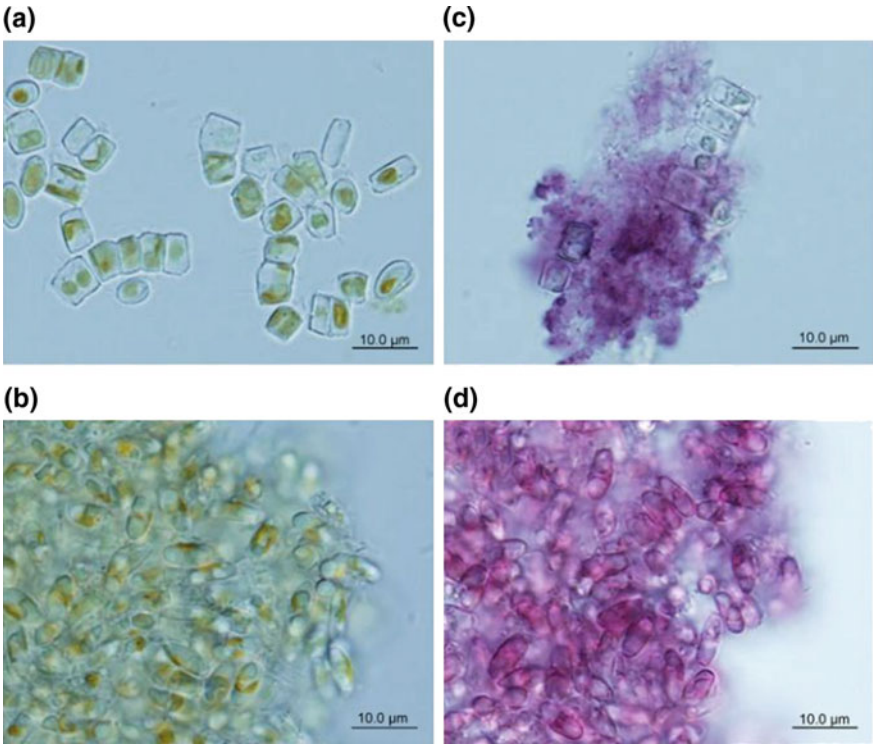

Fig. 2.8 Light microscope photographs of the diatom cells before (*left*) and 12 h after (*right*) tetrachloroaureate addition for **a**, **c** *Diadesmis gallica*, and **b**, **d** *Navicula atomus*. *Source* Schrofel et al. (2011). Copyright © 2011, Springer. Reproduced with permission

of gold nanoparticles. When the diatoms are grown in the presence of tetrachloroaurate, the diatoms reduces it to gold nanoparticles that are associated with the diatom frustules and exopolysaccharides (EPS) excreted by the diatoms. The size of the biosynthesized nanoparticles differed in each strain. Whereas *Diadesmis gallica* showed larger mean particle size (around 22 nm) and wider range of the size distribution, AuNPs synthesized by NA strain had smaller mean particle size (9 nm) and higher homogeneity in size (Schrofel et al. 2011).

2.3 Biomolecules Produced by Halophiles with Implications in Nanotechnologies

The synthesis of silver nanoparticles is attributed to the presence of peptides, amino acids, reducing sugars, and enzymes such as reductases and proteases. Thus, many biomolecules including proteins/enzymes/oligopeptides (Crespilho et al. 2009),

antibody/antigens (Haes et al. 2004; Pengo et al. 2007), biotin/streptavidin (Haes et al. 2004), and DNA/oligonucleotides/aptamers (Nykypanchuk et al. 2008) have been immobilized on the surface of nanoparticles to form noble metal nanoparticle–biomolecule conjugates. The similarity in size of nanoparticles and biomolecules makes them relatively easy to integrate.

The synthesis and applications of biomolecule-nanoparticle hybrid systems have been reviewed (Katz and Willner 2004). There have also been a number of articles that review different specific applications of biomolecule-nanoparticle hybrids, such as biosensing (Willner et al. 2007), probing cells (Roca and Haes 2008), delivery (Ghosh et al. 2008) and diagnosis (Baptista et al. 2008). For example, Katz and Willner (2004) reviewed advances in the synthesis of biomolecule (proteins or DNA)-nanoparticle (metal or semiconductor nanoparticles) conjugates as well as their application. Aubin-Tam and Hamad-Schifferli (2008) reviewed studies of the conjugation of protein and nanoparticles using a linker species. In addition, many specific applications have also been reviewed: biomolecule nanoparticle conjugates for the assembly of electrochemical biosensors (Willner et al. 2007), noble metal nanoparticle aggregates as tags for probing cells (Roca and Haes 2008), gold nanoparticles as non-toxic carriers for drug and gene delivery (Ghosh et al. 2008), and gold nanoparticles for application in clinical diagnosis (Baptista et al. 2008).

The development of biomolecule-nanoparticle systems has been very rapid, and many new materials have been reported. As a result, it is very imperative to review of recent research results including new synthesis methods, optical properties and applications. Conjugation of noble metal nanoparticles with biomolecules has mainly been achieved by one of four major mechanisms: electrostatic adsorption, direct chemisorption of thiol derivatives, covalent binding through bifunctional linkers, and specific affinity interactions (Zhang et al. 2012). Among these mechanisms, specific affinity interactions can be further classified into sub-categories including biotin-streptavidin binding, antibody-antigen conjugation, and complementary DNA association. These mechanisms are schematically described in Fig. 2.9.

Several biomolecules currently produced by these halophilic organisms, such as exopolysaccharides (EPS) and enzymes showed biological activity in harsh conditions. Combination of these biomolecules with various nanomaterials like thin-layers, nanotubes, nanospheres results in novel compounds possessing both biological properties of biomolecules and physico-chemical characteristics of nanomaterials. The present chapter deals with the main biomolecules produced by both halophilic microorganisms and bacteria revealing their potential implications in some nanotechnologies.

2.3.1 Exopolysaccharides from Halophiles

Exopolysaccharides (EPS) are often found surrounding the outermost structures of both prokaryotic and eukaryotic microbial cells. They may be closely associated

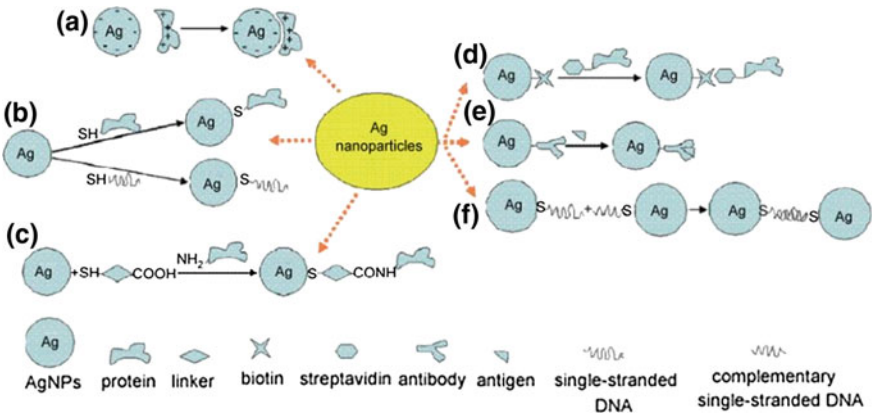

Fig. 2.9 Synthesis of biomolecule-nanoparticle systems by different routes: **a** assembly of nanoparticle (NP)–protein conjugates by electrostatic adsorption; **b** conjugation of biomolecules (proteins/DNA) on NPs through direct chemisorption of thiol derivation; **c** covalent binding through bifunctional linkers to form biomolecule-NP hybrids. *Source* Zhang et al. (2012). Copyright © 2012, Springer. Reproduced with permission

with the cell in the form of discrete capsules or else excreted as slime, unattached to the cell surface as such. EPSs exist in a wide variety of unique and often complex chemical structures and they are believed to provide self-protection against antimicrobial substances (Kumon et al. 1994), antibodies and bacteriophages (De Vuyst and Degeest 1999; Wingender et al. 1999) and/or afford adherence to other bacteria, animal and plant tissues or inert surfaces (Sutherland 2001), thus forming biofilms. Increasing interest is being generated in the study of these molecules because of their wide applications in food, pharmaceutical, petroleum and other industries (Dawes 1990; Sutherland 1990; Vandamme et al. 2002). Nevertheless, the strains used for the industrial production of EPS belong to a small number of taxa, such as *Xanthomonas campestris* (Evans et al. 1979), *Pseudomonas* (Jarman 1979), *Azotobacter* (Jarman et al. 1978), *Sphingomonas* (Lobas et al. 1992), *Alcaligenes* (Sutherland 1990).

Several halophilic microorganisms produce such exopolysaccharides in copious amounts, and therefore their commercial exploitation has been considered. The archaeon *Haloferax mediterranei*, excretes large amounts of anionic exopolysaccharides. The sulfated acidic heteropolysaccharide of *Haloferax* species has a high viscosity at low concentrations, its rheological properties are excellent and it is resistant to extremes of pH and temperature (Oren 2012). *Aphanothece halophytica*, a halophilic unicellular cyanobacterium found in the benthic cyanobacterial mats of solar salterns and in many other hypersaline lakes, is also known for its massive synthesis of polysaccharides. In solar salterns excessive polysaccharide production can have a negative impact on the salt production process, as explained above. However, a recent report on the immunomodulating properties of the sulfated polysaccharide of *A. halophytica* is of special interest: when administered orally in

mice, it significantly inhibited pneumonia induced by influenza virus H1N1 (Zheng et al. 2006). Among the halophilic representatives of the *Bacteria*, the *Halomonas* species (*H. maura*, *H. eurihalina*) shows considerable promise as a producer of large amounts of an extracellular polyanionic polysaccharide, a potent emulsifying agent that exhibits a pseudoplastic behaviour (Quillaguamán et al. 2007). The *H. maura* exopolysaccharide ('mauran') has also been shown to be an immunomodulator (Béjar et al. 1998; Arias et al. 2003; Llmas et al. 2006).

Polysaccharides are emerging as stabilizing and reducing agents for nanoparticles synthesis, however the commercial polysaccharides are not economically viable. Therefore, the exopolysaccharide from microbial origin such as bioflocculants are promising alternate for the synthesis and stabilization of nanoparticles. Polysaccharides have hydroxyl groups, a hemiacetal reducing end, and other functionalities that can play important roles in both the reduction (Mata et al. 2009) and the stabilization of metallic nanoparticles that creates vast opportunities for their utilization and potential mass production. Polysaccharide bioflocculants can be used for high-performance nanomaterials production, since they easily form a variety of liquid crystals in aqueous solutions and bioflocculant-mediated processes are highly profitable. Bacterial metabolites or products such as polysaccharides/bio-flocculants are now being used as reducing agents to synthesize inorganic nanoparticles. In most cases the synthesized nanoparticles are capped by reducing agents. AgNPs have were synthesized by using polysaccharide bioflocculant as reducing and stabilizing agent by Sathiyanarayanan et al. (2013). In their study, the polysaccharide bioflocculant produced by a halophilic bacterium *Bacillus subtilis* MSBN17 reduced silver nitrate to spherical AgNPs in reverse micelles. The electrostatic forces between the amino groups of the polysaccharide MSBF17 and the silver ions in the solution are proposed to be the driving force for the formation and stabilization of the silver nanoparticles. The carboxyl, hydroxyl and methoxyl groups of MSBF17 form a coating on the silver nanoparticles thereby stabilizing them. These nanoparticles exhibit anti-microbial activity against a host of pathogenic organisms. The AgNPs were spherical shaped (60 nm) and stable for 5 months (Sathiyanarayanan et al. 2013).

Besides the various inorganic and organic nanoparticles, the exopolysaccharides of the halophilic bacteria have also been utilized for fabrication of polymer hybrid nanomaterials. Raveendran et al. (2013b) reported the synthesis of an extremophilic bacterial sulfated polysaccharide based nanoparticle as a stable biocompatible material for drug delivery, evaluation of anticancer efficacy and bioimaging. Sulfated polysaccharides (SPSs) are gaining attention since last few decades because of their exceptionally best physico-chemical properties and bioactivities. Owing to their unique properties like stable structure, composition, fluid dynamics, extreme stability, biodegradability and biocompatibility, they are widely exploited in modern biotechnology and material science (Raveendran et al. 2013b). *Halomonas maura* is a moderately halophilic bacterium, which is capable of producing highly sulfated EPS residues into the external milieu (Fig. 2.10). As reported by Arias et al. (2003), *Halomonas* polysaccharides are rich in sulfate residues and hence posses various biological properties. Biologically active sulfated

Fig. 2.10 SEM micrograph of *Halomonas maura* showing the exopolysaccharide, MR, covering the cell wall. *Source* Raveendran et al. (2013c). Copyright © 2013, Elsevier. Reproduced with permission

polysaccharide produced by *H. maura* is called MR and it has exceptionally high sulfate content and uronic acid content. MR is a high molecular weight acidic polysaccharide with repeating units of mannose, galactose, glucose and glucuronic acid. It is highly anionic in nature due to the presence of sulfate and uronic acid moieties. Viscoelasticity, pseudoplasticity and thixotropic behavior of MR make it an ideal molecule for material science applications. Similarly rheological properties of MR are not easily affected by the presence of any salts, sugars, surfactants, lactic acid, and changes in pH and freeze thawing (Llamas et al. 2006). Another important striking property of MR is the ability to withstand various harsh conditions like temperature, freeze thawing, extreme pH values and salt conditions. High temperature over 55 °C has detrimental effect on viscosity, although it can regain its 70 % of its property on cooling to 25 °C (Arias et al. 2003). MR can form stable gels on binding to various metal ions that helps in efficient removal of toxic ions from the polluted environments and water. The unusually high sulfate content of MR contributes to immunomodulating and antiproliferative effects on human cancer cells (Llamas et al. 2006). The highly sulfated anionic exopolysaccharide, Mauran (MR) secreted by the halophilic bacterium *H. maura*, is well characterized and has been successfully used for generation of such hybrid nanomaterials. The high sulphate content imparts immunomodulating and anti-cancer properties to MR. Thus, MR can be used for various biomedical applications due to their biological and the physicochemical properties. MR-Chitosan (MR/CH) hybrid nanoparticles (Fig. 2.11) fabricated via the ionic-gelation technique when used for encapsulation of drugs exhibits controlled and sustained drug release and biocompatibility (Raveendran et al. 2013b).

Similarly, electrospun MR-polyvinyl alcohol (MR-PVA) nanofibre membranes (Fig. 2.12) boost the cellular adhesion, migration, proliferation and differentiation, properties desirable for tissue engineering applications (Raveendran et al. 2013c). Nanofibers synthesized from biocompatible and bioactive polymers are of great

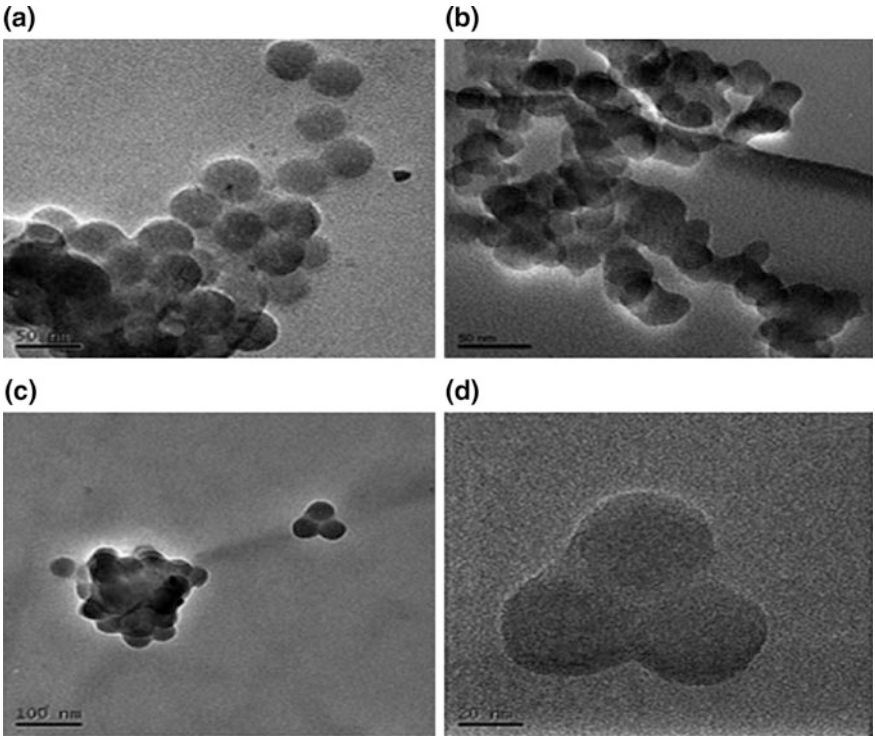

Fig. 2.11 TEM micrographs (**a**–**d**) depicting the morphology of the Mauran-Chitosan nanoparticles fabricated using the exopolysaccharide mauran secreted by the halophilic bacteria *Halomonas maura. Source* Raveendran et al. (2013b). Copyright © 2013, Elsevier. Reproduced with permission

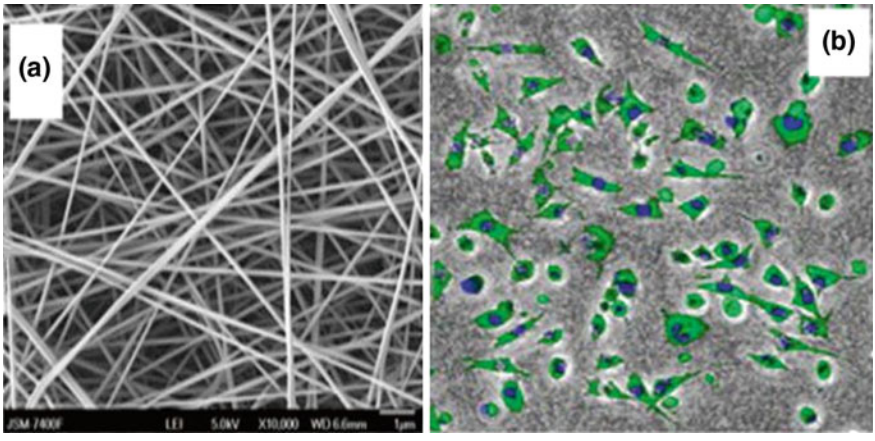

Fig. 2.12 SEM micrographs of the MR/PVA nanofi bres fabricated using the exopolysaccharide mauran (×10,000) (**a**). Confocal microscopy images of L292 cells attached and proliferating on MR/PVA nanofi bres (merged microtracker green and DAPI stained images) (**b**). *Source* Raveendran et al. (2013c). Copyright © 2013, Elsevier. Reproduced with permission

importance in the new generation biomedicine and nanotechnology. They are well established in the biomedical field for various applications like tissue engineering, wound dressing, drug delivery and enhanced cell adhesion (Vlierberghe et al. 2011; Dhandayuthapani et al. 2012). Recently optically transparent cellulose nanofibers have opened a wide scope of developing nanofiber matrices for enumerable applications even in the field of microelectronics (Nogi et al. 2009). Natural and synthetic polysaccharides are proven to be a good matrix material for generation of excellent tissue engineering scaffolds (Vlierberghe et al. 2011). Bacterial polysaccharide from moderately halophilic *Halomonas maura* based biocompatible nanofibers were produced for the first time via electrospinning technique by Raveendran et al. (2013c). In their study, mauran (MR), an extremophilic sulfated exopolysaccharide was extracted from *H. maura* and characterized for the application of nanofiber synthesis. Thin-uniform MR nanofibers were produced using homogenous solutions of poly (vinyl alcohol) (PVA) blended with different concentrations of MR. An average of 120 nm sized nanofibers were produced and tested for an enhanced cell growth under in vitro conditions in comparison with control. MR and MR/PVA nanofibers were found to be an excellent biomaterial for the migration, proliferation and differentiation of mammalian cells (Raveendran et al. 2013c).

Mauran may be used in augmenting the biocompatibility of quantum dots that are usually cytotoxic (Fig. 2.13). The nanocrystals, so-called quantum dots (QDs), are undisputedly excellent fluorescent markers for imaging and clinical diagnostics. However, their toxicity is always a perturbing issue and remains as the major hindrance for biocompatible imaging and other biomedical applications. Raveendran et al. (2014) demonstrated the extraction and application of an extremophilic bacterial polysaccharide, mauran (MR), from a moderately halophilic

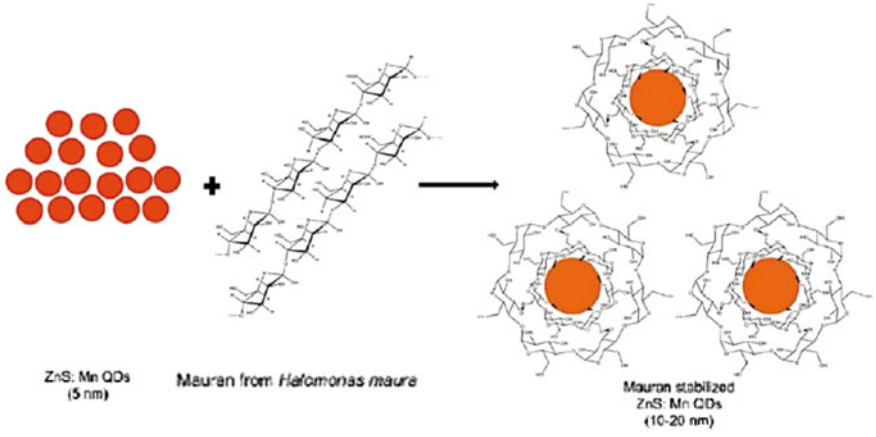

Fig. 2.13 Schematic representation of stabilization of QDs by Mauran, an exopolysaccharide secreted by *H. maura*. *Source* Raveendran et al. (2013b). Copyright © 2013, Elsevier. Reproduced with permission

bacterium called *Halomonas maura* in the stabilization of ZnS:Mn2+ QDs for the first time. Mauran was used as a natural polymer for bioconjugation to enhance the cellular acceptance and decrease the cytotoxicity of QDs while being used as a fluorescent marker for imaging purposes. Five nanometer-sized QDs were stabilized using an aqueous MR solution under ambient conditions to yield 10−20 nm-sized nanoparticles. MR conjugated with ZnS:Mn^{2+} QDs at a concentration of 0.05 mg resulted in a drastic increase in cell viability as compared to cell viability of bare QDs (Raveendran et al. 2014). Therefore, in addition to fabrication of polymer based nanomaterials, MR can also be used in conjugation with inorganic nanoparticles for enhancing their biological applications.

The water soluble exopolysaccharides extracted from the marine algae *Pterocladia capillacae, Jania rubins, Ulva faciata* and *Colpmenia sinusa* reduce silver ions to silver nanoparticles. In this work, a well-stabilized Ag-NPs solution with a concentration of 108 ppm was prepared using 30 mg of the prepared polysaccharides from *C. sinusa*, *J. rubins*, *U. faciata* and *P. capillacae* algae as reducing agents for silver ions as well as stabilizing agents for the formed AgNPs. The biosynthesized Ag nanoparticles are stable for six months and have the ability to be immobilized on the cotton fibers bringing a good antimicrobial property to the final textile product and rendering it to be used as an antiseptic dressing or bandage, which is in high demand for biomedical applications (El-Rafie et al. 2013).

EPS-gold and silica-gold bio-nanocomposites can be generated using the diatoms *Navicula atomus* and *Diadesmis gallica*. The diatoms when grown in presence of tetrachloroaureate, reduce it to gold nanoparticles that are associated with the diatom frustules and exopolysaccharides (EPS) excreted by the diatom cells. Scanning electron microscopy (SEM) and TEM showed that the nanoparticles were associated with the diatom frustules and extracellular polysaccharides (EPS) excreted by the diatom cells. Due to its accessibility, simplicity, and effectiveness, this method of nanocomposites preparation has great importance for possible future applications. The gold bio-nanocomposites may find applications in the field of catalysis (Schrofel et al. 2011).

2.3.2 Gas Vesicles

Buoyant gas vesicles are prokaryotic organelles that are widely distributed among bacterial and archaeal microorganisms and constitute protein nanoparticles (GVNPs) that may be engineered for biotechnological applications (DasSarma et al. 1994, 2010a, b; Shively et al. 2011; Cai et al. 2012). These organelles naturally promote flotation and increase the availability of light and oxygen to many aquatic microorganisms, especially those with photosynthetic or phototrophic capabilities. Water is excluded from the interior, a property that is thought to be a consequence of the hydrophobicity of the interior surface of the proteinaceous membrane. While the exact protein composition of the membrane has been difficult to ascertain due to its extreme stability against solubilization, production of these structures is easily

scaled-up and they are simple to purify by hypotonic lysis of the host and concentrate by flotation, enhancing their intrinsic value for biotechnological applications (Stuart et al. 2001, 2004). Gas vesicle nanoparticles (GVNPs), that may be engineered for various biotechnological applications are the buoyant gas vesicles widely distributed among bacteria and archaea. These organelles that naturally promote floatation are present in abundance in haloarchaea (Srivastava and Kowshik 2015). These vesicles are plasmid encoded with the genetic cluster gvp MLKJIHGFEDACNO involved in gas vesicle formation (DasSarma 1989; DasSarma and Arora 1997; DasSarma et al. 1987, 1994; Halladay et al. 1993). The proteins encoded by the gene clusters include the GvpA, J and M of Pfam 741 family, involved in gas vesicle membrane formation, and GvpF and L, coiled-coil protein (Pfam 6386) involved in the nucleation process of nanoparticles due to their self-associative properties (Jones et al. 1991; Shukla and DasSarma 2004). Genes corresponding to these proteins have been found in other organisms as well, with the exception of gvpC gene, which is found only in haloarchaea and cyanobacteria (van Keulen et al. 2005). In the haloarchaeon *Halobacterium* sp. NRC-1, the GvpC protein is hydrophilic and insertion mutations within this gene results in gas vesicles with altered shape and size (Fig. 2.14) (DasSarma et al. 2013). Thus, by genetic manipulation of gvpC gene, the gas vesicles may be made to express different proteins or display antigens, thereby increasing their applications in the field of biotechnology. A new *Halobacterium* sp. NRC-1 derived host strain and a series of smaller, more versatile plasmid expression vectors have been constructed. These represent a significantly improved genetic system for expression of GvpC-fusion proteins. For example, active *Gaussia princeps* luciferase enzyme can be fused to GvpC that would result in the expression of the luciferase enzyme on the surface of the GVNPs (DasSarma et al. 2013). Such GVNPs may be used for antigen display and vaccine development. These vesicle nanoparticles are stable biological structures resistant to degradation, devoid of nucleic acids, easy to harvest by lysis and flotation and are nontoxic. In preliminary studies, mice immunized with recombinant gas vesicles expressing an simian immunodeficiency virus (SIV) peptide elicited a strong antibody response and immune memory (Stuart et al. 2001). The potential for rapid, low-cost vaccine production, and increased safety make *Halobacterium* an excellent candidate for production of a vaccine vector.

2.3.3 Graphene Sheet

Graphene, the reduced form of graphene oxide (GO) is now being actively investigated for applications in areas including drug delivery and cellular imaging (Gilje et al. 2007; Sun et al. 2008) as well as nanoelectronics (Lee et al. 2011) molecular sensors, composite materials, and energy storage (Rao et al. 2009; Allen et al. 2010; Eda and Chhowalla 2010; Loh et al. 2010). In spite of many methods reported for producing graphene sheets, such as mechanical exfoliation of reduced GO (RGO), thermal expansion of graphitic oxide, (Eda and Chhowalla 2010) and

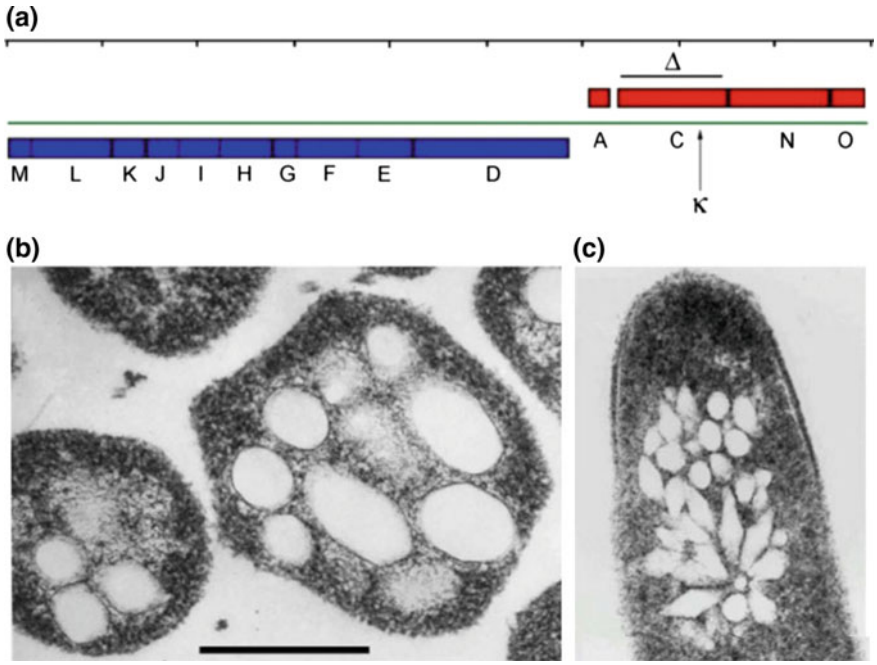

Fig. 2.14 *Halobacterium* sp. gas vesicle gene cluster and thin-sections. **a** Genetic map of the gas vesicle gene cluster from *Halobacterium*sp. NRC-1 pNRC100 is shown with genes transcribed rightward colored *red* and genes transcribed leftward colored *blue* (DasSarma et al. 2012). The scale is noted above (divided into kilobase pairs) and the positions of the *gvp*C deletion (Δ) and kappa insertion (κ) are indicated. **b** and **c** Thin sections of *Halobacterium* sp. NRC-1 (B) and SD109 (pFM104*gvp*C::κ1) mutant (**c**) viewed by transmission electron microscopy (*bar*, which is 325 nm long, applies to both **b** and **c**). Gas vesicles are visible as empty *oval* or *spindle-shaped* regions. Shapes observed reflect different planes of sectioning. *Source* DasSarma et al. (2013). Copyright © 2013, BioMed Central. Reproduced with permission

deoxygenation of graphene oxide via chemical reduction (Stankovich et al. 2007), a stable, cost-effective, and ecofriendly process for producing highly conductive graphene is proving to be elusive. Conventional methods for the preparation of graphene from dispersions from graphite oxide (Park and Ruoff 2009), involve either high temperatures or toxic and unstable gases. Recently, there have been several reports on the microbial reduction of GO to produce graphene. Many heterotrophic metal-reducing bacteria both facultative anaerobic and aerobic strains are capable of utilizing various organic compounds as terminal electron acceptors (Wu et al. 2005; Cho et al. 2012). Graphene sheets have been used for nanoparticle encapsulation (Myung et al. 2011).

Raveendran et al. (2013a) have successfully employed two strains of halophilic bacteria *H. eurihalina* ATCC 49336 and *H. maura* ATCC 700995 to convert GO to biocompatible graphene in a growth medium (Fig. 2.15). Microbial reduction experiment was performed under both aerobic and anaerobic conditions. At the end of

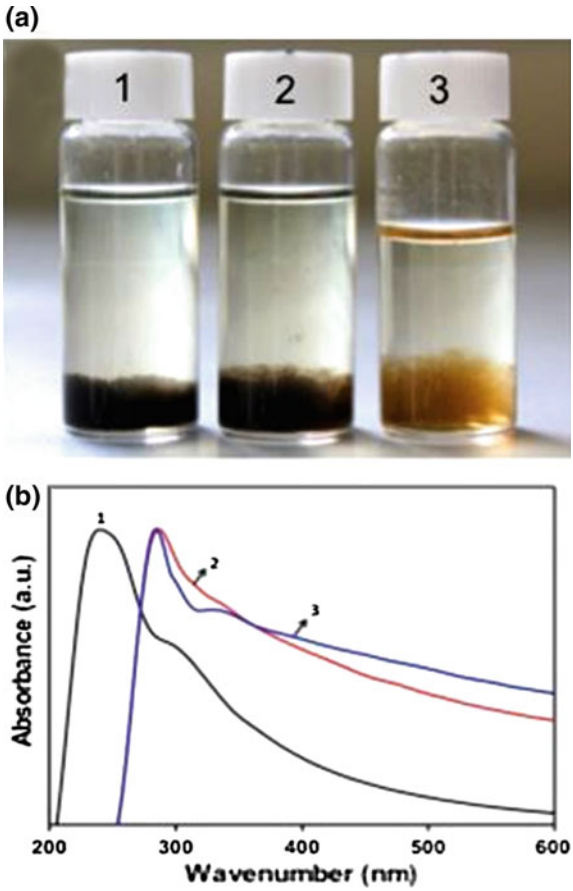

Fig. 2.15 **a** Extremophilic reduction of 10 times diluted concentration of GO—*a1*, *Halomonas eurihalina* reduced GO; *a2*, *Halomonas maura* reduced GO and *a3*, GO control; **b** UV–Vis spectrum—*b1*, GO spectrum; *b2*, ERGO spectrum; *b3*, MRGO spectrum. *Source* Raveendran et al. (2013a). Copyright © 2013, Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission

incubation, GO reduction was clearly seen from the change in the color of the GO in the medium, which changed from brown to a black precipitate settled at the bottom of the bottles (Fig. 2.15). It shows that the anaerobic reduction by *H. eurihalina* is much greater than that by the *H. maura*. Reports on biological synthesis of graphene by microorganisms are scarce. The microbially reduced GO sheet exhibits an increased conductivity as compared to chemically reduced GO and is bio-compatible. Such bio-compatible Graphene sheets may be used for green electronics and biological applications such as detection of cancer biomarkers, encapsulation of enzymes and nanoparticles (Myung et al. 2011; Raveendran et al. 2013a).

2.3.4 Halophilic Enzymes

Halophiles have been perceived as potential source of novel enzymes in recent years. Halophiles have been perceived as potential source of novel enzymes in

recent years. The interest emanates from their ability to catalyze efficiently under high salt and organic solvents The unique properties of halophilic enzymes such as the requirement of salt for stability and activity, the high resistance to several denaturation methods (Karan et al. 2012) and the ability to perform catalytic activity at low water activity or in organic solvents attracted the interest for research in this area (Tokunaga et al. 2008; Enache and Kamekura 2010; Oren 2010; DasSarma and DasSarma 2012; Karan et al. 2012). Halophilic enzymes are thought to remain active by having a predominance of negatively charged residues on the solvent-exposed surfaces of the protein. These negative charges attract water molecules and thereby keep the proteins hydrated so that they do not precipitate. It has also been shown that the hydrogen bonds formed between the negative side chains and the water molecules lead to the formation of a stable hydration shell. The increase in negative charges also results in an increase in ion-pair networks in halophilic enzymes (Bell et al. 1997; Britton et al. 2006; DasSarma and DasSarma 2012). The most well investigated haloezymes are hydrolases such as amylases (Amoozegar et al. 2003), lipases and esterases, xylanases, chitinases, proteases, cellulases, nucleases, etc. (Mellado and Ventosa 2003; Oren 2010; Moreno et al. 2013).

2.3.4.1 Proteases

Proteases are important hydrolytic enzymes which find significant applications in the biotechnology. Proteases have been immobilized on various supports like gold colloids [8], carbohydrate polymers (Zanphorlin et al. 2010; Zhao et al. 2010; Singh et al. 2011), polyvinyl alcohol beads (Hayashi et al. 1993) and microbial polysaccharides (Davidenko 1999). Recently attempts to immobilize protease on TiO_2, magnetic, bimetallic Ag-Au, silica nanoparticles have also been successful (Sadjadi et al. 2009; Jin et al. 2010; Soleimani et al. 2012). Halophilic proteases, apart from being highly salt stable, have also been recognized for their polyextremophilicity, as evident from their increased resistance to denaturation by higher temperatures, chemical reagents, detergents, chaotropic agents, organic solvents and extreme pH values (Karan and Khare 2011; Sinha and Khare 2014). Sinha and Khare (2015) explored the feasibility of using functionalized silica nanoparticles as an effective enzyme support for crude halophilic *Bacillus* sp. EMB9 protease to fabricate an active, stable, reusable enzyme preparation. The immobilization efficiency under optimized conditions was 60 %. Characterization of the immobilized preparation revealed marked increase in pH and thermal stability. It retained 80 % of its original activity at 70 °C while $t_{1/2}$ at 50 °C showed a five-fold enhancement over that for the free protease. The immobilized enzyme showed improved enzymatic and kinetic properties as well as reusability in comparison to the free enzyme. The thermally stable immobilized preparation was able to successfully hydrolyse whey proteins at high temperature with a high degree of hydrolysis.

2.3.4.2 Amylases

Amylases are important class of industrial enzyme finding wide scale applications in food, textile, paper, detergent, analytical chemistry, beverage, and pharmaceutical industry. Amylases have been characterized from many halophilic strains including *Natronococcus amylolyticus* (Kanai et al. 1995); *Haloferax mediterranei* (Perez-Pomares et al. 2003); *Haloarcula hispanica* (Hutcheon et al. 2005). *Halomonas meridiana* (Coronado et al. 2000); *Chromohalobacter* sp. TVSP 101 (Prakash et al. 2009); and *Nesterenkonia* sp. strain F (Shafiei et al. 2010). The production of amylases in halophiles is very low. However, conditions have been optimized in case of *Halomonas meridiana* (Coronado et al. 2000), *Halobacillus* sp. strain MA-2 (Amoozegar et al. 2003), and *Bacillus* sp. strain TSCVKK (Kiran and Chandra 2008) for enhancing the yield, yet maximum 3.2 U/mL could be attained.

The hydrolysis of starch to low molecular-weight products using α-amylase is one of the most important enzyme processes (Tanyolaç et al. 1998). In this regard, conversion of starch into sugars, syrups, and dextrins forms the major part of the starch processing industry. Immobilization of α-amylase on water-insoluble carriers seems to be the most promising way to obtain more stable and reuse forms of the enzyme (Cong et al. 1995). The immobilization of enzymes on solid support offers several advantages over the free enzyme, including easy recovery from the reaction medium, reusability, possibility of operation in continuous reactors, enhanced stability, and catalytic efficiency (Sheldon and Van Pelt 2013). There have been many reports on immobilization of α-amylase. Some examples involve reactive polymer films (Cordeiro et al. 2011), magnetic nanoparticles (Chen et al. 2012), mesoporous silica thin films (Bellino et al. 2010), and adsorption on zirconia (Reshmi et al. 2007). In recent years, nanostructured materials such as nanoporous media, nanofibers, nanotubes, and nanoparticles have emerged as amazingly effective enzyme support/matrix (Kim et al. 2006, 2008; Khan et al. 2013; Cipolatti et al. 2014). They provide the highest possible surface area for immobilization, enabling very high loading of enzyme on the support. This results in surprisingly high enzyme activities per unit volume (Ansari and Husain 2012).

Kumar and Khare (2015) utilize the α-amylase from moderately halophilic *Marinobacter* sp. EMB8 for nanoimmobilization and efficient starch hydrolysis. Amylase producer halophilic bacteria *Marinobacter* sp. EMB8 was isolated during screening of Indian saline habitats (Kumar et al. 2012). The α-amylase was purified and found to be salt and solvent stable. It was used for synthesis of industrially useful maltooligosaccharides (Kumar and Khare 2012). Kumar and Khare (2015) attempted to optimize production and immobilization on functionalized silica NPs for competitive yield and efficient application of α-amylase from *Marinobacter* sp. EMB8. Bacterial growth and enzyme production are greatly influenced by the nutritional factors (carbon and nitrogen sources, metal ions, etc.) and physical factors (pH, temperature, inoculation volume, and incubation time). Optimization of various culture parameters resulted in 48.0 U/mL amylase production, a 12-fold increase over that of unoptimized condition (4.0 U/mL). α-Amylase was

immobilized on 3-aminopropyl functionalized silica nanoparticles using glutaraldehyde as cross-linking agent. Optimization of various parameters resulted in 96 % immobilization efficiency. Starch hydrolyzing efficiency of immobilized enzyme was comparatively better. Immobilized α-amylase retained 75 % of its activity after 5th cycle of repeated use (Kumar and Khare 2015).

2.4 Conclusions and Future Perspectives

A variety of prokaryotic and eukaryotic halophilic microorganisms have been investigated with respect to their nanoparticle synthetic abilities. The vast biodiversity encountered in the prokaryotic world has been relatively less explored. Furthermore, most of the studies are related to the synthesis of silver nanoparticles, followed by those of gold. One reason for this could be the relative ease with which the noble metal ions of gold and silver are reduced. Microbial synthesis of nanoparticles of platinum, bismuth, cadmium (CdO, CdS, CdTe), antimony sulfide, copper oxide, zinc oxide, and titanium oxide described in Chap. 1, have been reported on fewer occasions on halophilic microorganisms.

The majority of the reports deal with application of novel biological systems in mediating the synthesis of nanoparticles, their characterization, and applications in the biomedical field, particularly as antimicrobial agents. However, there is a need to understand the mechanisms involved in the synthetic process. Another limitation of the studies is that the experiments have been conducted at laboratory scale and there are hardly any efforts for the scale-up of these processes. In the future, these shortcomings need to be addressed in an effective manner to harness the actual nanoparticle synthetic potential of the halophiles to their full extent.

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Chapter 3
Thermophiles and Psychrophiles in Nanotechnology

Abstract Some thermophiles and psychrophiles have developed the ability to resort to specific defense mechanisms to quell stresses like toxicity of heavy metal ions or metals. Some of them could survive and grow even at high metal ion concentrations and are capable of binding large quantities of metallic cations. Moreover, some of these microorganisms are able to synthesize nanoparticles. The remarkable ability of these group of microbes to reduce heavy metal ions make them one of the best candidates for nanoparticle synthesis. In this chapter, thermophilic and psychrophilic microorganisms used in nanoparticle biosynthesis are presented. The aim of chapter is to make a reflection on the current state and future prospects and especially the possibilities and limitations of the use of extremophiles in bio-based technique for industries.

3.1 Introduction

Thermophiles are heat-loving organisms, which not only tolerate high temperatures but also usually require these for their growth and survival. Unlike other types of bacteria, thermophiles can survive at much hotter temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures. Temperatures for their growth range from 50 °C to as high as 121 °C. Thermophiles are, for the most part, prokaryotic, and the most hyperthermophilic are archaea, which possess a large number of high-temperature adaptations. Only few eukaryotes are known to grow above 50 °C temperature, but some fungi grow in the temperature range 50–55 °C (Maheshwari et al. 2000). Several years ago Kristjansson and Stetter (1992), suggested a further division of the thermophiles and a hyperthermophile boundary (growth at and above 80 °C) that has today reached general acceptance. Thus, thermophiles are now classified into moderate thermophiles, extreme thermophiles, and hyperthermophiles. Most thermophilic bacteria characterised today grow below the hyperthermophilic boundary with some exceptions, such as *Thermotoga* and *Aquifex* (Stetter 1996; Takahata et al. 2001) while hyperthermophilic species are dominated by the Archaea.

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Habitats for the occurrence of thermophiles may be natural or man-made. Natural habitats that harbor a considerable variety of thermophilic microorganisms include terrestrial geothermal and volcanic areas and deep-sea hydrothermal vents (submarine hydrothermal vents) (Mehta and Satyanarayana 2013). Most of the currently known extreme thermophiles and hyperthermophiles have been recovered from these regions by culture-dependent as well culture-independent approaches. Geothermal and volcanic areas include terrestrial fumaroles (e.g., solfataras), terrestrial hot springs, and geysers. Other natural habitats include geothermally heated oil and petroleum reservoirs and sun-heated soils/sediments (Greene et al. 1997; Engle et al. 1995; Völkl et al. 1993; Ward et al. 1987; Zillig et al. 1980; Mehta and Satyanarayana 2013). Man-made thermophilic habitats include acid mine drainage and acidic effluents (Kelly and Wood 2000), self-heated compost piles (Fujio and Kume 1991; Tiquia 2005; Tiquia et al. 1996, 2002, 2005), biological wastes (Mehta and Satyanarayana 2013), and waste treatment plants (Mehta and Satyanarayana 2013). These are comparatively lower temperature habitats, as compared to the natural habitats, and are ideal for the isolation of moderate and extreme thermophiles.

As a prerequisite for their survival, thermophiles contain enzymes that can function at high temperatures. Thermophiles have been found to improve protein thermostability by expressing heat-shock chaperonins to assist protein folding, prevent protein denaturation, and, in the case of small heat-shock protein "holdases," disaggregate amyloids (Trent 1996; Luo and Robb 2011). Protein folding is also aided by moving charged residues to the interior of the protein, effectively neutralizing the surface charge (Fukuchi et al. 2003). At high temperatures, neutral proteins have lower dielectric properties and desolvation penalties (Thomas and Elcock 2004). In addition, more densely packed protein folding and exposed hydrophobic shells minimize regions where high-temperature water can destabilize the protein (Vetriani et al. 1998). Interior salt and disul fi de bridges further improve thermostability (Robb and Maeder 1998). High temperatures promote the degradation of nucleic acids, so many thermophiles upregulate DNA repair pathways in order to preserve genetic stability (Toueille and Sommer 2011).

In industrial applications with thermophiles and thermostable enzymes, isolated enzymes are today dominating over microorganisms (Tiquia-Arashiro 2014; Tiquia and Mormile 2010; Satyanarayana et al. 2013). An enzyme or protein is called thermostable when a high defined unfolding (transition) temperature (Tm), or a long half-life at a selected high temperature, is observed. A high temperature should be a temperature above the thermophile boundary for growth (>55 °C). Most, but not all proteins from thermophiles are thermostable. Extracellular enzymes generally show high thermostability, as they cannot be stabilised by cell-specific factors like compatible solutes (Santos and da Costa 2002). The discovery and use of thermostable enzymes in combination with recombinant production and development using site-directed and enzyme evolution technologies, have erased some of the first identified hinders (e.g. limited access and substrate specificity) for use in

industrial biocatalysis. Recently, thermophilic microorganisms have been explored to synthesize metallic nanoparticles with well-defined chemical composition, size, and morphology.

Psychrophiles are a class of extremophiles that have the ability to live in extremely low temperature conditions. About 75 % of the Earth's biosphere, including polar, deep ocean, and atmospheric habitats, is permanently cold, and 70 % of the surface of the planet has a temperature between 1 and 5 °C (Feller and Gerday 2003; Cavicchioli 2006). Thus, the existence of psychrophiles, or organisms that live in these cold environments, is intuitive. Psychrophiles are commonly divided into two categories: stenopsychrophiles and eurypsychrophiles. Stenopsychrophiles, or obligate psychrophiles, can only survive at temperatures below 15 °C, while eurypsychrophilic or mesotolerant, organisms grow optimally below 15 °C but can also survive at higher temperatures (Feller and Gerday 2003; Cavicchioli 2006). Psychrophiles are those which have an optimum growth temperature below 15 °C and cannot grow at temperature beyond 20 °C. Psychrotolerant microorganisms, also known as psychrotrophs are those which can live in cold conditions, but have a higher optimum growing temperature, much greater than 20 °C. Many organisms remain metabolically active at temperatures well below freezing (Koshima 1984). Psychrophilic microorganisms, including the bacterium *Psychromonas ingrahamii*, the archaeon *Methanosarcina baltica*, and the fungus *Humicola marvinii*, metabolize and reproduce optimally at temperatures below 15 °C and, in some cases, have been reported to survive with greatly reduced metabolism down to −20 °C (Von Klein et al. 2002; Auman et al. 2006; Kumar et al. 2007; Weinstein et al. 1997).

These cold-loving microorganisms are commonly found in Polar region, deep sea, mountains, glaciers, fresh and marine waters, polar and high alpine soils. Psychrophiles face many challenges in this environments, including membrane rigidity, protein misfolding, and slower reaction rates. Many psychrophilic microorganisms, such as the alga *Chlamydomonas* sp. ICE-L, have been found to increase membrane fluidity by incorporating more unsaturated fatty acids into the phospholipid bilayer (Zhang et al. 2011). Methanogens also express a variety of unique protein adaptations designed to assist both anabolic and catabolic metabolism (Von Klein et al. 2002; Kumar et al. 2007). For example, a psychrophilic α-amylase was found to have a higher turnover rate (kcat), lower activation energy ($\Delta G\ddagger$), and lower change in enthalpy ($\Delta H\ddagger$) than mesophilic and thermophilic amylases (Feller and Gerday 2003). This reduction in activation energy is partially achieved through increased flexibility in the psychrophilic enzyme's active site, which reduces the substrate-enzyme complex binding energy and activation energy (Roulling et al. 2011). Eurypsychrophilic methanogens have also been found to possess temperature-dependent transcription factors that respond to variations in temperature by changing proteome composition (Goodchild et al. 2004). Finally, extracellular polysaccharide substances (EPS) and teichoic acid are cryoprotectants, and intracellular fumarate and glycerol are chaotropes commonly produced upon cold shock (Rice et al. 2008; Marx et al. 2009; Chin et al. 2010).

Psychrophiles became an important resource for bioprospecting because of its unique cold adaptations, which helped them successfully live in such frigid living conditions. Psychrophiles are successful in surviving such extreme conditions by optimizing various basic cell processes like enzyme function, nutrient transport and cell membrane function. The most important of these adaptations, which has immense potential to be exploited, are the production of polyunsaturated fatty acids and cold adaptive enzymes. The membranes and proteins of these microbes have a special property of increased structural flexibility that enhance the catalytic function and the presence of unsaturated fatty acids help in easy nutrient cell transportation, due to better fluidity. When temperature drops, psychrophiles produce cold shock or antifreeze proteins that enhance the activity of enzymes by improving enzyme kinetics and stabilizing microtubules. Psychrophilic enzymes have the advantage of having a low temperature optimum for activity with enhanced specific activity at low temperatures and rather high thermostability. The useful applications of cold active enzymes are widespread to a large number of industries such as the textile industry, the brewing and wine industry and the food and dairy industry. Psychrophiles are also a good source of polyunsaturated fatty acids, which can be extensively used in pharmaceutical industry for developing new therapeutic agents.

Microorganisms possess remarkable ability to reduce heavy metal ions and are one of the best candidates for nanoparticle synthesis. For instance, some thermophiles and psychrophiles have developed the ability to resort to specific defense mechanisms to quell stresses like toxicity of heavy metal ions or metals. Some of them could survive and grow even at high metal ion concentrations and are capable of binding large quantities of metallic cations. Moreover, some of these microorganisms are able to synthesize nanoparticles. The remarkable ability of some psychrophiles to reduce heavy metal ions make them one of the best candidates for nanoparticle synthesis. In this chapter, thermophilic and psychrophilic microorganisms used in nanoparticle biosynthesis (Table 3.1) are presented. The aim of chapter is to make a reflection on the current state and future prospects and especially the possibilities and limitations of the use of extremophiles in bio-based technique for industries.

3.2 Synthesis of Nanoparticles by Thermophiles

3.2.1 Thermophilic Bacteria

3.2.1.1 *Geobacillus* spp.

The genus *Geobacillus* comprises a group of Gram-positive thermophilic bacteria, including obligate aerobes, denitrifiers, and facultative anaerobes that can grow over a range of 45–75 °C. Originally classified as group five *Bacillus* spp., strains of *Bacillus stearothermophilus* came to prominence as contaminants of canned food

Table 3.1 Thermophiles in biosynthesis of nanoparticles

Thermophile	Nanoparticle	References
Bacteria		
Geobacillus stearothermophilus	Ag, Au	Fayaz et al. (2010)
Geobacillus sp.strain ID17	Au	Correa-Llantén et al. (2013)
Geobacillus wiegelii Strain GWE1	Se	Correa-Llantén et al. (2014)
Thermomonospora sp.*	Au	Ahmad et al. (2003a)
Thermomonospora sp. 67 Th*	Au	Kalabegishvili et al. (2013)
Ureibacillus thermosphaericus	Ag	Juibari et al. (2011)
Thermoactinomycete sp. 44 Th	Au	Kalabegishvili et al. (2013)
Thermus scotoductus SA-01	Au	Erasmus et al. (2014)
Thermincola ferriacetica strain Z-0001	Fe_2O_3	Koksharov et al. (2009)
Caldicellulosiruptor saccharolyticus	Pd	Shen et al. (2015)
Thermoanaerobacter sp.BKH1	SiO_2	Show et al. (2015)
Thermoanaerobacter sp.	CdS	Moon et al. (2013)
Thermoanaerobacter TOR-39	Zinc ferrite	Yeary et al. (2011)
Thermoanaerobacter sp. TOR-39	L-substituted magnetites	Moon et al. (2007)
Thermoanaerobacter ethanolicus TOR-39	Metal-substituted magnetite nano-crystals	Roh et al. (2006, 2007)
Thermoanaerobacter sp. X513	Cu	Jang et al. (2015)
Archaea		
*Sulfolobus islandicus***	Ag	Kalabegishvili et al. (2015)
Fungi		
Humicola sp.	Ag	Syed et al. (2013)
Humicola sp.	CeO_2	Khan and Ahmad (2013)
Humicola sp.	Gd_2O_3	Khan et al. (2013)

*Thermoalkaliphilic
**Thermoacidophilic

and soon became the organism of choice for comparative studies of metabolism and enzymology between mesophiles and thermophiles. More recently, their catabolic versatility, particularly in the degradation of hemicellulose and starch, and rapid growth rates have raised their profile as organisms with potential for second-generation (lignocellulosic) biorefineries for biofuel or chemical production (Hussein et al. 2015).

Geobacillus stearothermophilus

Biogenic synthesis of silver and gold nanoparticles by *Geobacillus stearothermophilus* was explored by Fayaz et al. (2010). The exposure of *G. stearothermophilus* cell free extract to the metal salts leads to the formation of stable silver and gold nanoparticles in the solution. The silver and gold nanoparticles have absorption maxima at 423 and 522 nm respectively. The TEM micrograph revealed the formation of polydispersed particles in the case of silver nanoparticles and monodispersed particles with respect to the gold nanoparticles. High stability of the nanoparticle solution could be attributed to the secretion of certain capping proteins by the bacterium in the reaction mixture (Fayaz et al. 2010).

Geobacillus sp. strain ID17

The production of gold nanoparticles by the thermophilic bacterium *Geobacillus* sp. strain ID17 is reported by Correa-Llantén et al. (2013). The strain was isolated from the Deception Island, Antarctica. The Deception Island is a complex strato-volcano with a "horseshoe" shape whose central part has a caldera structure. This volcanic island has been very active during the last century: fumarolic emissions, thermal springs and hot soils are evidence of Deception Island's continuing activity (Caselli et al. 2004), making it an interesting site for isolating new thermophilic bacteria. In order to study the biocatalytic reaction that takes place in the reduction of Au^{3+}, crude extracts of ID17 were assayed for their ability to reduce $HAuCl_4 \times 3H_2O$. Extracts from ID17 were able to catalyze the NADH-dependent reduction of Au^{3+} (Fig. 3.1a, b). NADH-dependent Au^{3+} reductase activity was dropped approximately in 98 % with SDS or proteinase K treatment, reflecting the enzymatic nature of this reaction. The activity was associated to at least four bands present in non denaturing gel PAGE corresponding to proteins in free cells extracts. One of these bands is present in high amount, based on the intensity of the band in a non denaturing gel PAGE (Fig. 3.1c). These results strongly suggest that the biosynthesis of gold nanoparticles by *Geobacillus* sp.strain ID17 is mediated by enzymes and NADH as a cofactor for this biological transformation. The intracellular localization and particles size were verified by TEM showing two different types of particles of predominant quasi-hexagonal shape with size ranging from 5 to 50 nm. The majority of them are between 10-20 nm in size (Correa-Llantén et al. 2013).

Geobacillus wiegelii Strain GWE1

Geobacillus wiegelii, strain GWE1, an aerobic thermophile belonging to genus *Geobacillus*, isolated from a drying oven (Correa-Llantén et al. 2014). This thermophile has the ability to reduce selenite evidenced by the change of color from colorless to red in the culture (Fig. 3.2). The SeNPs have a defined spherical shape

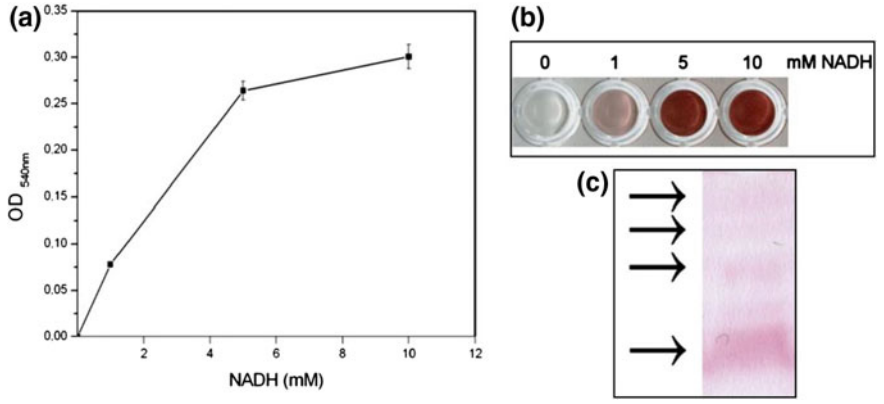

Fig. 3.1 Evidence of enzymatic Au^{+3} reduction in ID17. **a** OD at 540 nm using different concentrations of NADH (1, 5 and 10 mM); **b** NADH dependence for the $HAuCl_4$ reduction concentration; colour intensity indicates gold nanoparticles formation; **c** Zymogram analysis of enzymes (*indicated with arrows*) involved in Au^{+3} reductase activity. *Source* Correa-Llantén et al. (2013). Copyright © 2013, BioMed Central. Reproduced with permission

and a selenium elemental state. Size and shape of SeNPs can be modulated by pH and temperature. Size distribution ranged from 40 to 160 nm, where 70 % of nanoparticles have less than 100 nm in size. The size of all nanoparticles was less than 100 nm at pH 4.0; over 50 % of nanoparticles have less than 100 nm at pH 5.0; at pH 6.0 and 8.0 over 90 % of nanoparticles have less than 100 nm in size (Correa-Llantén et al. 2014). At neutral pH (7.0) nanoparticles reach a size around 120 nm and only 20 % of them were less than 100 nm. Nanoparticles did not show a significant difference in size when they were incubated between 0 and 3 h at 60 °C (Correa-Llantén et al. 2014). Meanwhile at 80 °C the nanoparticles suspension lost its homogeneity. A change in size was observed from 0 h of incubation at 80 °C,

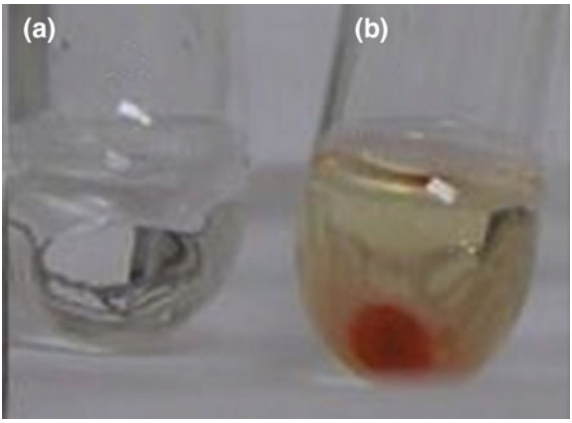

Fig. 3.2 Enzymatic selenite reduction by crude extract of *Geobacillus wiegelii* GWE1 and NADH. *Reddish color* in tube (**b**) indicates SeNPs formation. Tube (**a**) is a control without NADH. *Source* Correa-Llantén et al. (2014). Copyright © 2014, World Academy of Science, Engineering and Technology

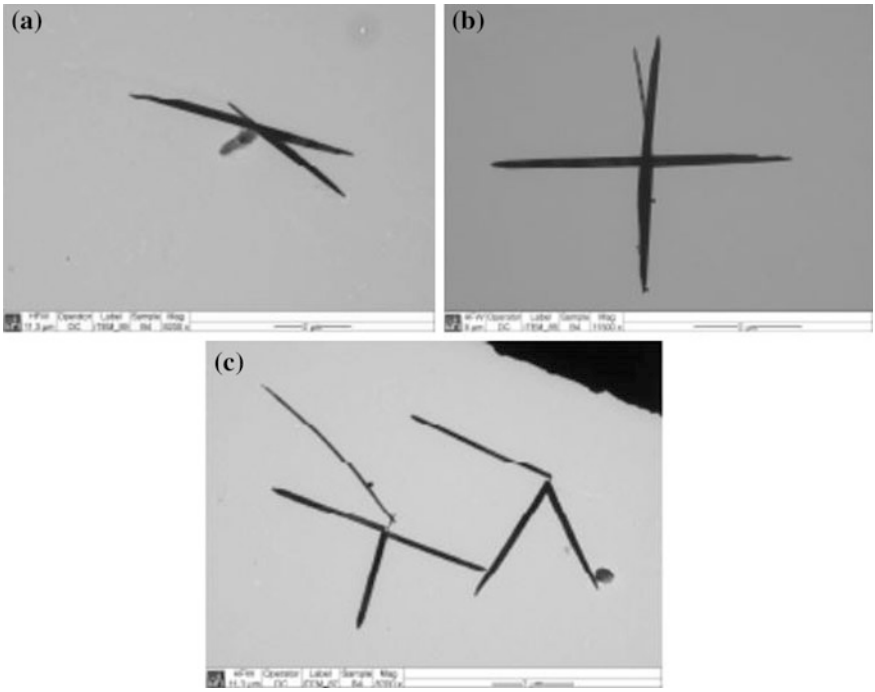

Fig. 3.3 Nanoparticles incubated for 3 h at 100 °C. **a**, **b** and **c** larger structures formed called nanorods. *Source* Correa-Llantén et al. (2014). Copyright © 2014, World Academy of Science, Engineering and Technology

observing a size range between 40 and 160 nm, with 20 % of them over 100 nm. Meanwhile after 3 h of incubation at size range changed to 60–180 nm with 50 % of them over 100 nm. At 100 °C the nanoparticles aggregate forming nanorod structures (Fig. 3.3). These results indicate that is possible to modulate size and shape of biologically synthesized nanoparticles by modulating pH and temperature (Correa-Llantén et al. 2014).

3.2.1.2 *Thermomonospora* sp.

Ahmad and group have performed a series of studies on bacterial synthesis of gold nanoparticles. In one such study, they used the extremophilic actinomycete *Thermomonospora* sp. to efficiently synthesize monodisperse gold nanoparticles (Ahmad et al. 2003a). By comparing this with their earlier work on gold nanoparticle synthesis from a fungus, *Fusarium oxysporum* (Ahmad et al. 2003b) they postulated that reduction of metal ions stabilization of the gold nanoparticles occurs by an enzymatic process. Furthermore, they attributed the synthesis of monodisperse gold

nanoparticles by *Thermomonospora* sp. to extreme biological conditions (i.e., alkaline and slightly elevated temperature conditions) (Ahmad et al. 2003a).

3.2.1.3 *Ureibacillus Thermosphaericus*

Ureibacillus thermosphaericus showed high potential for silver nanoparticle biosynthesis with extracellular mechanism and selected for the biosynthesis optimization (Juibari et al. 2011). Biosynthesis reactions were conducted using the culture supernatant at different temperatures (60–80 °C) and silver ion concentrations (0.001–0.1 M). Figure 3.4. shows the results of visual and spectral analysis of biosynthesis optimization reactions conducted at different silver nitrate concentrations (0.001 and 0.01 M) and temperatures (60, 70, and 80 °C). Visual analysis revealed that the intensity of color change enhanced with the increasing in temperature and ion concentration The maximum nanoparticle biosynthesis was achieved at 0.01 M $AgNO_3$ and 80 °C. However, ion concentration seemed to have a more significant effect on particle size, since silver particles were the main products at the higher ion concentrations such as 0.1 M $AgNO_3$. The average size of AgNPs at the silver ion concentration of 0.001 M and different temperatures of 60, 70 and 80 °C was 57, 29 and 13 nm, respectively (Fig. 3.4b). Whereas, these values

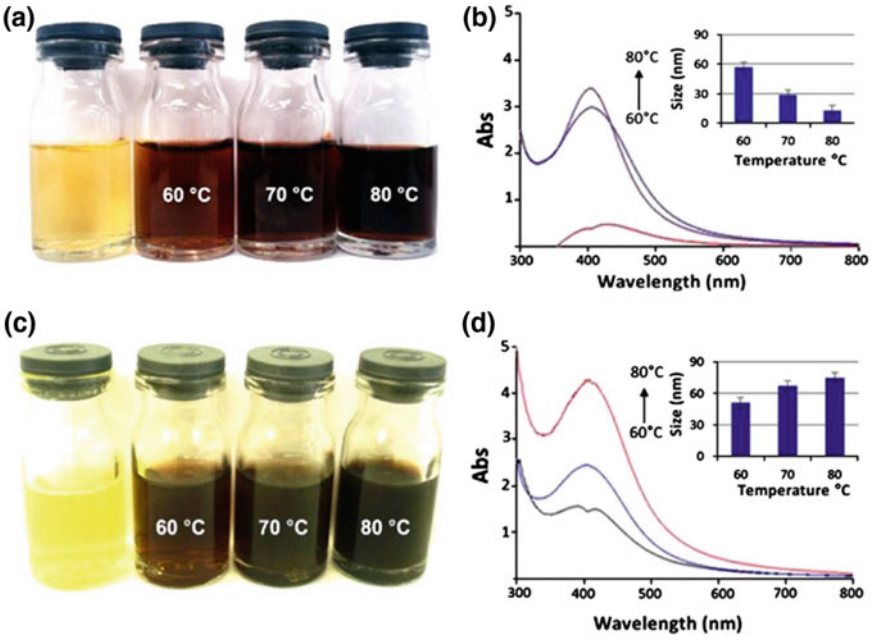

Fig. 3.4 Comparison of change in color intensity and UV–Vis spectrum of biosynthesis reaction conducted at different silver nitrate concentrations and temperatures, 0.001 M of $AgNO_3$ (**a**, **b**) and 0.01 M of $AgNO_3$ (**c**, **d**) Juibari et al. (2011). Copyright © 2011, Elsevier. Reproduced with permission

showed an increasing trend; 51, 67, and 75 nm, respectively, when the silver ion concentration 0.01 M was used (Fig. 3.4d). The increasing trend observed in the average particle size at the silver ion concentration of 0.01 M $AgNO_3$ by increasing the temperature could be the result of increasing in the secondary reduction process which is a common phenomenon in high ion concentrations (Juibari et al. 2011). The results obtained showed that pure spherical nanoparticles in the range of 10–100 nm were produced, and the maximum nanoparticle production was achieved using 0.01 M $AgNO_3$ at 80 °C. The findings of this study confirmed the great biocatalyzing potential of the extremophilic *U. thermosphaericus* supernatant for intensified biosynthesis of silver nanoparticle at elevated temperatures and high silver ion concentrations. These results demonstrated the great advantage of thermophilic bacteria as biocatalysts such as *U. thermosphaericus* for enhanced nanoparticle biosynthesis over currently developed biocatalysts which obtained from mesophilic strains and plant cells (Juibari et al. 2011).

3.2.1.4 *Thermoactinomycete* sp. 44 Th

The cultivation of *Thermoactinomycete* sp. 44 Th for preparation of samples containing Au nanoparticles was carried out using $HAuCl_4$ 10^{-3} M. AuNPs were formed after 3–4 days of reaction. The nanoparticles size distributions range from 5 to 60 nm (Kalabegishvili et al. 2013).

3.2.1.5 *Thermus scotoductus* SA-01

Thermus scotoductus SA-01 has the ability to reduce Au(III) and produce nanoparticles, making it a suitable candidate for the production of AuNPs (Erasmus et al. 2014). Physico-chemical parameters have influence on particle size with lower pH and higher temperatures resulting in larger particles and in contrast, higher pH and lower temperatures produce smaller particles. Gold reduction primarily occurred in the cell envelope which is strong evidence for gold-specific reduction process (Erasmus et al. 2014). An ABC transporter, peptide-binding protein of *T. scotoductus* SA-01, able to reduce and synthesize gold nanoparticles was purified to homogeneity. Even though this type of protein is not a classical oxidoreductase, a cysteine–disulphide bridge electron shuttle mechanism is likely involved in reducing Au(III). Moreover, the protein also acts as nucleation seed sites that initiate and direct nanoparticle synthesis. Through manipulation of physico-chemical parameters, it is clear that particle formation can be influenced in terms of size, shape and number of particles formed. However, since biological Au(III) reduction and nanoparticle synthesis is a complex process, manipulations of single parameters are unlikely to result in the best conclusive results. This is witnessed by a lack of particle monodispersity (with the exception of small spherical particles) when evaluating any of the parameters. Varying and investigating multiple parameters simultaneously will

likely shed light on the way forward to controlling and directing *T. scotoductus* SA-01 AuNP synthesis (Erasmus et al. 2014).

3.2.1.6 *Thermincola ferriacetica* Z-0001

Thermincola ferriacetica Z-0001 is an anaerobic iron-reducing bacterium that uses Fe^{3+}-hydroxide as an electron acceptor, and acetate as an electron donor for anaerobic growth (Koksharov et al. 2009). Koksharov et al. (2009) studied the Fe_2O_3 synthesis and the electro magnetic resonance spectra of Fe_2O_3 nanoparticles related to the reduction metabolism of the dissimilatory bacterium *Thermincola ferriacetica* Z-0001. The magnetization of the biologically-induced nanoparticles increases with time. However, the Fe_2O_3 nanoparticles obtained were not stable during later storage under open air conditions (Koksharov et al. 2009).

3.2.1.7 *Caldicellulosiruptor saccharolyticus*

Shen et al. (2015) examined the of coupling palladium (Pd) nanoparticle synthesis and H_2 production by *Caldicellulosiruptor saccharolyticus* for wastewater treatment under extreme thermophilic conditions. Na_2PdCl_4 was added to cell cultures to achieve a final Pd Concentration of 50 mg/L. Methyl orange (MO) and diatrizoate were chosen as the contaminants in water. In the cultures with, and without, Pd, MO (100 mg/L) was degraded within 30 min and in over 6 h, respectively. Diatrizoate (20 mg/L) was degraded within 10 min in cultures with Pd (Shen et al. 2015). The degradation of MO and diatrizoate were both enhanced by Pd. The removal of MO was the result of the combined action of hydrogen, hydrogenase and Pd^0 nanoparticles. Pd^0 particles also played an essential role in the removal of diatrizoate. The Pd^0 particles were well dispersed by cells of *C. saccharolyticus* and showed a better catalytic activity than chemical Pd^0 without dispersant The diameter of most Pd^0 particles formed in the presence of cells is under 100 nm. These Pd^0 particles are polyporous and homogeneous (Shen et al. 2015).

3.2.1.8 *Thermoanaerobacter* Spp.

Thermoanaerobacter BKH1

A green technique of silica nanoparticles (SiO_2-NPs) formation by using a thermophilic bacterium *Thermoanaerobacter* BKH1 as biological template is demonstrated by Show et al. (2015). SiO_2-NPs are synthesized from inorganic (magnesium tri-silicate), and organic (tetraethyl orthosilicate) precursor with the help of *Thermoanaerobacter* BKH1. TEM image of a specimen revealing the separations of SiO_2-NPs from the BKH1 template with few residual particles over the surface is demonstrated in Fig. 3.5a. Similar residual particles are seen in

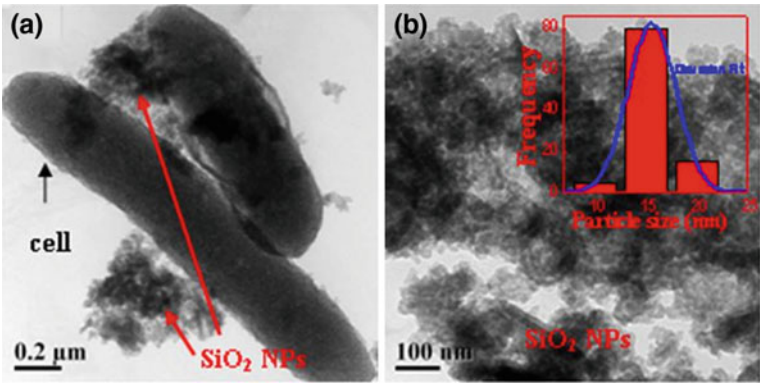

Fig. 3.5 TEM micrograph of **a** separation of the SiO_2-NPs from bacterium cell by centrifuge, **b** separated SiO_2-NPs and histogram with Gaussian curve fitting (*inset*) shows the particle size distribution where the average particles size is 15 ± 5 nm (n = 100). *Source* Show et al. (2015). Copyright © 2015, Elsevier. Reproduced with permission

Fig. 3.5b. The amorphous nature of the SiO2-NPs is also confirmed from TEM images as well. The biologically synthesized NPs are in regular spherical shapes. The histogram of the particle size distribution is shown as inset in Fig. 3.5b. The average particle size was around 15 ± 5 nm. The synthesized SiO_2-NPs can be utilized in various biomedical applications, such as, killing of unwanted bacteria with UV irradiation and subsequently use as an optical probe in medical diagnosis by using the visible emission (Show et al. 2015). The Zeta potential of the biologically derived SiO_2-NPs reveals stability of the synthesized nanoparticles in dispersed medium and impedes agglomeration. It can be logically asserted that silica leaching proteins, for instance bioremediase, may be involved in the formation of silica nanoparticles as a consequence of bacterial activity (Show et al. 2015).

Thermoanaerobacter X513

Moon et al. (2013) reported a microbially facilitated synthesis of cadmium sulfide (CdS) nanostructured particles (NP) using anaerobic, metal-reducing *Thermoanaerobacter* sp. The extracellular CdS crystallites were <10 nm in size with yields of ~3 g/L of growth medium/month with demonstrated reproducibility and scalability up to 24 L (Fig. 3.6). During synthesis, *Thermoanaerobacter* cultures reduced thiosulfate and sulfite salts to H_2S, which reacted with Cd^{2+} cations to produce thermodynamically favored NP in a single step at 65 °C with catalytic nucleation on the cell surfaces. Photoluminescence (PL) analysis of dry CdS NP revealed an exciton-dominated PL peak at 440 nm, having a narrow full width at half maximum of 10 nm. A PL spectrum of CdS NP produced by dissimilatory sulfur reducing bacteria was dominated by features associated with radiative

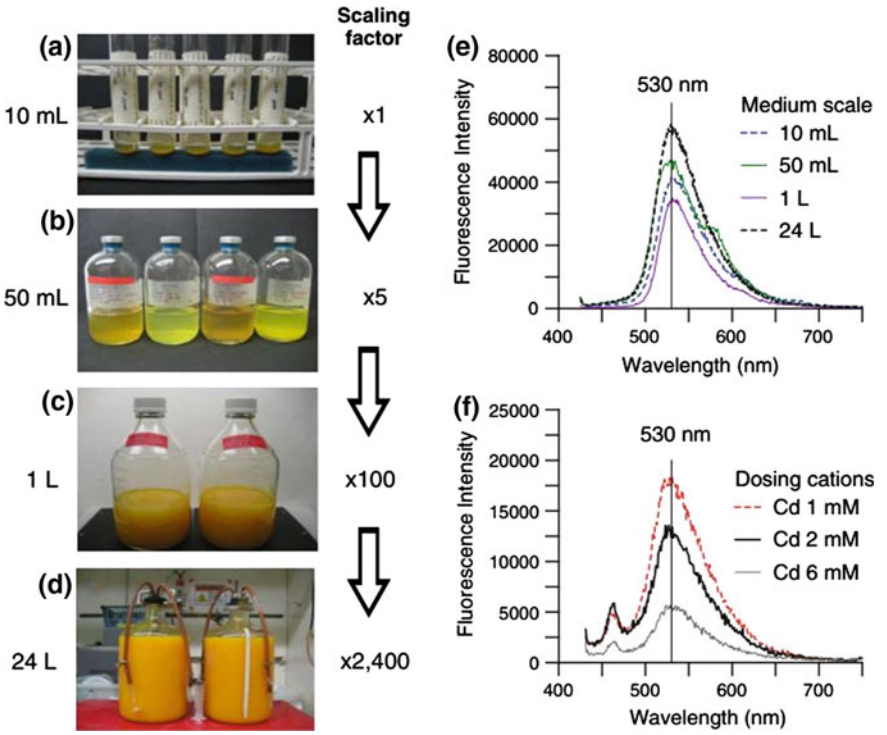

Fig. 3.6 Scale-up experiment for CdS NP from 10 mL to 24 L; **a** dosing 0.09, 0.27, 0.44, 0.62, and 0.89 mM of Cd^{2+} to 10 mL; **b** pairs of control and microbial samples of 50 mL (*left*, with cysteine S; *right*, with thiosulfate); **c** pulsed dosing of 0.4 and 0.8 mM/day for 10 days to 1 L; **d** pulsed dosing of 0.4 mM/day for 10 days to parallel reactors of 24 L. Fluorescence comparison for the scale-up experiment, **e** e dosing of Cd 0.4 mM/day into varying medium scales of 10 mL, 50 mL, 1 L, and 24 L of medium for 10 days; **f** single dosing of 1, 2, or 6 mM Cd^{2+} into the same 10 mL medium volume. *Source* Moon et al. (2013). Copyright © 2013, Elsevier. Reproduced with permission

exciton (Moon et al. 2013). CdS NPs of controlled size and crystal structure with high yield and size uniformity are produced. Controlling factors such as cell mass, dosing concentrations, type of precursors, and the basal medium composition using appropriate microbial populations as a reducing force are critical in producing copious CdS NP (Moon et al. 2013). Advantages of CdS NP synthesis using *Thermoanaerobacter* X513 include: (1) a relatively simple procedure without complicated steps (Tong and Zhu 2006), (2) low energy consumption at near room temperature compared to traditional methods requiring 250–300 °C (Yu and Peng 2002) or high mechanical energy like ball-milling up to 30 h (Patra et al. 2011), (3) easily tunable synthetic conditions for size control without implementation of additional steps like anion exchange chromatography (Kang et al. 2008),

(4) semi-continuous production without sacrificing the culture and anaerobic system, (5) minimizing the use of organic solvents and hazardous precursors (Flenniken et al. 2004), and (6) no need for post-treatment such as a lengthy dialysis (Zhang et al. 2004a).

Thermoanaerobacter TOR-39

Synthesis of Zinc Ferrite

A biological method to produce nanometer sized Zn-ferrites from *Thermoanaerobacter* TOR-39 was described by Yeary et al. (2011). Variability of particle size within sample is obvious in the TEM (Fig. 3.7). Figure 3.7a indicated large crystalline entities of 500 nm in size while Fig. 3.7b revealed well defined particles closer to 10 nm. Figure 3.7c shows a TEM image from different sample set showing uniformed well developed crystalline particles.

Highest amounts of zinc substitution, is shown in Fig. 3.7d. The bacteria produced consistent particles of 30–50 nm in size at an incubation temperature of 65 °C (Yeary et al. 2011). Microbially-mediated synthesis of nm-sized Zn-ferrites resulted in enhanced magnetic properties relative to chemically produced nanoparticles. The increased magnetic properties may have been related to lower fabrication temperatures of the bacterial process. Furthermore, properties of

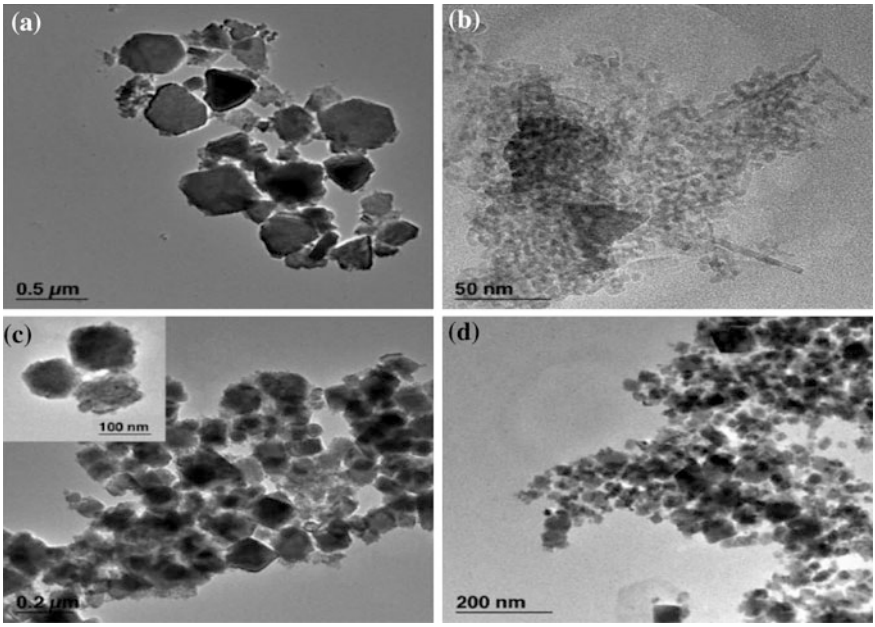

Fig. 3.7 TEM micrographs of representative Zn-ferrites. *Source* Yeary et al. (2011). Copyright © 2011, Elsevier. Reproduced with permission

microbially synthesized zinc ferrites vary systematically with Zn content (Yeary et al. 2011). Cation site location is often influenced by synthesis temperature, therefore, the biosynthesis of Zn-ferrite occurring at a much lower temperature compared to other methods may lead to different phenomena. Formation of Zn-ferrite with the incubation at ambient or slightly above room temperature may encourage a different distribution of zinc ions between octahedral and tetrahedral sites relative to what is observed in processes that take place at elevated temperatures. It is therefore possible that low temperature incubation may enhance the magnetic properties of bacterially synthesized Zn-ferrite (Yeary et al. 2011).

Synthesis of Lanthanide-Substituted Magnetite Nanoparticles

Moon et al. (2007) proposed a new mixed-lanthanide precursor method as compared to the traditional direct addition technique. Lanthanide (Nd, Gd, Tb, Ho and Er)-substituted magnetites, $LyFe_{3-y}O_4$ were microbially produced using lanthanide-mixed precursors, $L_xFe_{1-x}OOH$, where x = 0.01–0.2. By combining lanthanides into the akaganeite precursor phase, some of the toxicity is mitigated, enabling the microbial formation of L-substituted magnetites using a metal reducing bacterium, *Thermoanaerobacter* sp. TOR-39 (Moon et al. 2007). The employment of L-mixed precursors enabled the microbial formation of L-substituted magnetite, nominal composition up to $L_{0.06}Fe_{2.94}O_4$, with at least tenfold higher L-concentration than could be obtained when the lanthanides were added as soluble salts. This mixed-precursor method can be used to extend the application of microbially produced L-substituted magnetite, while also mitigating their toxicity (Moon et al. 2007).

Thermoanaerobacter ethanolicus

Thermoanaerobacter ethanolicus is a species of thermophilic, anaerobic, non-spore-forming bacteria. This bacterium were first isolated from hot springs in Yellowstone National Park. The bacteria ferment sugars into ethanol and carbon dioxide more than other anaerobes, hence the species name ethanolicus. The growth range of *T. ethanolicus* is 37–77 °C and pH 4.4–9.9, with the optimum growth temperature at around 70 °C (Wiegel and Ljungdahl 1981).

The extracellular synthesis of magnetite nanoparticles based on the reduction of iron oxyhydroxides to magnetite nanocrystals by *Thermoanaerobacter ethanolicus* TOR 39 was elucidated by Roh et al. (2006). Magnetite yield of up to 20 g/L per day was observed in 20-L vessels. *T. ethanolicus* TOR 39 reduced iron oxyhydroxides in the presence of metals (i.e. Co, Cr, Mn, Ni) and produced metal-substituted magnetite nanoparticles. The particles for sharp well-formed octahedral crystals. These crystals are in the single-domain size range of 30–100 nm. The magnetites formed using akaganeite with Cr, Mn and Ni showed well crystalline magnetite crystals (Roh et al. 2006). The biologically facilitated formation of metal-substituted magnetite does not require the reduction agents and the addition of exogenous electron

carrier substances such as humic acids. These biologically mediated reactions for mineral synthesis represent a novel way to make a number of inorganic materials and are potentially useful for the synthesis of nm-sized ferromagnetic materials (Roh et al. 2006). Magnetic nanoparticles synthesized by *T. ethanolicus* TOR-39 may be useful for improved magnetorheological (MR) fluids and ferrofluids for applications in active damping and for advanced power transmission devices such as fluid clutches (Roh et al. 2006).

Roh et al. (2007) examined the influence of Mn ion on the microbial synthesis of magnetite nanoparticles by *Thermoanaerobacter ethanolicus* TOR 39. The reductive biotransformation of an akaganeite (β-FeOOH) and Mn-substituted (2–20 mol%) akaganeite ($Fe_{1-x}Mn_xOOH$) by *T. ethanolicus* TOR-39 was investigated under anaerobic conditions at pH 7–8. *T. ethanolicus* TOR-39 reduced akaganeite and formed nm-sized magnetite using lactate or glucose as an electron donor. The Mn-mixed akaganeite as a magnetite precursor enabled production of microbially-synthesized Mn-substituted magnetite nanoparticles by *T. ethanolicus* TOR-39 at 60 °C rather than synthesizing a mixture of Mn-carbonate (Roh et al. 2007). Microbial formation of metal-substituted Fe(II)-minerals such as magnetite, siderite, and green rust is influenced by foreign ions (e.g. Mn), microorganisms, and incubation temperature. This method provide a means to mitigate the metal toxicity problem by incorporating or pre-alloying the substituting species into the colloidal metal oxyhydroxide phase. Besides reducing the potential toxicity, this method has further benefit of enhancing the uniform, intimate mixing of the different metal species in the final product by providing an intimately mixed starting material. This method may also allow the incorporation of metal species whose low aqueous solubility would limit the amount of the species that could be carried as soluble ions in the culture medium. Moreover, if several of the metal species are each reducible by the bacteria, the invention may help to produce a single-phase product that suppress the formation of two separate product phases. This mixed precursor method can therefore be used to extend the application for bacterial synthesis fields where there is a need for economically low-energy consumable microbial production of nm-sized materials that should involve toxic or inhibitory element to bacterial growth or by product formation in addition to magnetite synthesis (Roh et al. 2007).

Thermoanaerobacter sp. X513

Elemental copper nanoparticles with a bimodal distribution of 3 and 70 nm diameters were synthesized from inexpensive oxidized copper salts by an extracellular metal-reduction process using anaerobic *Thermoanaerobacte*r sp. X513 bacteria in aqueous solution (Jang et al. 2015). FTIR spectra indicated that chelating and capping agents coated the NP surfaces despite heterogeneous bacterial organic matter produced during the microbial activity (Jang et al. 2015). These coatings protected the surface from air oxidation under aqueous and dry lm conditions. The chelating agent, NTA, effectively facilitated the growth of particles and limited the

size to 70 nm. Fatty acids and amines capped Cu NPs without causing an increase in size, stabilized them against oxidation and agglomeration in aqueous solution and enabled the formation of the most stable elemental Cu NP film. Compared to previously reported Cu NP syntheses, this biological process substantially reduced the requirement for hazardous organic solvents and chemical reducing agents, while reducing the levels of Cu oxide impurities in the product. This process was highly reproducible and scalable from 0.01 to 1 L batches (Jang et al. 2015).

3.2.2 Thermophilic archaea

3.2.2.1 *Sulfolobus islandicus*

The hyperthermophiles from the order *Sulfolobales-Sulfolobus* spp., grow optimally at temperatures above 80 °C and pH values below 3 (O'Connor and Shand 2002). They are metabolically dependent on sulfur oxidation when sulfur acts as the final electron acceptor. Some strains of *Sulfolobus* have technical potential for metal extraction from ore (Huber and Prangishvili 2006). The potential of the thermoacidophilic archaeon *Sulfolobus islandicus* cells to serve as macromolecular template for the formation of silver nanoparticles (AgNPs) was demonstrated by Kalabegishvili et al. (2015). At the reaction in silver nitrate solution with *S. islandicus* suspension, the aggregation of the AgNPs from Ag(I) to Ag(0) by biomolecules, proteins and enzymes of *S. islandicus.* Sonication increases nanoparticle formation as it increases total surface of the biomass by its fragmentation. The *S. islandicus* suspension grown for 4 days was sonicated at 35 kHz for 10 min, then the aqueous solution of $AgNO_3$ with a concentration of 10^{-3} M was added to this suspension. The AgNPs synthesis was carried out at 75 °C. The stable formation of small sized AgNPs in the biomass of *S. islandicus* takes place in a few hours. In the first 20 h of reaction of the *S. islandicus* biomass with silver nitrate aqueous solution, the formation of AgNPs in the range of 10–50 nm takes place. The sizes increase further with time, reaching 10-50 nm (25 nm on average). The number of AgNPs also increases with time (Kalabegishvili et al. 2015).

3.2.3 Thermophilic Fungi

3.2.3.1 *Humicola* sp

Silver Nanoparticle Synthesis

Syed et al. (2013) elucidated the biosynthesis of silver nanoparticles by the thermophilic fungus *Humicola* sp. The fungus when reacted with Ag^+ ions reduces the

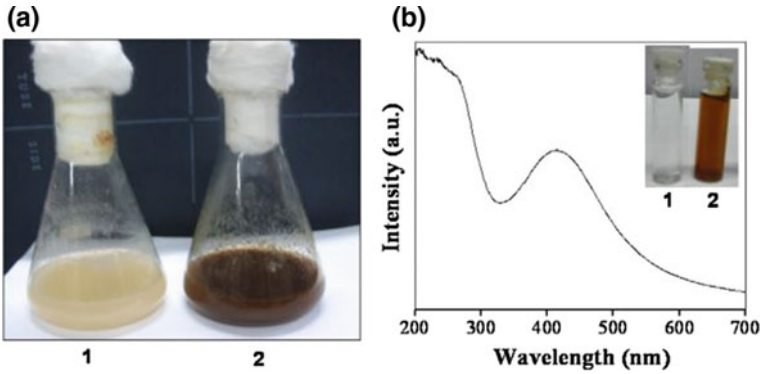

Fig. 3.8 **a** Conical flasks with fungus (Humicola sp.) before exposure (*1*) and after (*2*) exposure to $AgNO_3$ ions. **b** UV–Vis spectrum for the silver nanoparticles. Inset shows filtrate of the control flask (*1*) and filtrate of treated flask (*2*). *Source* Syed et al. (2013). Copyright © 2013, Elsevier. Reproduced with permission

precursor solution and leads to the formation of extracellular nanoparticles. The uniqueness of this study is that the investigators achieved superior control over the size of these nanoparticles, focusing upon them to be in the size range of 5–25 nm, so that these AgNPs when employed in biomedical applications will not block the glomerulus of the kidneys and will easily pass through urine within a short period of time. Figure 3.8a shows two conical flasks with the fungal biomass before (1) and after (2) exposure to 1 mM $AgNO_3$ solution at temperature 50 °C and pH 9 for 96 h under shaking condition. Formation of silver nanoparticles is clearly demonstrated by the change in color from yellow to brown. After filtration, it was observed that the aqueous solution contained the silver nanoparticles, characterized by an intense brown color (Fig. 3.8b). The AgNPs synthesised are non-toxic to cancer and normal cells up to concentrations of 50 µg/ml and thus will find various applications in drug and targeted drug delivery systems (Syed et al. 2013).

Cerium Oxide Nanoparticle Synthesis

Khan and Ahmad (2013) reported for the first time, the bio-inspired synthesis of biomedically important cerium oxide (CeO_2) nanoparticles using the thermophilic fungus *Humicola* sp. The fungus Humicola sp. when exposed to aqueous solutions of oxide precursor cerium (III) nitrate hexahydrate ($CeN_3O_{96}H_2O$) results in the extracellular formation of CeO_2 nanoparticles containing Ce(III) and Ce(IV) mixed oxidation states, confirmed by X-ray Photoemission Spectroscopy (XPS). The formed nanoparticles are naturally capped by proteins secreted by the fungus and thus do not agglomerate, are highly stable, water dispersible and are highly

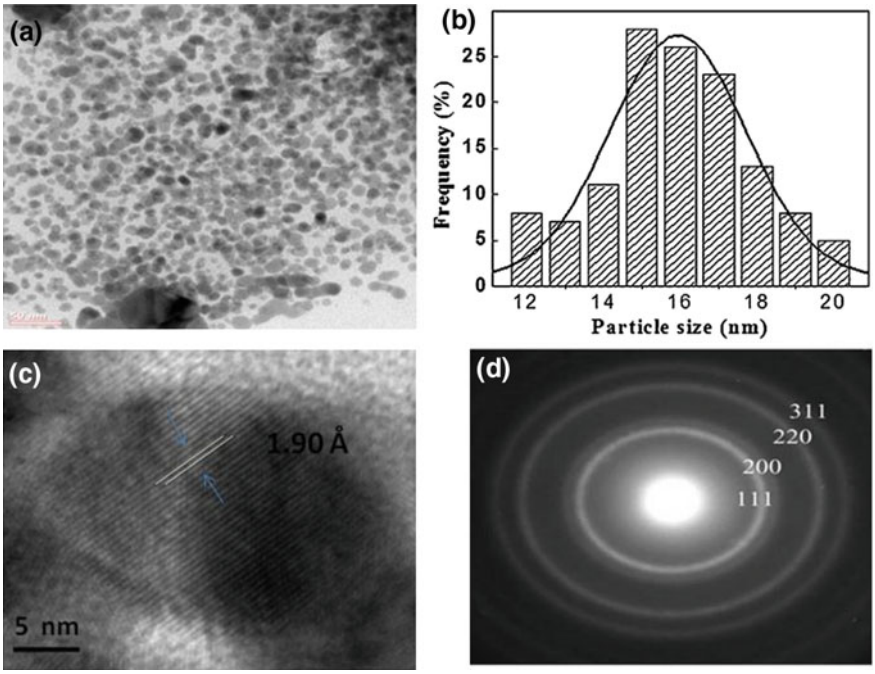

Fig. 3.9 **a** TEM micrograph recorded from drop-cast films of CeO_2 nanoparticle solution formed by the reaction of cerium nitrate with the fungal biomass of *Humicola* sp. for 96 h. **b** Particle size distribution determined from TEM microgaph. **c** HR-TEM image of CeO_2 nanoparticles showing inter planar distance. **d** Selected Area Electron Diffraction (SAED) pattern recorded from the CeO_2 nanoparticles. *Source* Khan and Ahmad (2014). Copyright © 2014, Elsevier. Reproduced with permission

fluorescent as well. The CeO_2 nanoparticles are polydispersed and spherical in shape (Fig. 3.9a). Particle size distribution analysis in Fig. 3.9b reveals that the particles are in the range of 12–20 nm with 16 nm as an average diameter. Figure 3.9c represents the HR-TEM image of one of the CeO_2 nanoparticles showing inter planar distance or 'd' value which was estimated to be 1.90 Å and corresponds to the {2 2 0} plane of CeO_2 nanoparticles. Selected Area Electron Diffraction pattern in Fig. 3.9d indicates that the CeO_2 nanoparticles are polycrystalline in nature (Khan and Ahmad 2013). Khan and Ahmad (2013) have shown a very simple fungus based method for the synthesis of biomedically important cerium oxide (CeO_2) nanoparticles. Capping protein which is involved in the capping of nanoparticles makes them water dispersible and can prove very important for their clinical applications such as treatment of diseases which involve ROS production.

Gadolinium Oxide Nanoparticle Synthesis

Gadolinium oxide nanoparticles are very important as nuclear, electronic, laser, optical, catalyst and phosphor materials (Hussein 1994; Bhattacharyya and Agrawal 1995; Chen 1996; Gündüz and Uslu 1996). Many organic compounds use Gd_2O_3 for their dimerization (Gündüz and Uslu 1996). It is also used in imaging plate neutron detectors, as neutron reactors (Gündüz and Uslu 1996), and as an additive in ZnO_2 to enhance its toughness (Bhattacharyya and Agrawal 1995; Chen 1996). Gd_2O_3 has several potential applications in biomedicine, too. For example, it is used in magnetic resonance imaging, since it exhibits superparamagnetism and involves T1 relaxation, and can be useful as a multimodal contrast agent for in vivo imaging (Bridot et al. 2007). It can also be easily doped with other lanthanides and exploited as a fluorescent tag, thus replacing other fluorescent organic molecules. Chemical and physical protocols for the of Gd_2O_3 nanoparticles are limited. The most common methods are the thermal decomposition of precursor salts, mechanochemical processing, milling and calcinations (Matijević and Hsu 1987; Mazdiyasni and Brown 1971; Rowley and Parkin 1993). Unfortunately, these methods give agglomerated particles, occur at high temperatures, and employ harsh environments, thus rendering it difficult to find any usage of Gd_2O_3 nanoparticles in biomedical applications.

Khan et al. (2014) showed that the thermophilic fungus *Humicola* sp. can be used for the synthesis of Gd_2O_3 nanoparticles at 50 °C. As $GdCl_3$ is dissolved in water along with fungal biomass, $GdCl_3$ ionizes to Gd^{3+} and $3Cl^{-}$. The Gd^{3+} ions are then attracted toward anionic proteins, which are secreted by *Humicola* sp in solution. Reductase enzymes present in the anionic protein fraction act on Gd^{3+} and convert it to Gd^{2+}. Oxidase enzymes, which are also secreted by the fungus in the solution mixture, act on these Gd^{2+} ions resulting in the formation of Gd_2O_3 nanoparticles. The $GdCl_3$ NPs articles are irregular in shape, presenting an overall quasi-spherical morphology. Particle size distribution analysis of Gd_2O_3 nanoparticles confirmed that the nanoparticles are in the range of 3–8 nm with an average size of 6 nm. Since Gd_2O_3 nanoparticles have proved their value in site specific drug delivery systems for cancer therapy, Khan et al. (2014) extended the work of biosynthesis of Gd_2O_3 nanoparticles to bioconjugation with taxol. Bioconjugation of taxol with gold and iron oxide nanoparticles has also been reported (Gibson et al. 2007; Hwu et al. 2009). Taxol is one of the most important anticancer drugs used for breast, ovarian and lung cancers. The potent anticancer effect of taxol is mainly attributed to its mechanism of action. It stabilizes microtubules by preventing their depolymerization Khan et al. (2014). However, taxol is a hydrophobic drug and less specific to certain tumors due to its low solubility in water. To counter these problems, we carried out the bioconjugation of chemically modified taxol with biocompatible Gd_2O_3 nanoparticles, which may result in an enhancement of the hydrophilicity of taxol and may render it more potent in killing tumor/cancer cells Khan et al. (2014).

3.3 Thermophilic Enzymes in Nanotechnology

3.3.1 Immobilization of Thermozymes with Magnetic Nanoparticles

Thermophilic microorganisms represent a novel source of highly active enzymes with attractive features for industrial bioprocesses due to their adaptability and stability under extreme conditions (Demirjiana et al. 2011). Thermoenzymes from these microorganisms allow to perform reactions at high temperatures, which result in lower medium viscosity, increased substrate diffusion coefficients and fewer microbial contamination risks (Zhou et al. 2013). Thermostable enzymes from extreme thermophiles have led to a special focus due to their intrinsic thermostability and resistance to denaturing physical and chemical factors (Li et al. 2010a). However, to carry out these bioprocesses under preparative conditions, immobilization procedures are required to enable biocatalyst recovery and reusability. Immobilized enzymes are drawing significant attention for potential commercial applications as biocatalysts by reducing operational expenses and by increasing process utilization of the enzymes. Immobilized enzymes can be recycled by utilizing the physical or chemical properties of the supporting material. Several nanoparticles have been employed to improve traditional enzyme immobilization methods in order to enhance loading, activity and stability of enzymes and to reduce the biocatalyst costs in industrial biotechnology (Abad et al. 2005; Yiu and Keane 2012). In particular, MNP-based immobilization of enzymes presents several advantages, including (i) high surface-to-volume ratio offered by nanosize magnetic beads, (ii) good dispersibility, (iii) easy separation of enzymes from the reaction mixture, and (iv) reuse by applying an external magnetic field (Johnson et al. 2011). One of the crucial points in protein immobilization on nanoscale solid surfaces is that structural modifications may occur, which could affect protein activity and stability to different extents depending on the protein and the conjugation strategy (Occhipinti et al. 2011). For this reason, there has been an increasing interest in developing new reliable approaches for the immobilization of enzymes on magnetic nanoparticles (Johnson et al. 2008; Li et al. 2010b; Yu et al. 2012). However, although great efforts have been made for this purpose, the actual effect of immobilization on enzyme functionality is still poorly understood. Furthermore, not many studies involving enzymes from extremophile microorganisms have been carried out so far.

3.3.1.1 Immobilization of α-Amylase

Magnetic nanoparticles has been used for efficient immobilization of lysine-tagged α-amylase (BACΔNC-*Lys*7) from thermophilic *Bacillus* sp. strain TS-23 (Chen et al. 2012). The carboxylated magnetic nanoparticles were prepared by the simple co-precipitation of Fe^{3+}/Fe^{2+} in aqueous medium and then subsequently modified

with adipic acid. Transmission electron microscopy micrographs showed that the carboxylated magnetic nanoparticles remained discrete and had no significant change in size after the binding of BACΔNC-*Lys*7. Free enzyme was active in the temperature range of 45–70 °C and had an optimum of 60 °C, whereas the thermal stability of BACΔNC-*Lys*7 was improved as a result of immobilization. The immobilized BACΔNC-*Lys*7 could be recycled 20 times without a significant loss of the amylase activity and had a better stability during storage with respect to free enzyme. Taken together, the magnetic nanoparticles coated with this functional moiety can be a versatile platform for the effective manipulation of various kinds of engineered proteins (Chen et al. 2012). Surface immobilization of the enzyme on the magnetic nanoparticles increase the thermal stability, probably by increasing its molecular rigidity, thus preventing any undesirable change in the tertiary molecular structure due to heating (Chen et al. 2012).

3.3.1.2 Immobilization of Superoxide Dismutase

Superoxide dismutase (SOD) is one of the most important metalloenzymes for aerobic and anaerobic organisms in the first line of defense against oxidative stress, catalyzes the disproportionation of superoxide anion (O_2^-) to O_2 and H_2O_2 (Miller and Sorkin 1997). Due to its enzymatic activity, SOD has been widely applied in the medical treatment, cosmetic, food, agriculture, and chemical industries (Pugliese and Pugliese 2002; Zhang et al. 2004b; Kumar et al. 2006). Thermostable Fe/Mn-SODs have been identified in thermophiles including, *Aquifex pyrophilus* (Lim et al. 1997), *Chloroflexus aurantiacus* (Lancaster et al. 2004), Sulfolobus solfataricus (Yamano and Maruyama 1999), *Aeropyrum pernix* (Yamano et al. 1999), *Pyrobaculum aerophilum* (Whittaker and Whittaker 2000), *Thermus aquaticus* (Sato and Harris 1977), and *Thermus thermophilus* (Li et al. 2010a; Liu et al. 2011). These thermostable SODs have the potentials to be widely used in industry. Superparamagnetic nanoparticles, due to the remarkable properties such as superparamagnetism, high field irreversibility, and high saturation field, have shown great potential applications in various fields (Andrade et al. 2011), especially in biomedicine and bioengineering such as magnetic separation (Gupta and Gupta 2005; Lucena et al. 2011), tumor hyperthermia (Ito et al. 2005), magnetic resonance imaging (De et al. 2008), magnetically assisted site-specific drug delivery (Neuberger et al. 2005; McCarthy et al. 2007), and biomolecule immobilization (Saiyed et al. 2007).

Song et al. (2012) immobilized the thermostable Mn-SOD from *Thermus thermophilus* on supermagnetic nanoparticles. The thermostable Mn-SOD from *T. thermophilus* was covalently bound to the supermagnetic 3-APTES-modified Fe_3O_4@SiO_2 nanoparticles using glutaraldehyde method to prepare the Mn-SOD bound magnetic nanoparticles. The immobilization proof of Mn-SOD on the 3-APTES-modified Fe_3O_4@SiO_2 nanoparticles was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), X-ray diffraction (XRD), transmission electron microscopy (TEM), and vibrating sample magnetometer (VSM) observation (Song et al. 2012). By comparison with the free Mn-SOD, it

was found that the immobilized Mn-SOD on nanoparticles exhibited better resistance to temperature, pH, metal ions, enzyme inhibitors, and detergents. The results showed that the immobilized Mn-SOD on nanoparticles could be reused ten times without significant decrease of enzymatic activity (Song et al. 2012).

3.3.1.3 Immobilization of Trelahose Synthase

Trehalose synthase (TreS) catalyzes the reversible interconversion of trehalose (glucosyl-alpha,alpha-1,1-glucose) and maltose (glucosyl-alpha1-4-glucose) (Woo et al. 2010; Schiraldi et al. 2002; Zdziebło and Synowiecki 2006). Trehalose can find applications in medicine, pharmacy, cosmetics and food chemistry as sweeter, stabilizer, antifreeze agent and humectants (Roser 1991; Richards et al. 2002). This non-reducing disaccharide protects cell proteins and biological membranes against physical and chemical stresses such as freezing, high temperature, dehydration and high osmotic pressure (Crove and Crove 2000; Benaroudi et al. 2001; Higashiyama 2002; Elbein et al. 2003). Furthermore, the mild sweetness of trehalose, its low cariogenicity, good solubility in water, high water retention capabilities, stability, reduction of water activity, depression of freezing point and protein protection properties make it a valuable food ingredient (Richards et al. 2002). Trehalose is resistant to hydrolysis at low pH values and even at elevated temperatures. Moreover, this disaccharide does not caramelize and undergo Maillard's reaction.

The immobilization on magnetic nanoparticles of the recombinant trehalose synthase from thermophilic bacterium *Deinococcus geothermalis* was demonstrated by Panek et al. (2013). In their study the gene encoding trehalose synthase from Recombinant trehalose synthase from D. *geothermalis* was covalently immobilized on magnetic support obtained by co-precipitation of Fe^{3+} and Fe^{2+} salts. The mean diameter of the prepared nanoparticles determined by SEM analysis was 30 nm. However, the size distribution of the particles seems to be wide. Before the immobilization magnetic support was silanized using 3-aminopropyltriethoxysilane which allows coupling the enzyme via glutaraldehyde. The catalytic activity of enzyme immobilized using glutaraldehyde is dependent on the amount of crosslinking reagent applied during enzyme fixation as well as other factors, such as immobilization time and enzyme concentration in the media. The most active preparations (0.134 U/g of support) were obtained at glutaraldehyde concentration of 10 mM. Increase of glutaraldehyde concentrations from 15 mM to 30 mM has no influence on the amount of bound protein. At higher glutaraldehyde concentration of 30 mM the enzyme activity decreased considerably to 63.4 % of the maximal value and was 0.084 U/g of support. The reaction of enzyme coupling is fast and preparations of the immobilized trehalose synthase achieved maximal activity after 0.5 h of the process. Further increase of immobilization time did not influence on enzyme activity. The obtained immobilized preparation has specific activity of 0.134 U/g support when measured at 40 °C using maltose as substrate (Panek et al. 2013). Immobilization process was almost fully completed after 30 min of the reaction at 30 °C. The highest immobilization yield of the enzyme

was achieved at glutaraldehyde concentration of 10 mM. No significant differences in optimal pH and temperature were observed upon immobilization. The immobilized trehalose synthase exhibited mass transfer limitation, which is reflected by higher KM and activation energy values. In addition, immobilized trehalose synthase was easily separated from the reaction medium by an external magnetic field and retained 82 % of its initial activity after successive twelve repeated batches reaction. Stability during the operation of immobilized trehalose synthase was investigated by determination of residual activity of the enzyme after repeated batch reactions carried out for 1 h each. After each run the immobilized enzyme was recovered from the reaction mixture by magnetic separation and washed. This experiment shows the high stability of the immobilized trehalose synthase. No remarkable changes were observed on the adsorption capacity and activity recovery of the trehalose synthase during twelve reaction cycles which retained about 80 % of initial activity (Panek et al. 2013). This is the first report on such immobilization of trehalose synthase from *D. geothermalis*.

3.3.1.4 Immobilization of Carboxypeptidase

Carboxypeptidases are enzymes that remove amino acids one residue at a time from the C-termini of polypeptide chains. Three types of enzyme have been characterized which differ in the rate at which they release particular amino acids. Carboxypeptidases A (CPA) (from pancreas) release most rapidly amino acids with an aromatic or large aliphatic side chain, while carboxypeptidases B (CPB) (also from pancreas) release the basic amino acids lysine and arginine very much faster than any of the other common protein amino acids. Plants contain carboxypeptidase C, which will liberate the amino acid proline as well as being able to release many of the other protein amino acids (Ambler 1967).

Sommaruga et al. (2014) developed a nanobioconjugate of carboxypeptidase from the hyperthermophilic archaeon *Sulfolobus solfataricus* CP*Sso* immobilized on silica-coated magnetic nanoparticles, which exhibited enhanced stability in aqueous media at room temperature as well as in different organic solvents (Fig. 3.10). CP*Sso* is a heat- and pressure-resistant zinc-metalloprotease consisting of four identical 43 kDa subunits (Colombo et al. 1995; Bec et al. 1996; Tortora and Vanoni 2004; Occhipinti et al. 2006). The catalytic and kinetic mechanisms of CP*Sso* have been well established and were confirmed by a 3D model that was developed and validated in the past years (Occhipinti et al. 2003; Sommaruga et al. 2008). CP*Sso* exhibits nonconventional catalytic properties that are useful in several synthetic processes. First, it removes any amino acid from the C-terminus of short peptides, with the sole exception of proline, and also hydrolyzes N-blocked amino acids, thus acting as an aminoacylase (Sommaruga et al. 2008). Second, despite its remarkable thermophilicity, it maintains a significant fraction of its maximal activity even at room temperature. Finally, CP*Sso* maintains a significant activity in solvent mixtures even at high content of organic fraction (Colombo et al. 1992). These peculiar properties highlight the biotechnological potential of this enzyme, in

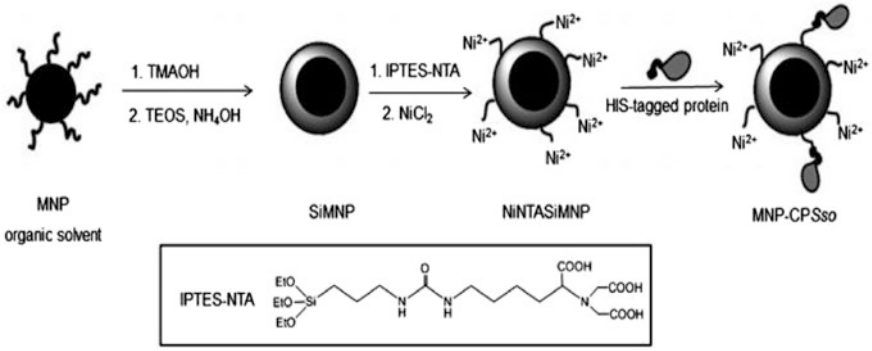

Fig. 3.10 Procedure of CP*Sso* immobilization on NiNTASiMNP. *Source* Sommaruga et al. (2014). Copyright © 2014, BioMed Central. Reproduced with permission

particular to achieve the synthesis of N-blocked amino acid in organic media. The nanobioconjugate was obtained by affinity-oriented immobilization of His-tagged CPSso on silica-coated magnetic nanoparticles functionalized with Ni-NTA groups (NiNTASiMNP). The procedure for the synthesis of NiNTASiMNP is illustrated in Fig. 3.10 and the resulting functionalized Fe_3O_4@silica core-shell nanoparticles gave a stable dispersion in aqueous environment, as determined by dynamic light scattering analysis. Following the immobilization, CP*Sso* acquired distinctly higher long-term stability at room temperature compared to the free native enzyme, which, in contrast, underwent extensive inactivation after 72 h incubation, thus suggesting a potential utilization of this enzyme under low energy consumption. CP*Sso* conjugation also resulted in a significantly higher stability in organic solvents at 40 °C, which made it possible to synthesize N-blocked amino acids in remarkably higher yields compared to those of free enzyme. Improved stability of MNP-CP*Sso* in organic solvent is relevant to possible industrial applications of the enzyme as a biocatalyst in synthetic reactions carried out in organic environment. CP*Sso* could be an ideal candidate as a biocatalyst for the synthesis of N-blocked amino acids in water-cosolvent mixtures following the thermodynamic method (Sommaruga et al. 2014).

3.4 Synthesis of Nanoparticles by Psychrophiles

3.4.1 Synthesis of Nanoparticles by Psychrophilic Bacteria

3.4.1.1 *Morganella Psychrotolerans*

Ramanathan et al. (2011) showed for the first time that by controlling the growth kinetics of *Morganella psychrotolerans*, a silver-resistant psychrophilic bacterium,

Table 3.2 Psychrophiles in biosynthesis of nanoparticles

Psychrophile	NP	References
Bacteria		
Morganella psychrotolerans	Ag	Ramanathan et al. (2011)
Phaeocystis antarctica	Ag	Shivaji et al. (2011)
Pseudomonas proteolytica	Ag	Shivaji et al. (2011)
Pseudomonas meridiana	Ag	Shivaji et al. (2011)
Arthrobacter kerguelensis	Ag	Shivaji et al. (2011)
Arthrobacter gangotriensis	Ag	Shivaji et al. (2011)

the shape anisotropy of silver nanoparticles can be achieved (Table 3.2). This is particularly important considering that there has been no report that demonstrates a control over shape of Ag nanoparticles by controlling the growth kinetics of bacteria during biological synthesis. This is most possibly because most of the previous studies in this field had only reported the outcomes of exposure of Ag^+ ions to bacteria, without making any deliberate efforts to control the bacterial growth kinetics to achieve shape control. To this end, recent study by Fayaz et al. (2009) in which the effect of temperature on the size of Ag nanoparticles formed during a fungus-mediated biosynthesis process was investigated is particularly notable. It is also notable that controlling reaction kinetics and altering reaction parameters via photochemical, microwave, and ultrasound-assisted techniques are known to achieve anisotropic growth in conventional chemical synthesis.

Ramanathan et al. (2011) utilized *Morganella psychrotolerans* as a model organism, which is a close relative of silver resistant bacteria *Morganella morganii*. *M. morganii* RP42 strain was recently reported for its specificity toward sustaining high concentrations of Ag^+ ions via extracellular synthesis of spherical Ag nanoparticles (Parikh et al. 2008). Ramanathan et al. (2011) chose *M. psychrotolerans* as a model organism for controlling shape anisotropy of Ag nanoparticles due to its tolerance for lower temperature (psychros: cold) and its capability to grow at a wider temperature range of typically 0–30 °C with 20 °C as the optimum temperature (Emborg et al. 2006). At the optimum growth temperature of 20 °C, predominantly spherical Ag nanoparticles of ca. 2–5 nm diameter along with relatively few nanoplates of 100–150 nm edge length were observed during TEM imaging (Fig. 3.11b) (Ramanathan et al. 2011). However, in contrast to previous biosynthesis studies, when *M. psychrotolerans* bacteria was used in this study for biosynthesis of Ag nanoparticles at temperatures different from its optimum growth temperature, formation of Ag nanoplates was observed (Fig. 3.11a, c and d). For instance, at 25 °C, which is 5 °C higher than the optimum growth temperature of bacteria, a mixture of triangular and hexagonal nanoplates along with spherical nanoparticles was obtained (Fig. 3.11a). Similarly, at 15 °C, which is 5 °C lower than the optimum growth temperature, again a mixture of nanoplates and spherical particles was obtained (Fig. 3.11c). Further reduction in bacterial physiological

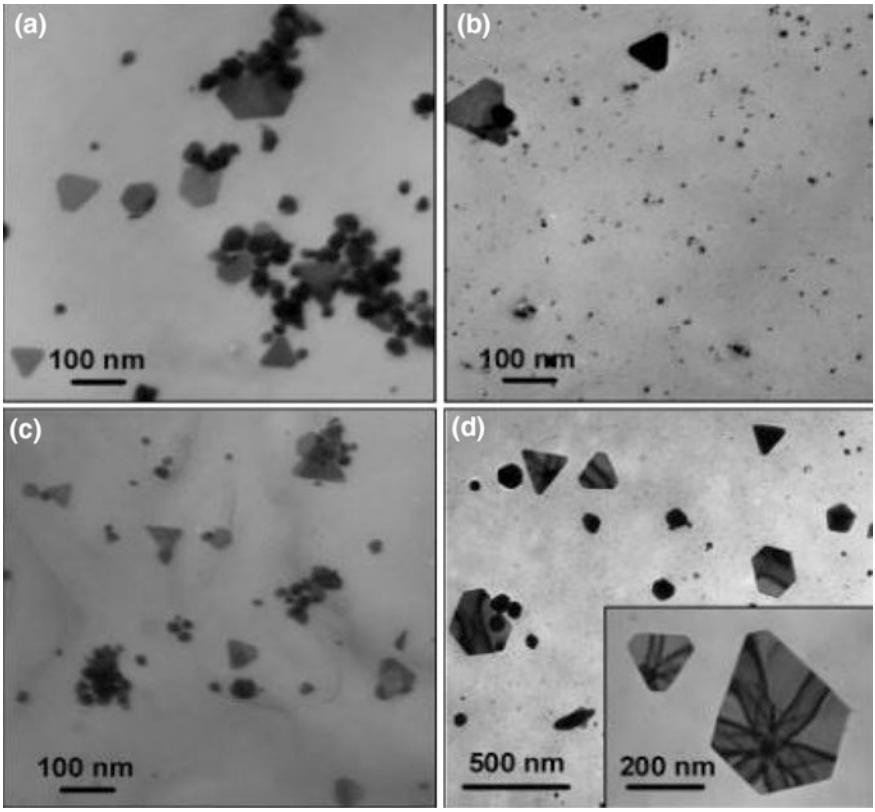

Fig. 3.11 TEM images of biogenic Ag nanoparticles biosynthesized by *M. psychrotolerans* at **a** 25, **b** 20, **c** 15, and **d** 4 °C after 20 h, 24 h, 5 days, and 15 days of reaction, respectively. *Source* Ramanathan et al. (2011). Copyright © 2011, American Chemical Society. Reproduced with permission

activity and growth by reducing its growth temperature to 4 °C results in a significant increase in the number of nanoplates, whereas only a relatively smaller proportion of spherical nanoparticles were formed (Fig. 3.11d) (Ramanathan et al. 2011). It is however notable that although the proportion of spherical Ag particles formed at 4 °C is lower than that observed at other temperatures; the spherical particles formed at 4 °C are larger in size (ca. 70-100 nm). Typically, *M. psychrotolerans* was found to synthesize Ag nanoplates with 50-150 nm edge length at 25 and 15 °C; however, biosynthesis at 4 °C resulted in larger nanoplates with 150-450 nm edge length (Ramanathan et al. 2011). The possibility to achieve nanoparticle shape control by using a green biosynthesis approach is expected to open up new exciting avenues for eco-friendly, large-scale, and economically viable shape-controlled synthesis of nanomaterials (Ramanathan et al. 2011).

3.4.1.2 *Phaeocystis antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana, Arthrobacter kerguelensis*, and *Arthrobacter gangotriensis*

Cell-free culture supernatants of five psychrophilic bacteria *Phaeocystis antarctica*, *Pseudomonas proteolytica, Pseudomonas meridiana, Arthrobacter kerguelensis,* and *Arthrobacter gangotriensis* were used to synthesize AgNPs (Shivaji et al. 2011). AgNPs synthesized are spherical in shape and the average size of the AgNPs varied from minimum of 6.2 ± 2.4 nm to a maximum of 12.2 ± 5.7 by TEM. The cell-free culture supernatants formed AgNPs only if the reaction was incubated in the light. These results are similar to the earlier observations using culture supernatants of *B. subtilis* (PTCC 1023), *L. acidophilus* (PTCC 1608), *K. pneumoniae* (PTCC 1053), *E. coli* (PTCC 1399), *E. cloacae* (PTCC 1238) Staphylococcus aureus (PTCC 1112) and *Candida albicans* (PTCC 5011) (Minaeian et al. 2008; Natarajan et al. 2010), but differ from that of Saifuddin et al. (2009) who demonstrated that the culture supernatant of *B. subtilis* produces AgNPs in the dark. Two other studies dealing with extracellular biosynthesis of AgNPs using culture supernatants of bacteria did not mention whether the synthesis was achieved in the light or dark (Shahverdi et al. 2007; Kalimuthu et al. 2008). It is still not known as to how light or darkness influences the formation of AgNPs. Shivaji et al. (2011) observed that AgNPs formed using cell-free supernatants of *A. kerguelensis* and *P. antarctica* were stable up to 8 months if stored in dark but not in the light. The cell free culture supernatants of *A. kerguelensis* and *P. antarctica* started synthesis of AgNPs after 2 h of incubation. This rate was faster than that reported in most of the earlier studies, where synthesis was slow as in *B. licheniformis* (Kalimuthu et al. 2008), *B. subtilis* (Saifuddin et al. 2009) and *F. oxysporum* (Durán et al. 2005) but was comparable to the synthesis in *Morganella* sp. (Parikh et al. 2008) where it took place in 1 h. Further, AgNPs from *A. kerguelensis* were more stable (no change up to 24 h) compared to the AgNPs from *P. antarctica*, as judged by the decrease in the characteristic peak at 410 nm and formation of a new peak at 524 nm by 6 h, probably due to the aggregation of AgNPs. The ability of the cell-free culture supernatant of *A. kerguelensis* to synthesize AgNPs did not depend on the temperature (8, 22 and 30 °C), pH (pH 5, 7 and 10) or the phase of growth (early log, mid log and late log phase of growth) of the culture or on the temperature at which the AgNPs were synthesized. In contrast, when the cell-free culture supernatant of *P. antarctica* was used, the stability of the AgNPs was compromised irrespective of the growth temperature of the bacterium (8, 22 and 30 °C), when the culture was grown at pH 7 and the AgNPs were synthesized either at 25 or 37 °C. These study indicates that the factors in the cell-free culture supernatants that facilitate AgNPs synthesis and stability vary from bacterial species to species (Shivaji et al. 2011). The AgNPs exhibited antibacterial activity and this confirms earlier studies on the bactericidal activity of silver nanoparticles (Sondi and Salopek-Sondi 2004; Li et al. 2005; Panacek et al. 2006).

3.5 Psychrophilic Enzymes in Nanotechnology

Pioneering studies of psychrophiles at the molecular level were mainly focused on cold-active enzymes because this aspect was regarded as a prerequisite to the environmental adaptation (Feller 2003, 2008; Feller and Gerday 2003). It has been shown that the high level of specific activity at low temperature of cold-adapted enzymes is a key adaptation to compensate for the exponential decrease in chemical reaction rates as the temperature is reduced. Such high biocatalytic activity arises from the disappearance of various noncovalent stabilizing interactions, resulting in an improved flexibility of the enzyme conformation. It should be noted that this adaptive feature is genetically encoded within the protein sequence and results from a long-term selection. As a general picture, psychrophilic enzymes are all faced to a main constraint, to be active at low temperatures, but the ways to reach this goal are quite diverse. The main functional and structural adaptive properties of cold-active enzymes as well as the recent advances related to their synthesis, folding and biotechnological applications are presented by Feller (2013), and Margesin and Feller (2010).

3.5.1 Pectate Lyase

Pectate lyase is one of the enzymes of the pectinase group of enzymes. The enzyme exhibits a β-elimination mechanism in the cleavage of α-1,4-glycosidic bonds of polygalacturonic acid producing unsaturated Δ 4,5 bond at the non reducing end of the polysaccharide and generates 4,5-unsaturated oligogalacturonates, which results in the formation of a double bond between C4 and C5 at the non-reducing end via E2 elimination mechanism and an elimination of CO_2 (Mukhopadhyay et al. 2012). The pectate lyase enzymes have enormous applications in the textile, food and beverages and paper industries, in wastewater treatment and bioremediation. These enzymes have the capacity to degrade pectin, which is a component of plant biomass, which pectin is often the raw material for industrial usage. Pectate lyase also has the ability to degrade or the waste material obtained after utilization of plant biomass. The basic principle of solid state functionalization at nanoscale had first been explored by Bhattacharyya et al. (2014). The theoretical insight of lipid functionalized SWNT self-assembly (nanorope design) which serve as a molecular machine has been reported previously by Bhattacharyya et al. (2013) for developing a reusable glucose sensor.

Mukhopadhyay et al. (2015a) investigated the use of nanoparticles to stabilize a psychrophilic pectate lyase (optimally active at 10 °C) at a temperature as low as 4 °C. Pectate lyase from a pyscrophilic bacterium was supplemented with calcium hydroxyapatite nanoparticles (NP-PL) as a substitute for Ca, (the cationic activator of this enzyme) and entrapped in single walled nanotube (SWNT) based molecular self-assembly. The retention of enzymatic activity (more than 70 %) after repeated freezing and thawing at 25 °C in presence of the specific nanoparticle offers a novel

use of such nano-enzyme systems. The activity and stability of PL was enhanced both at temperatures as low as 4 °C and as high as 80 °C in presence of NP and SWNT. The enzyme could be repeatedly released and re-trapped (in SWNT based molecular self-assembly) while retaining significant activity (Mukhopadhyay et al. 2015a). The immobilized PL (in SWNT based molecular self-assembly), retained its activity after repeated freezing and thawing. This unique capability of SWNT to activate and stabilize a cold active enzyme at temperatures much lower or higher than its optimal range may be utilized for processes that require bio-conversion at low temperatures while simultaneously allowing for shifts to higher temperature (Mukhopadhyay et al. 2015a).

3.5.2 *Laccase*

Laccases catalyze the removal of a hydrogen atom from the hydroxyl group of ortho and para-substituted monophenolic and polyphenolic substrates and from aromatic amines by one-electron abstraction, to form free radicals, capable of undergoing further depolymerization, repolymerization, demethylation or quinone formation (Gianfreda et al. 1999). Laccase enzymes have enormous applications in the textile, dye and paper industries, in wastewater treatment and bioremediation. These enzymes have the capacity to degrade lignin (in lignocellulose) thereby enhancing its potential as raw material for industrial usage. There has been a limited study of psychrophilic pectinolytic, cellulolytic and xylanase secreting bacteria. In fact, reports on psychrophilic laccase secreting bacteria in literature are lacking. A marine Antarctic, psychrotrophic bacterium (*Pseudoalteromonas haloplanktis*, strain ANT/505), isolated from sea ice covered surface water from the Southern Ocean, showed pectinolytic activity on citrus pectin agar. Isolated enzymes (pelA and pelB) from this strain represented the first pectate lyase isolated and characterized from a cold-adapted marine bacterium (Truong et al. 2001). Reports on pectinases from cold-adapted microorganisms have been restricted to psychrotrophic spoilage bacteria such as different strains of *Pseudomonas fluorescens* (Schlemmer and Ware 1987).

Mukhopadhyay et al. (2015b) demonstrated a simple nanotechnology based immobilization technique for imparting psychrostability and enhanced activity to a laccase from a psychrophilic bacterium obtained from Himalayan (Pindari glacier) soil. Laccase from the psychrophilic bacterium was supplemented with copper oxide nanoparticles (NP) corresponding to copper (NP-laccase), the cationic activator of this enzyme and entrapped in single walled nanotube (SWNT). The activity and stability of laccase was enhanced both at temperatures as low as 4 °C and as high as 80 °C in presence of NP and SWNT. The enzyme could be released and re-trapped (in SWNT) multiple times while retaining significant activity. Laccase, immobilized in SWNT, retained its activity after repeated freezing and thawing. This unique capability of SWNT to activate and stabilize cold active enzymes at temperatures much lower or higher than their optimal range may be utilized for

processes that require bio-conversion at low temperatures while allowing for shifts to higher temperature if so required (Mukhopadhyay et al. 2015b).

3.6 Future Perspectives

Major drawbacks associated with the biosynthesis of nanoparticles using thermophiles and psychrophiles include tedious purification steps and poor understanding of the mechanisms. For instance, the extraction and purification of the produced metal nanoparticles from the extremophilic microbes (intracellular or extracellular synthesis) for further applications are not well investigated. Many bacterial species have been reported to synthesize nanoparticles very quickly and efficiently but intracellularly. However, because of the current unavailability of an efficient method for intracellular NP recovery and associated agglomeration issues (Shakibaie et al. 2010), researchers cannot utilize the potential of these highly efficient microorganisms and are more focused on extracellular NP-synthesizing microorganisms. In order to release the intracellularly produced NPs, additional processing steps such as ultrasound treatment or reaction with suitable detergents are required (Sonkusre et al. 2014). This can be exploited in the recovery of precious metals from mine wastes and metal leachates (Iravani 2014). Biomatrixed metal nanoparticles could also be used as catalysts in various chemical reactions (Castro et al. 2014). This will help to retain the nanoparticles for continuous usage in bioreactors. Physicochemical methods including freeze-thawing, heating processes, and osmotic shock can be used to extract the produced NPs from the cells. But, it seems that these methods may interfere with the structure of NPs, and aggregation, precipitation, and sedimentation could happen. These may change the shape and size of NPs and interfere with the suitable properties of them. Moreover, enzymatic lysis of the microbial cells containing intracellular NPs can be used, but this method is expensive and it cannot be used in up-scalable and industrial production of NPs. It seems that surfactants and organic solvents can be used for both extraction and stabilization of NPs, but these chemical materials are toxic, expensive, and hazardous. It should be noted that in case of extracellular production of nanoparticle, centrifuge could be used for extraction and purification of NPs, but aggregation might happen (Iravani 2014; Sonkusre et al. 2014).

The elucidation of the biochemical pathways is necessary to develop a rational approach to nanoparticles biosynthesis. A number of issues need to be addressed from the nanotechnology and microbiological points of view before such biosynthetic procedures can compete with the conventional protocols. Frequently encountered in the biosynthesis of nanoparticles are the challenges to control the shape and size of the particles; to achieve the monodispersity in solution phase, and to scale up for production level processing. Furthermore, little is known about the mechanistic aspects, and information in this regard is necessary for economic and rational development of nanoparticle biosynthesis. These important technical challenges must be overcome before this bio-based method with extremophiles will be a successful and competitive alternative for industrial synthesis of nanoparticles.

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Chapter 4
Alkaliphiles and Acidophiles in Nanotechnology

Abstract Acidophiles and alkaliphiles have been exploited for the synthesis of nanoparticles. The nanomaterial synthesizing biocomponents of these microorganisms have an added advantage of providing excellent stability to the nanomaterial being synthesized. Some produce biomolecules such as proteins, peptides and a special class of metal-binding molecules referred to as phytochelatins that are used for the in vitro stabilization of synthesized nanomaterials. This chapter provides an overview of the many acidophilic and alkalophilic microorganisms capable of synthesizing nanoparticles.

4.1 Introduction

Organisms that thrive at the extremes of pH are classified as either acidophiles, which exhibit optimal growth below pH 3, or alkaliphiles, which grow optimally at pH greater than 9 (Rothschild and Mancinelli 2001; Wiegel 2011). Acidophiles and alkaliphiles have been discovered in habitats all over the world. Acidophiles thrive in sites of acid mine drainage, solfataric fields, acidothermal hot springs and fumaroles, coal spoils, and bioreactors. These environments feature low pH values, temperatures ranging from 25 °C to over 90 °C, pressures up to 5 MPa, low salinity, some heavy metals, and either anaerobic or aerobic conditions (Seckbach and Libby 1970; Hallberg and Lindström 1994; Golyshina et al. 2000; He et al. 2004; Ferris et al. 2005; Yoshida et al. 2006; Hallberg et al. 2010; Reeb and Bhattacharya 2010). Acidophiles use a variety of pH homeostatic mechanisms that involve restricting proton entry by the cytoplasmic membrane and purging of protons and their effects by the cytoplasm. To help maintain ΔpH, acidophiles have a highly impermeable cell membrane to restrict proton influx into the cytoplasm (Konings et al. 2002). Because the membrane proton permeability determines the rate at which protons leak inward, the balance between proton permeability, proton influx through energetic and transport systems, and the rate of outward proton pumping determines whether a cell can sustain an appropriate proton motive force (PMF). An example of a highly impermeable cell membrane is the archaeal-specific

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structures composed of tetraether lipids (as opposed to the ester linkages found in bacterial and eukaryal cell membranes), which have been identified in *Thermoplasma acidophilum* (Shimada et al. 2002), *Ferroplasma acidiphilum* YT and Y-2, *Ferroplasma acidarmanus* (Macalady and Banfield 2003), *Sulfolobus solfataricus* (van de Vossenberg et al. 1998a) and *Picrophilus oshimae* (van de Vossenberg et al. 1998b). Some acidophilic bacteria incorporate ω-alicyclic fatty acids into the membrane in order to increase acid resistance (Chang and Kang 2004), although these acid-resistant lipids can lose structural integrity at neutral pH (van de Vossenberg et al. 1998a). In addition, the genome of some bacterial species contains significant cell membrane biosynthetic enzyme diversity, which has been postulated to allow membrane adaptation to fluctuating acidic pH (Baker-Austin and Dopson 2007). The second strategy is to reduce H^+ influx through transmembrane channels. At low extracellular pH, *Acidithiobacillus ferrooxidans* upregulates the expression of Omp40, a channel with a smaller pore size. Another adaptive mechanism is the generation of a Donnan potential through the accumulation of monovalent cations in the cytoplasm. The high intracellular cation concentration generates a positive charge gradient $\Delta\psi$, thereby inhibiting H^+ in flux despite the favorable concentration gradient. K^+/H^+ antiporters with stoichiometries of >1:1 are often employed to promote the formation of this Donnan potential. These antiporters, as well as ATP-dependent H^+ pumps, which are found in acidophiles of all three domains, also serve to promote the efflux of H^+ and resist cytoplasmic acidification (Matin 1990; Baker-Austin and Dopson 2007; Das et al. 2009; Enami et al. 2010).

Acidophiles are most widely distributed in the bacterial and archaeal domains (Johnson and Hallberg 2003; Baker-Austin and Baker 2007) and contribute to numerous biogeochemical cycles including the iron and sulfur cycles (Druschel et al. 2004). In the production of acid mine drainage (AMD), the release of metal-rich, acidic effluents can cause considerable environmental damage such as the contamination of drinking water. One important effect of acidophiles lies in their biotechnological application for metal extraction from ores (Rohwerder et al. 2003), and this sustainable biotechnological process is becoming increasingly important (Chang and Kang 2004) because of its reduced and containable pollutant outputs (Golyshina and Timmis 2005). Acidophiles could also be a source of gene products; for example, acid-stable enzymes with applications as lubricants and catalysts (van den Burg 2003).

The term "alkaliphile" is used for microorganisms that grow optimally or very well at pH values above 9 but cannot grow or grow only slowly at the near-neutral pH value of 6.5. Alkaliphiles include prokaryotes, eukaryotes, and archaea. Many different taxa are represented among the alkaliphiles, and some of these have been proposed as new taxa. Alkaliphiles can be isolated from normal environments such as garden soil, although viable counts of alkaliphiles are higher in samples from alkaline environments. Alkaliphiles have also been found to proliferate in alkalithermal hot springs, shallow hydrothermal systems, sewage, and hypersaline soda lakes, such as Mono Lake, CA, USA, and Lake Elementaita in the Kenyan-Tanzanian Rift Valley. The conditions at these locations include a wide range of temperatures but usually

feature high pH and moderate to high concentrations of dissolved salts (Xu et al. 1999; Hoover et al. 2003; Ma et al. 2004; Kanekar et al. 2012). Alkaliphiles live in an environment with a low [H^+], so the challenge for these organisms is to continuously neutralize the cytoplasm and encourage H^+ influx to drive ATP synthesis. The first and most prominent adaptation is the use of Na^+/H^+ and K^+/H^+ antiporters to move H^+ into and monovalent cations out of the cell. The anaerobic alkaliphiles *Natranaerobius thermophilus* and *Desulfovibrio vulgaris* typically utilize NhaC (Na^+/H^+) antiporters, while the aerobic alkaliphiles *Bacillus pseudofirmus* and *Synechococcus elongatus* primarily express $Na^+(K^+)/H^+$ antiporters of the CPA-1 and CPA-2 families (Krulwich et al. 2009; Mesbah et al. 2009; Mesbah and Wiegel 2011). Alkaliphiles have made a great impact in industrial applications. Biological detergents contain alkaline enzymes, such as alkaline cellulases and/or alkaline proteases that have been produced from alkaliphiles. The current proportion of total world enzyme production destined for the laundry detergents market exceeds 30 %. Another important application is the industrial production of cyclodextrin with alkaline cyclomaltodextrin glucanotransferase. This enzyme reduced the production cost and paved the way for its use in large quantities in foodstuffs, chemicals and pharmaceuticals. Besides these applications, there are other possible applications in food and waste treatment industries.

Acidophiles and alkaliphiles have been exploited for the synthesis of nanoparticles. The nanomaterial synthesizing biocomponents of these microorganisms have an added advantage of providing excellent stability to the nanomaterial being synthesized. The stabilization is achieved through a single unit of biomolecule that caps the synthesized nanomaterial. Biomolecules such as proteins, peptides and a special class of metal binding molecules referred to as phytochelatins are used for the in vitro stabilization of synthesized nanomaterials. In case of bacterial and fungal nanoparticles synthesis, stabilization has been attributed to specific proteins either found intracellularly or released into the medium extracellularly (Mukherjee et al. 2001a; Ahmad et al. 2002). After the synthesis of nanomaterials, these stabilizing proteins are supposed to neutralize the charges over the metallic nanomaterial by providing appropriate seal form a stable structure the nanomaterials. The interaction of protein with different metals has been well documented in the literature and thorough study of critical stability constants of the reactants supports the finding that proteins are responsible for stabilization of nanomaterials. Metal-protein bonds provide the molecule more stability as compared to its free form (Martell and Smith 1974). On comparing the critical stability constants with reference to proteinaceous structure with free amino group, sulfhydryl and disulfide group, the entities with free amino groups provide more stability to the metal species on nanoscale. In case of yeast and algae, stabilization is brought about by a special class of peptides called phytochelatins (Dameron et al. 1989; Gekeler et al. 1998). Phytochelatins are functional analogues of metallothioneins consisting of three amino acids namely glycine, cysteine and glutamine. Stabilization is achieved through the binding of metal ions by thiolate coordination to form metal complexes (Cobbett 2000). This chapter provides an overview of the many acidophilic and alkalophilic microorganisms capable of synthesizing nanoparticles.

4.2 Synthesis of Nanoparticles by Acidophiles

4.2.1 Synthesis of Nanoparticles by Acidophilic Bacteria

4.2.1.1 Acidithiobacillus Ferrooxidans

Acidithiobacillus ferrooxidans is a chemolithotrophic acidophilic aerobe that takes in ferrous or reduced inorganic sulfur compounds as energy source. The study by Yan et al. (2012) suggested that *A. ferrooxidans* is able to synthesize intracellular electron dense magnetite (Table 4.1). In comparison with fastidious magnetotactic bacteria, *A. ferrooxidans* can grow without the drastic regimes of oxidative stress, and the mass cultivation of these bacteria has been easily available. Yan et al. (2010, 2012) investigated the biocompatibility of Fe_3O_4 nanoparticles formed by *A. ferrooxidans* as a bioactive substance delivery carrier. The Fe_3O_4 nanoparticles formed by *A. ferrooxidans* were isolated by a method combining ultracentrifugation and magnetic separation (Yan et al. 2012). MTT test, hemolysis assay and micronucleus test were carried out to evaluate in vitro cytotoxicity, blood toxicity and genotoxicity of magnetosomes, respectively. Under these conditions, the Fe_3O_4 nanoparticles showed no cytotoxic, genotoxic and hemolytic effects up to 4.0 mg/ml indicating good biocompatibility of these biological nanoparticles. The Fe_3O_4 nanoparticles exhibit special properties such as ferrimagnetism, nanoscale size, membrane bound structure, and good biocompatibility. Based on these special characterizations, magnetosomes are emerging as promising tools for various biotechnological and biomedical applications. They appear to be promising immobilization of bioactive substances including enzymes, antibodies and biotin (Yan et al. 2012).

4.2.1.2 Lactobacillus acidophilus

Lactobacillus acidophilus is a species of gram positive bacteria in the genus *Lactobacillus*. *L. acidophilus* is a homofermentative, microaerophilic species, fermenting sugars into lactic acid, and grows readily at rather low pH values (below pH 5.0) and has an optimum growth temperature of around 37 °C (Bâati et al. 2000). *L. acidophilus* is best known as a probiotic. The exact mechanism of the probiotic effect is still under investigation. Adherence to the epithelium of the GI tract is thought to play an important role in the probiotic effect of *L. acidophilus*. Studies show that the exact mechanism of adherence varies from strain to strain. Possible mechanisms include protein and carbohydrate mediated adherence. Although both types of mechanisms have been demonstrated in vitro neither has successfully been demonstrated in vivo. Because the GI tract is a constantly changing environment it is difficult to mimic the environment in vitro. Further studies with biopsies of intestinal tissues are necessary to confirm adherence and retention of *L. acidophilus* in the GI tract. However, studies that include such

Table 4.1 Acidophiles in biosynthesis of nanoparticles

Acidophile	Nanoparticle	References
Bacteria		
Acidithiobacillus ferrooxidans	Fe_3O_4	Yan et al. (2012)
Lactobacilus acidophilus	Ag	Rajesh et al. (2015)
Lactobacilus acidophilus 01	Ag	Namasivayam et al. (2010)
Lactobacilus acidophilus	Se	Rajasree and Gayathri (2015)
Lactobacillus acidophilus DSMZ 20079T	CdS	El-Raheem et al. (2012)
Pilimelia columellifera subsp. *pallida* SL19	Ag	Golinska et al. (2015)
Pilimelia columellifera subsp. *pallida* SL24	Ag	Golinska et al. (2015)
Thiobacillus thioparus	Fe_3O_4	Elcey et al. (2014)
Actinobacteria C9	Ag	Anasane et al. (2016)
Actinobacteria SF23	Ag	Anasane et al. (2016)
Streptacidiphilus sp. strain CGG11n	Ag	Railean-Plugaru et al. (2016)
Arthrobacter nitroguajacolicus	Au	Dehnad et al. (2015)
Archaea		
*Sulfolobus islandicus**	Au	Kalabegishvili et al. (2014)
*Sulfolobus islandicus**	Ag	Kalabegishvili et al. (2015)
*Sulfolobus acidocaldarius**	Ag	Bartolome et al. (2012), Selenska-Pobell et al. (2011)
Fungi		
Verticillium luteoalbum	Au	Mukherjee et al. (2001a)
Verticillium sp.	Ag	Sastry et al. (2003), Mukherjee et al. (2001b)
Verticillium sp.	Fe_3O_4	Bharde et al. (2006)
Bipolaris nodulosa	Ag	Saha et al. (2010)
Pichia jadinii (formerly *Candida utilis*)	Au	Gericke and Pinches (2006)
Fusarium oxysporum	Au	Mukherjee et al. (2002)
Fusarium oxysporum	Ag	Senapati et al. (2004), Dhandhukia et al. (2012), Birla et al. (2013), Korbekandi et al. (2013), Husseiny et al. (2015)
Fusarium oxysporum	Au-Ag alloy	Senapati et al. (2005)
Fusarium oxysporum	$SrCO_3$	Rautaray et al. (2004)

(continued)

Table 4.1 (continued)

Acidophile	Nanoparticle	References
Fusarium oxysporum	CdS	Ahmad et al. (2002)
Fusarium oxysporum	Fe_3O_4	Bharde et al. (2006)
Fusarium oxysporum	ZrO_2	Bansal et al. (2004)
Fusarium oxysporum	SiO_2	Bansal et al. (2005), Kannan et al. (2015)
Fusarium oxysporum	TiO_2	Bansal et al. (2005)
Fusarium oxysporum	$BaTiO_3$	Bansal et al. (2006)
Fusarium oxysporum	CdSe	Kumar et al. (2007)
Aspergillus niger	Ag	Kumar et al. (2008)
Aspergillus fumigatus	Ag	Bhainsa and D'Souza (2006), Navazi et al. (2010)
Aspergillus flavus	Ag	Moharrer et al. (2012), Vigneshwaran et al. (2006)
Aspergillus clavatus	Ag	Saravanan and Nanda (2010), Verma et al. (2010)
Aspergillus temeri	Ag	Kumar et al. (2012)
Aspergillus terreus	Au	Baskar et al. (2014)
Aspergillus terreus	Ag	Li et al. (2012)
Aspergillus niger	CuO	Etefagh et al. (2013)
Aspergillus niger	Zn	Noorbatcha and Salleh (2014)
Aspergillus oryzae	Ag	Phanjom and Ahmed (2015)
Aspergillus spp.	Zn	Pavani et al. (2011)
Aspergillus spp.	Pb	Pavani et al. (2012)
Aspergillus spp.	Cu	Pavani et al. (2013)
Aspergillus tubingensis	MgO	Raliya et al. (2014)
Penicillium sp.	Ag	Zhang et al. (2009), Hemath et al. (2010)
Penicillium fellutanum	Ag	Kathiresan et al. (2009)
Penicillium purpurogenum	Ag	Nayak et al. (2010)
Penicillium nalgiovense	Ag	Maliszewska et al. (2014)
Penicillium atramentosum KM	Ag	Sarsar et al. (2015)
Penicillium citrinum	Ag	Honary et al. (2013)
Penicillium chrysogenum	Au	Sheikhloo and Salouti (2011)
Penicillium purpurogenum NPMF	Ag	Nayak et al. (2011)
Penicillium nalgiovense AJ12	Ag	Maliszewska et al. (2014)
Penicillium aurantiogriseum	CuO	Honary et al. (2012)
Penicillium citrinum	CuO	Honary et al. (2013)
Penicillium waksmanii	CuO	Honary et al. (2012)

*Thermoacidophilic

biopsies are rare. In vitro, NCFM specifically shows a protein mediated response. The NCFM that demonstrated adherence did not appear to have a polysaccharide layer, which may be significant to its ability to adhere. Further study is needed to confirm mechanisms in vivo (Sanders and Klaenhammer 2001). When *L. acidophilus* is co-cultivated with other organisms, *L. acidophilus* has repeatedly been shown to inhibit the growth of competing microbes. It is thought that *L. acidophilus* produces a variety of antimicrobial compounds including organic acids, hydrogen peroxide, diacetyl and bacteriocins. The activity of these compounds is evident in the laboratory, but the in vivo role of these compounds is less clear. This is an area of active research. For instance, human fecal samples show a correlation between a reduction in pH and an increase in short chain fatty acids with higher fecal counts of Lactobacilli and bifidobacteria (which is another species that exhibits a probiotic effect). In the laboratory strain NCFM demonstrated antagonistic activity against common foodborne disease agents such as *Staphylcoccus aureus*, *Salmonella typhimurium*, and enteropathogenic *Escherichia coli* (Sanders and Klaenhammer 2001).

Synthesis of Ag Nanoparticles

L. acidophilus, which acts both as reducing and capping agent was observed to reduce silver ions into silver nanoparticles within 24 h of reaction time under room temperature (Rajesh et al. 2015). The presence of stable spherical-shaped silver nanoparticles in the size range of 4 to 50 nm were observed. The AgNPs synthesised are dispersed and stable due to the proteins secreted by organism, which act as a capping agent. These AgNPs showed effective antibacterial activity towards *Klebsiella pneumoniae*. The mechanism of the silver nanoparticle bactericidal activity is discussed in terms of its interaction with the cell membrane of bacteria by causing cytolysis and leakage of proteins and carbohydrates. In the cell, silver ions may deactivate cellular enzymes, DNA and proteins by reacting with electron-donating groups such as thiol (SH) groups and generate reactive oxygen species (Feng et al. 2000; Lok et al. 2006). Thus, it is reasonable to infer that there is a high chance for generating novel antimicrobials using *L. acidophilus* biogenic nanoparticles. Rajesh et al. (2015) recorded the protein and reducing sugar leakage at maximum of 60 mg/mL after 2 and 4 h of exposure This clearly explains the formation of pits in the cell membrane of *K. pneumoniae* resulting in the oozing out of the reducing sugars and proteins from the cytoplasm of bacterial cell (Rajesh et al. 2015).

The biological synthesis of AgNPs by *L. acidophilus* (strain 01) was also demonstrated by Namasivayam et al. (2010). The nanoparticle solution is extremely stable for more than six months with no signs of aggregation even at the end of this period. The SEM micrograph showed spherical nanoparticles with the size range of 45–60 nm. Silver nanoparticles synthesized by dried biomass of *L. acidophilu*s 01 strain was evaluated against toxicity of genomic DNA isolated from bacteria (*E. coli*), fungi (*Beauveria bassiana*), algae (*Scenedesmus acutus*) and human

blood. Silver nanoparticles did not show any distinct effect on the genomic DNAs of the tested organisms. The genomic DNAs of the tested samples did not show fragmentation or degradation, which clearly reveals the non-toxicity of AgNPs on genomic DNA and its best compatibility with genetic material of the organisms (Namasivayam et al. 2010).

Synthesis of Se Nanoparticles

Rajasree and Gayathri (2015) describe a biosynthesis of highly stable SeNPs using *L. acidophilus*. Selenium is a non-metallic element that exhibits many properties such as high thermal conductivity, superconductivity, and catalytic activities. It is also one of the key elements for maintaining the health of mammalian animals because of its anti-oxidative and pro-oxidative effects (Salata 2004). After the incubation of L acidophilus with sodium selenite, the culture media displayed a time-dependent color change, indicating the reduction of sodium selenite. Biosynthesized selenium were spherical in shape with size range of 20–250 nm. The nanoparticles were also analyzed for antimicrobial activity against pathogenic fungi (*Aspergillus niger* and *Candida albicans*) by well diffusion method. The zone of inhibition ranged from 4 to 10 mm. These results indicate that the SeNPs synthesized have strong antimicrobial activity (Rajasree and Gayathri 2015).

Synthesis of CdS Nanoparticles

Synthesis of CdS by *L. acidophilus* has been reported (El-Raheem et al. 2012). The synthesis was performed at room temperature and CdS nanoparticles were formed within 24 h. Ultraviolet (UV)–visible spectroscopy study revealed the build-up of absorption band at 362.5 nm for assisted synthesis of CdS nanoparticles. Individual nanoparticles as well as few aggregates having the size of 2.5–5.5 nm were found (El-Raheem et al. 2012).

4.2.1.3 Actinobacteria

Actinobacteria is a phylum of Gram-positive bacteria with high guanine and cytosine content in their DNA (Ventura et al. 2007). The G + C content of *Actinobacteria* can be as high as 70 %, though some may have a low G + C content (Ghai et al. 2012). They can be terrestrial or aquatic (Servin et al. 2008). Although understood primarily as soil bacteria, they might be more abundant in freshwaters (Ghai et al. 2011). *Actinobacteria* is one of the dominant bacterial phyla and contains one of the largest of bacterial genera, *Streptomyces* (Hogen 2010). Analysis of glutamine synthetase sequence has been suggested for phylogenetic analysis of Actinobacteria (Hayward et al. 2009). Actinobacteria have long been known to produce valuable natural products (streptomycin, the second antibiotic to

be developed comes from such a bacterium). Acidic forest soils are among of extreme environments that contain high numbers of acid-loving actinobacteria. Actinobacteria from extreme habitats are a rich source of new compounds for healthcare (Bull 2010). Little is known about the metabolic properties of acidophilic actinomycetes, though they are common in acidic habitats (Williams et al. 1971; Khan and Williams 1975; Goodfellow and Dawson 1978; Seong et al. 1993). Most isolates have been considered to be streptomycetes (Labeda et al. 2012) and a few shown to produce acid stable enzymes (Williams and Flowers 1978; Williams and Robinson 1981). Ayuso-Sacido and Genilloud (2005) studied the presence of NRPS (nonribosomal peptide synthetases) and PKS-I gene sequences, which are responsible for production of secondary metabolites from various actinobacteria. In *Pilimelia columellifera* subsp. pallida they found only the NRPS genes.

Pilimelia columellifera

Biological synthesis of AgNPs by acidophilic actinomycetes *Pilimelia columellifera* SL19 and *Pilimelia columellifera* SL24 strains isolated from pine forest soil (pH < 4.0) was reported by Golinska et al. (2015). The synthesis of AgNPs was observed by change in colour of cell filtrate from light-yellow to dark-brown upon treatment with $AgNO_3$ after 24 h of incubation, and by UV–visible spectroscopy where narrow peaks with a maximum absorbance at 425 and 430 nm were recorded, indicating that the nanoparticles were spherical and monodispersed. The particles were of nano-size, polydispersed and mainly spherical in shape, mostly occurring as individual particles but a few aggregates are also present. The AgNPs synthesized by *P. columellifera* SL19 and *P. columellifera* SL24 strains showed average sizes of 12.7 and 15.9 nm, respectively The AgNPs from *P. columellifera* SL19 and *P. columellifera* SL24 showed size distribution in the range of 4–36 nm. Antibacterial activity of biogenically synthesized AgNPs was evaluated against five clinical bacteria such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.* The AgNPs synthesized from *P. columellifera* SL19 strain showed the highest antibacterial activity against *S. aureus*, followed by *B. subtilis, P. aeruginosa* and *E. coli.* Similarly, AgNPs from *P. columellifera* SL24 demonstrated higher activity against *P. aeruginosa*, compared to *S. aureus.* Among tested pathogens, the lowest activity of AgNPs from SL24 was found against *K. pneumoniae* (Golinska et al. 2015). The activity of antibiotic was enhanced, when tested in combination with silver nanoparticles synthesized from both actinobacterial strains (Golinska et al. 2015).

Arthrobacter Nitroguajacolicus

Dehnad et al. (2015) described the extra- and intracellular synthesis of AuNPs by *Arthrobacter nitroguajacolicus* isolated from the soil of Andalian gold mine located in Northwestern Iran. The average size of nanoparticles is 40 nm and the size of

gold nanoparticles outside the cell is often larger than the nanoparticles stored inside the cell. This non-pathogenic bacterium is able to synthesize gold nanoparticles within 24 h (Dehnad et al. 2015).

Actinobacteria C9 and *Actinobacteria* SF23

The extracellular biosynthesis of AgNPs by acidophilic actinobacteria (SF23, C9) was shown by Anasane et al. (2016). The actinobacterial strain SF23 was isolated from partially fermented (F) layer of soil collected from inland dune of the Southern slope of the pine forest in Poland and C9 was recovered from mineral (C) layer of a spruce soil of Hamsterley Forest, County Durham, UK. 6 The pH of soils was found to be <4.0. The formation of silver nanoparticles (AgNPs) by actinobacterial strains (SF23 and C9) was observed by change in colour of cell filtrate from colourless to dark-brown after challenging with 1 mM $AgNO_3$ and 24 h of incubation. Later, the synthesised AgNPs were detected by UV–visible spectroscopy and sharp narrow peaks were observed at 432 nm for SF23 and 427 nm for C9 strains. The sharp peak predicted that nanoparticles were spherical and monodispersed. Nanoparticle tracking analysis (NTA) confirmed the average size of AgNPs synthesised from SF23 and C9 (both *P. columellifera* subsp. pallida) were of 30 nm and 28 nm, respectively. The concentration of synthesised AgNPs from both strains was found to be 6.08×10^6 and 6.16×10^8 particles per ml, respectively. The TEM analysis displayed that the AgNPs were nanosized, dispersed and spherical in shape, mostly as individual particles but at some places as aggregates. The AgNPs synthesised from SF23 revealed size in the range of 4–36 nm. The AgNPs synthesised by C9 showed the size in the range of 8–60 nm. The obtained results indicated formation of different-size nanoparticles. The biosynthesised AgNPs were screened against fungi-causing superficial mycoses such as *Malassezia furfur*, *Trichophyton rubrum*, *Candida albicans* and *C. tropicalis.* The highest antifungal activity of AgNPs from SF23 and C9 against *T. rubrum* and the least against *M. furfur* and *C. albicans* was observed as compared to other tested fungi. The biosynthesised AgNPs were found to be potential anti-antifungal agent against fungi-causing superficial mycoses.

Streptacidiphilus sp. strain CGG11n

Silver nanoparticles have successfully synthesized from *Streptacidiphilus* sp. strain CGG11n (Railean-Plugaru et al. 2016). Like many acidophilic members of the phyla *Actinobacteria*, this acidophilic isolate from the mineral horizon of the spruce forest soil at Hamsterley Forest has many advantages over other nanoparticle producing species. These include (1) high tolerance of acidophilic environments towards metals, (2) high intracellular metal uptake capabilities (Volesky and Holan 1995; Dias et al. 2002) and (3) extracellular secretion of different enzymes with optima below those of neutrotolerant actinobacteria (Williams and Flowers 1978; Williams and Robinson 1981), and it can be extended to the synthesis of

nanoparticles of different chemical composition, shapes and sizes. The formation of silver nanoparticles can be primarily distinguished through the visible observation in the change of solution color (Mohanta and Behera 2014). The formation of silver nanoparticles can be primarily distinguished through the visible observation in the change of solution color from yellow to brown. A typical silver nanoparticle absorption band in the visible region (λ = 250–700 nm) has been observed at λ = 420 ± 5 nm, which clearly indicates the formation of silver nanoparticles in the solution (Zaki et al. 2011). The observation of the peak in this region, provided by the surface plasmon resonance, is well documented for various metal nanoparticles with a size ranging from λ = 2 to 100 nm (Karthik et al. 2013). The EDX spectra showed bands characteristic for the groups present in peptides and proteins. Some reports suggest that proteins are able to form bonds with AgNPs through free amino groups (Gole et al. 2001; Rajasekharreddy et al. 2010). Proteins can bind to nanoparticles either through free amine groups or cysteine residues and through the electrostatic attraction of negatively charged carboxyl groups in enzymes, present in the cell wall of bacteria or fungi, which form a coat for covering the particles to prevent agglomeration and to lead toward the stabilization of the silver nanoparticles (Sastry et al. 2003; Vigneshwaran et al. 2007). Moreover, Railean-Plugaru et al. (2016) observed the stretching vibration of carbonyl amino groups recognized as amide I and amide II, appeared due to amide linkages of the protein, which are commonly responsible for the reduction process (Bhat et al. 2011). The presence of hydroxyl and carbonyl groups onto studied AgNPs indicates that the proteins secreted by actinobacteria to the medium could participate in reduction of Ag+. The synthesized silver bionanoparticles from *Streptacidiphilus* sp. strain CGG11n possess potent inhibitory effect that offers valuable contribution to pharmaceutical associations. The AgNPs synthesized by *Streptacidiphilus* CGG 11n strain, are rather small sized (average 16 nm). Thus, they have higher toxicity on bacterial pathogens, as these nanoparticles probably diffused more easily than the larger ones (Panacek et al. 2006; Mohan et al. 2007).

4.2.1.4 *Thiobacillus thioparus*

The ability of the iron-reducing bacterium, *Thiobacillus thioporus* to synthesize magnetite nanoparticles was shown by Elcey et al. (2014). The bacterial strain was isolated from an iron ore mining site and was characterized for its ability to impart magnetic properties under laboratory conditions. Growth of the organism and the magnetite production were optimized under different ranges of pH, temperature and substrate concentrations. *T. thioporus* was enriched in 9 K medium with graded doses of $FeSO_4$. Higher concentration of $FeSO_4$ (4 %) contributed an acidic environment to the medium, which favored the growth of the organism and in turn the accumulation of cell biomass. As the growth advanced in the medium the isolated cells capable of utilizing ferrous ions showed magnetotactic properties. The reduction occurred within 24 h. The endogenic magnetite particles were separated and collected in phosphate buffer after the lysis of the cells The clear suspension in

phosphate buffer exhibited a glowing property under magnetic field proved that the magnetite nanoparticles were monodispersed in the solution upon lysis (Elcey et al. 2014). The distinct glowing property was at par with chemically co-precipitated magnetite nanoparticles in suspension. Temperature is a key factor which decides the growth of organisms and its metabolism. The result showed that the organisms could survive under a range of temperatures 4–48 °C. But the optimum temperature was recorded as 28 °C. This temperature also enhances the production of magnetosomes (Elcey et al. 2014). The nanoparticles produced by *T. thioporus* were analyzed using SDS-PAGE to confirm the protein coating in comparison with the co-precipitated magnetite nanoparticles alone, as well as the particles coated with bacterial protein. The result proved that the purified particles synthesized by isolated bacterial strains have a protein coating as evidenced on the stained polyacrylamide gel. The glowing property of the solution under magnetic field and aggregation of the particles at the edge of the wells in the absence of protein coat showed the presence of monodisperse magnetite nanoparticles in the preparation (Elcey et al. 2014).

4.2.2 Synthesis of Nanoparticles by Acidophilic Archaea

Sulfolubus species belong to the order *Sulfolobales*, which grow optimally at temperatures above 80 °C and pH values below pH 3. (Huber and Prangishvili 2006). *Sulfolobales* are metabolically dependent on sulfur: heterotrophic or autotrophic, their energy comes from the oxidation of sulfur and/or cellular respiration in which sulfur acts as the final electron acceptor. For example, *Sulfolobus tokodaii* is known to oxidize hydrogen sulfide to sulfate intracellular (Brock et al. 1972). *Sulfolobus acidocaldarius* is used to leach copper and iron from ore. Metal extraction efficiency increased proportionally with increasing temperature up to 80 °C. Also viruses infecting members of the *Sulfolobales* have high technological potential (Basta and Prangishvili 2007; Steinmetz et al. 2008).

4.2.2.1 *Sulfolobus islandicus*

Synthesis of Au Nanoparticles

The synthesis of AuNPs by cells of the thermoacidophilic archaeon *Sulfolobus islandicus* for technological purposes has been studied by (Kalabegishvilli et al. 2014). Whole cells of *S. islandicus* were incubated in chloroauric acid ($HAuCl_4$) 1–3 mM aqueous solution at pH $\sim$ 2 and temperature 75 °C with shaking. The samples of cell suspensions with gold nanoparticles were taken for UV–Vis spectrometry. In the biomass of *S. islandicus*, the extracellular as well as intracellular formation of gold nanoparticles of spherical shape with sizes in the range of 20–80 nm (50 nm average) takes place. The growing formation of gold

nanoparticles in the biomass of *S. islandicus* takes place mainly at few hours. The sonication of biomass *S. islandicus* can be used for intensification AuNPs production processes. The gold total concentrations in the biomass *S. islandicus* show that in the first days the metal ions are mainly adsorbed onto the surface of microorganisms and then they slowly transported into the cells (Kalabegishvilli et al. 2014).

Synthesis of Ag Nanoparticles

Silver nanoparticle production of *S. islandicus* interacting with $AgNO_3$ aqueous solution has been shown for the first time by Kalabegishvilli et al. (2015). The AgNPs are formed within several hours. The AgNPs produced by *S. islandicus* are crystalline in nature and are mainly intracellular. The particle size ranged from 10 to 10 nm, and increase to 10–50 nm over several days of reaction. Sonication of the biomass has no effect in intensifying the process of nanoparticle synthesis *S. islandicus* (Kalabegishvilli et al. 2015).

4.2.2.2 *Sulfolobus acidocaldarius*

Selenska-Pobell et al. (2011) have shown that AuNPs could be successfully grown onto the naturally thiol-containing proteinaceous surface layer (S layer) of *Sulfolobus acidocaldarius* without any chemical functionalization by thiol groups. In their present work, the S-layer protein, called SlaA (Veith et al. 2009), of the acidothermophilic crenarchaeon *Sulfolobus acidocaldarius* was used as a matrix for the synthesis and stabilization of Au nanoparticles. Their rationale for choosing this archaeal S-layer was, on one hand, the indigenous presence of two sulfur-containing cysteine residues per protein monomer (König et al. 2007). These residues provide thiol groups which can stimulate the binding of Au(III) and the efficacy of the nanoclusters formation. On the other hand, as mentioned above, the presence of thiol groups in the Au nanoclusters may influence their magnetic properties. The archaeal SlaA-layer possesses p3-symmetry and exhibits extreme stability to high acidity and temperatures as well as to proteases and detergents (Mark et al. 2006; Engelhardt 2007; König et al. 2007). The latter property allows the isolation of empty SlaA-layer ghosts, with the shape of the cells, by using standard purification procedures (Mark et al. 2006; König et al. 2007). These ghosts can be mechanically disrupted via sonication in monolayer SlaA-layer sheets, which are comparable to those obtained usually from bacteria (Mark et al. 2006). It was demonstrated that metals bind to the inner, facing the cells, side of the S-layers (Creamer et al. 2007; Selenska-Pobell and Merroun 2010), which is negatively charged (Pum et al. 1989). In the case of *S. acidocaldarius*, this means that Au(III) should be deposited and reduced inside the thiol containing SlaA-ghosts. This process seems advantageous for Au(0) nanoclusters formation in comparison to the previously described procedure when monolayers of thiol-free bacterial S-layer sheets were used.

4.2.3 *Synthesis of Nanoparticles by Acidophilic Fungi*

In recent years, fungi have been explored for nanoparticles synthesis. Fungi are more advantageous compared to other microorganisms in many ways. Fungal mycelial mesh can withstand flow pressure and agitation and other conditions in bioreactors or other chambers compared to plant materials and bacteria. These are fastidious to grow and easy to handle and easy for fabrication. The extracellular secretions of reductive proteins are more and can be easily handled in downstream processing. And also, since the nanoparticles precipitated outside the cell is devoid of unnecessary cellular components, it can be directly used in various applications. Fungi can accumulate metals by physicochemical and biological mechanisms including extracellular binding by metabolites and polymers, binding to specific polypeptides, and metabolism-dependent accumulation. In some cases, as it have been described for extracellular reduction of metallic ions by bacteria, an enzymatic process seems to be responsible for nanoparticle production. Protein assays indicate that an NADH-dependent reductase, is also the main responsible factor of biosynthesis processes in fungi. This reductase gains electrons from NADH and oxidizes it to NAD^+. The enzyme is then oxidized by the simultaneous reduction of metal ions.

4.2.3.1 *Verticillium* spp.

Verticillium is a genus of fungi in the division *Ascomycota*, and are an anamorphic form of the family Plectosphaerellaceae. The genus used to include diverse groups comprising saprobes and parasites of higher plants, insects, nematodes, mollusc eggs, and other fungi, thus the genus used to have a wide-ranging group of taxa characterised by simple but ill-defined characters. The genus, currently thought to contain 51 species (Kirk et al. 2008) may be broadly divided into three ecologically based groups-mycopathogens, entomopathogens (Zare and Gams 2001) and plant pathogens and related saprotrophs (Barbara and Clewes 2003). However, the genus has undergone recent revision into which most entomopathogenic and mycopathogenic isolates fall into a new group called *Lecanicillium*. The genus now includes the plant-pathogenic species *V. dahliae, V. longisporum, V. albo-atrum, V. nubilum*, and *V. tricorpus*.

Synthesis of Au Nanoparticles

Mukherjee et al. (2001a) demonstrated that exposure of the acidophilic fungus, *Verticillium* sp. (AAT-TS-4), to aqueous $AuCl_4^-$ ions results in reduction of the metal ions and formation of gold nanoparticles of around 20 nm diameter. The gold nanoparticles are formed on both the surface and within the fungal cells (on the cytoplasmic membrane) with negligible reduction of the metal ions in solution.

The size of the gold nanoparticles was thus determined to be about 25 nm. The number of AuNPs is higher on the cytoplasmic membrane than on the cell wall. A very small percentage of larger AgNPs is observed within the cytoplasm. Selected area diffraction analysis of a single gold particle revealed diffuse rings with lattice spacings in excellent agreement with those expected for gold. The large number of the gold nanoparticles on the membrane surface is striking because very often in bacteria, not more than 10 ± 15 nanoparticles are observed in one cell (Klaus et al. 1999). Of note is that the size distribution of gold nanoparticles produced using *Verticillium* sp. is much narrower than that observed for silver particles produced in bacteria (Klaus et al. 1999). The AuNPs on the cytoplasmic membrane are mostly spherical but that there are a few triangular and hexagonal particles. A large, quasi-hexagonal gold particle is observed within the cytoplasm (Mukherjee et al. 2001a).

Synthesis of Ag Nanoparticles

The acidophilic fungus *Verticillium* sp. when challenged with $AgNO_3$ leads to their reduction and accumulation as silver nanoparticles within the fungal biomass (Sastry et al. 2003). The appearance of a dark brown colour in the fungal biomass after reaction with Ag^+ ions is a clear indicator of the reduction of the metal ions and formation of silver nanoparticles in the fungal biomass. The growth of silver nanoparticles occurred only within the fungal biomass and not extracellularly, and is an interesting feature of this particular fungus. While there is no evidence of absorption in the spectral window 400–800 nm in the case of the as-harvested fungal cells, the fungal cells exposed to Ag^+ ions show a distinct and fairly broad absorption band centred at ca. 450 nm. The presence of the broad resonance indicates an aggregated structure of the silver particles in the film. A possible mechanism for the presence of silver nanoparticles in the fungal biomass could be the extracellular reduction of the Ag^+ ions in solution followed by precipitation onto the cells. The exact mechanism leading to the intracellular formation of silver nanoparticles is not fully understood. Sastry et al. (2003) speculate that since the nanoparticles are formed on the surface of the mycelia and not in solution, the first step involves trapping of the Ag^+ ions on the surface of the fungal cells possibly via electrostatic interaction between the Ag^+ and negatively charged carboxylate groups in enzymes present in the cell wall of the mycelia. Thereafter, the silver ions are reduced by enzymes present in the cell wall leading to the formation of silver nuclei, which subsequently grow by further reduction of Ag^+ ions and accumulation on these nuclei. The TEM results indicate the presence of some silver nanoparticles on the cytoplasmic membrane as well as within the cytoplasm (Sastry et al. 2003). It is possible that some Ag^+ ions diffuse through the cell wall and are reduced by enzymes present on the cytoplasmic membrane and within the cytoplasm. It may also be possible that some of the smaller silver nanoparticles diffuse across the cell wall to be trapped within the cytoplasm (Sastry et al. 2003).

4.2.3.2 *Bipolaris nodulosa*

The potential of a *Bipolaris nodulosa* to produce anisotropic silver nanoparticles using its mycelia free media (MFM) (Saha et al. 2010). After addition of aqueous $AgNO_3$ (1 mM), the mycelia free media showed a gradual change in colour at room temperature with time from yellowish to light pink, reddish brown and finally to dark brown within 24 h. The reduction of silver was subjected to spectral analysis by using the UV–Vis spectrophotometer. This showed an absorbance peak around 420 nm which was specific for silver nanoparticles. Laser diffraction studies revealed that particles were monodisperse in nature and the size range from 10 to 60 nm. Antimicrobial tests were performed against *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus vulgaris, Escherichia coli* and *Micrococcus luteus.* Silver nanoparticles at a concentration of 100 μg/ml showed a range of specificity towards its antimicrobial activity (Saha et al. 2010).

4.2.3.3 *Pichia Jadinii* (Formerly *Candida utilis*)

The possibility to manipulate the size and shape of gold nanoparticles by altering key growth parameters was investigated by Gericke and Pinches (2006). One of the most promising results was obtained with the yeast, *Pichia jadinii* (formerly *Candida utilis*). The biomass turned dark purple within a few hours after exposure to $HAuCl_4$, while the solution remained colourless, an indication of intracellular nanoparticle synthesis. Various particle morphologies, which included spherical, triangular, hexagonal and other shapes were present in all three cultures (Gericke and Pinches 2006). Large variations in particle size were observed and these varied from a few to approximately 100 nm in diameter. The images suggested that the spherical particles tended to be smaller than the hexagonal and triangular shaped particles. The pH was found to be an important parameter affecting gold nanoparticle synthesis. Particles formed at pH 3 were predominantly spherical in shape, relatively uniform in size, with the majority of the particles having less than 10 nm in diameter. Nanoparticles synthesised at pH 5 included small spherical particles, similar to those dominating at pH 3. In addition a large number of bigger particles with well-defined shapes, including triangles, hexagons, spheres and rods also occurred at this pH. The shapes of the particles formed at pH 7 were similar to those formed at pH 9 and included small spherical particles as well as bigger particles with irregular, undefined shapes (Gericke and Pinches 2006). These results are supported by previous studies suggesting that optimum gold accumulation by microbial cells normally occurs in the pH range of 2–6 (Nakajima 2003) and test work performed with *Lactobacillus* showed that changes in the pH could have an effect on the size distribution of gold nanoparticles (Nair and Pradeep 2002). The variety in the shapes of particles formed at the different pH levels indicates that changes in this parameter would play an important role during optimisation of a process controlling particle morphology. The rate of formation of the nanoparticles is related to the incubation temperature and increased temperature levels allowed

particle growth at a faster rate (Gericke and Pinches 2006). At the lower temperatures, the majority of nanoparticles formed after 1 h exposure to the gold solution were spherical with an average diameter of less than 10 nm. Further incubation for 24 h led to the number of smaller particles decreasing, whereas the number of larger particles, exhibiting well-defined shapes, increased. At 50 °C, no difference could be detected in the size and morphology of particles produced after 1 and 24 h exposure to gold and very few small spherical particles were present. The size of the nanoparticles can be controlled by operating at low temperatures, which would allow particle formation at a slower rate (Gericke and Pinches 2006).

4.2.3.4 *Fusarium* spp.

Among fungi, *Fusarium oxysporum* is the only fungus, which has been completely explored and exploited to the maximum for the production of various nanoparticles. It extracellularly synthesized various nanoparticles like gold, silver, bimetallic Au–Ag alloy, silica, titania, zirconia, quantum dots, magnetite, strontianite, Bi_2O_3 and barium titanate. In 2002, Mukherjee et al. reported the synthesis of spherical and triangular gold nanoparticles in the size range of 20–40 nm. FTIR spectrum showed the presence of amide (I) and (II) bands from carbonyl and amine stretch vibrations in proteins respectively. Electrophoresis revealed the protein of molecular mass between 66 kDa and 10 kDa involved in nanoparticles stabilization. Zirconia nanoparticles were produced by cationic proteins secreted by F. oxysporum when incubated with ZrF_6^{-2} anions. The protein of molecular weight 24–28 kDa was found to be responsible for the formation of zirconia nanoparticles (Bansal et al. 2004). The particles were overall quasi-spherical in shape with size range between 3 and 11 nm. Senapati et al. (2004, 2005) showed the formation of extracellular silver nanoparticles and bimetallic gold–silver (Au–Ag) alloy nanoparticles by *F. oxysporum*. Because of their unique electronic and structural properties, Au–Ag alloy nanoparticles can be used in biomedical applications. Strontianite ($SrCO_3$) crystals of needle-like morphology with higher order quasi-linear superstructures with aqueous Sr^{+3} ions were reported (Rautaray et al. 2004). Since the source of carbonate ions that reacted with strontium ions was the fungus itself. This procedure is called total biological synthesis. Similarly, it also produced silica and titania nanoparticles with SiF_6^{-2} and TiF_6^{-2} anionic complexes, which resulted in the synthesis of crystalline titania in room temperature and calcinations at 300 °C for crystallization of silica (Bansal et al. 2005). In addition, it also produced ternary oxide, barium titanate nanoparticles ($BaTiO_3$) of irregular quasi-spherical morphology with an average size of 4 ± 1 nm at room temperature. SAED confirmed the crystalline nature of tetragonal phase of nanoparticles, which exhibited a well-defined ferroelectric–paraelectric transition at room temperature which can be useful in microelectronics (Bansal et al. 2006). With the mixture of salts $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ after 24 h, it produced crystalline magnetite nanoparticles with single-domain characteristics. These particles were quasi-spherical in shape with 20–50 nm in size (Bharde et al. 2006). Similarly, upon incubation with $CdCl_2$ and $SeCl_4$, it produced

highly luminescent water soluble quantum dots (CdSe) with SPR band at 370 nm at room temperature. These particles were stable with ~6–7 ns fluorescence half life and showed polydispersed spherical morphology with 9–15 nm size. Although *Fusarium* species can produce various nanoparticles widely, it is not applicable to all *Fusarium* sp. *F. moniliforme*, which produces reducing components, could not be able to form silver nanoparticles upon incubation with silver ions (Durán et al. 2007). Furthermore, the deposition of bismuth was reported on the periphery of *Yersinia enterocolitica* 8081c cultures in log phase growth with bismuth subsalicylate (BSS) (Nadeau et al. 1992). Very recently, it was also shown that *F. oxysporum* produced optoelectronic material Bi_2O_3 nanocrystals in the size between 5 and 8 nm extracellularly with quasi-spherical morphology and good tunable properties. When bismuth nitrate was added as precursor, the as-synthesized nanocrystals were in monoclinic and tetragonal phases (Uddin et al. 2008).

Kumar et al. (2007) have demonstrated enzymatic synthesis of silver nanoparticles using -NADPH-dependent nitrate reductase purified from *F. oxysporum*. Protein assays indicate that an NADH-dependent reductase, is the main responsible factor of biosynthesis processes. The enzymatic route of in vitro synthesis of silver nanoparticles by NADPH-dependent nitrate reductase from *F. oxysporum* with capping peptide, phytochelatin was demonstrated recently and the mechanistic aspect was explained (Durán et al. 2005). Apart from enzymes, quinine derivatives, such as naphthoquinones and anthraquinones, also act as redox centers in the reduction of silver nanoparticles. A similar finding was also reported in the reduction of gold(III) chloride to metallic gold by NADPH-dependent sulfite reductase and phytochelatin.

4.2.3.5 *Aspergillus* spp.

Biosynthesis of Ag NPs using Aspergillus niger isolated from soil was reported by Kumar et al. (2008). Cell filtrate of *A. niger* was treated with 1 mmol/L silver nitrate and placed on a rotary shaker at 120 rpm and 25 °C in the ark. When treated with silver nitrate solution *Aspergillus flavus* accumulated Ag NPs on the surface of its cell wall after 72 h. The average size of the NPs was calculated as 8.92 nm. These Ag NPs are found to have a characteristic absorption peak at 420 nm and emission peak at 553 nm (Vigneshwaran et al. 2006). Extracellular biosynthesis of Ag NPs using *Aspergillus fumigatus* was investigated (Navazi et al. 2010). Silver nanoparticles can be mycosynthesized extracellularly using Aspergillus clavatus. (Saravanan and Nanda 2010; Verma et al. 2010). Silver nanoparticles were synthesized using a reduction of aqueous Ag ion with the culture supernatants of *Aspergillus terreus* (Li et al. 2012). Mycosynthesized AgNPs were polydispersed spherical particles ranging in size between 1 and 20 nm and could efficiently inhibit a variety of plant pathogenic fungi and bacteria. Antibacterial action of Ag NPs against *Escherichia coli, Candida albicans* and *Pseudomonas fluorescence* was revealed using a disc-diffusion technique. Similarly, the NPs showed antimicrobial activity against fungal and bacterial strains (Jaidev et al. 2010). An environmental

friendly process for the synthesis of Ag NPs using a fungus *Aspergillus tamarii* has been investigated (Kumar et al. 2012). The scanning electron microscope (SEM) result showed the distribution of spherical Ag NPs ranging from 25 to 50 nm. Raliya and Tarafdar (2014) reported the synthesis of zinc, magnesium and titanium NPs by using six *Aspergillius* species belonging to *A. flavus, A. terreus, A. tubingensis, A. niger, A. fumigatus* and *A. oryzae* by employing various precursor salts of sulphates, nitrates, chlorides and oxides. The authors also optimized the factors responsible for more production of monodispersed Zn, Mg and Ti NPs.

4.2.3.6 *Penicillium* spp.

Nanoparticles produced by *Penicillium* possessed a negative zeta potential and were fairly stable at a pH value above 8 due to electrostatic repulsion (Zhang et al. 2009). *Penicillium* sp. could effectively myco-reduce and nucleate $AuCl_4^-$ ions, and intracellular biosynthesis of size-controlled gold NPs after exposure to $HAuCl_4$ solution. In vitro biosynthesis of Ag NPs was achieved by *Penicillium fellutanum* using $AgNO_3$ as a substrate isolated from coastal mangrove sediment (Kathiresan et al. 2009). An eco-friendly process for the synthesis of nanomaterials using *Penicillium brevicompactum* WA 2315 and *Penicillium purpurogenum* NPMF has been attempted, respectively (Nayak et al. 2010). The green synthesis of Ag NPs by the cell-free filtrate of *Penicillium nalgiovense* AJ15 was reported by Maliszewska et al. (2014). The authors claimed that Ag NPs synthesis by the *P. nalgiovense* AJ15 cell free filtrate is a non-enzymatic process and the proteins containing cysteine play a significant role in the reducing of silver ions. In another example, Singh et al. (2014b) reported the synthesis of Ag NPs by an endophytic *Penicillium* sp. isolated from healthy leaves of *Curcuma longa* (turmeric). Honorary et al. (2012) proposed a green process for the extracellular production of copper oxide nanoparticles from *Penicillium aurantiogriseum*, *Penicillium citrinum* and *Penicillium waksmanii*. The results show the presence of secreted proteins from the fungi through the culture, which are capable of hydrolyzing metal precursors to form metal oxides extracellularly.

4.3 Synthesis of Nanoparticles by Alkaliphiles

4.3.1 Spirulina platinensis

Spirulina platensis is a free floating filamentous cyanobacterium characterised by cylindrical, multicellular trichomes in an open, left-hand helix. It occurs naturally in tropical and subtropical lakes with high pH and high concentrations of carbonate and bicarbonate. *S. platensis* is one of the most widely used microorganisms in the biotechnology of nutrition, pharmaceutics, and medicine. It is the world's richest

natural source of vegetable proteins, amino acids, vitamins, essential fatty acids, beta carotene, iron, and other biologically active beneficial substances (Dillon et al. 1995). It is also a source of potent antioxidants including spirulans, selenocompounds, phenolic compounds, and phycobiliproteins (Dillon et al. 1995). *S. platensis* is often used as a matrix for pharmaceuticals as well as a biologically active food additive for humans and animals (Doshi et al. 2007; Kim et al. 2007). It accumulates essential elements (Se, I, Cr and others) and produces complexes easily assimilated by the human organism in sufficient quantity. It may be hypothesized that the biomass of *S. platensis* with gold and silver nanoparticles has great potential for medical applications. The synthesis of nanoparticles by *S. platensis* has been studied elsewhere (Govindaraju et al. 2008; Sadowski 2010).

S. platensis effectively produced gold and silver nanoparticles by interacting with aqueous solutions of chloroauric acid ($HAuCl_4$) and silver nitrate ($AgNO_3$), respectively (Kalabegishvili et al. 2013a) (Table 4.2). The gold and silver nanoparticles formed by algal biomass are crystalline in nature and are produced mostly extracellularly. In general, they proved to have spherical shapes and sizes in the range of 5–40 nm. Total concentrations of gold and silver determined in the biomass showed that on the first day the metal ions were rapidly adsorbed mostly into the cell surface and then slowly transported into bacterial cells (Kalabegishvili et al. 2013a). The experiments carried out using a method of equilibrium dialysis confirmed the importance of surface processes in the synthesis of metal

Table 4.2 Alkaliphiles in biosynthesis of nanoparticles

Alkaliphiles	Nanoparticle	References
Bacteria		
Spirulina platinensis	Ag, Au	Kalabegishvili et al. (2013a)
Spirulina platinensis	Ag	Sharma et al. (2015), Ahmed et al. (2015), Tsibakhashvili et al. (2010)
Bacillus sp.	Ag	Tayde (2012)
Nostoc sp.	Ag	Ahmed et al. (2015)
Thermomonospora sp.*	Au	Ahmad et al. (2003)
Thermomonospora sp. 67 Th*	Au	Kalabegishvili et al. (2013b)
Streptomyces sp. *211A*	Ag	Tsibakhashvili et al. (2010)
Pseudomonas alcaliphila	Se	Zhang et al. (2011)
Bacillus licheniformis	Au	Singh et al. (2014a)
Bacillus licheniformis	CdS	Shivashankarappa and Sanjay (2015)
Bacillus licheniformis JS2	Se	Dhanjal and Cameotra (2011)
Bacillus licheniformis	Ag	Kalimuthu et al. (2008)

*Thermoalkaliphilic

nanoparticles. The use of ultrasound for the sonicating *Spirulina* biomass increased the nanoparticles production yield. The concentrations of some toxic elements in the *Spirulina* platensis biomass did not exceed permissible levels, and the obtained nanomaterials turned out to have great potential, especially for medicine and pharmacology (Kalabegishvili et al. 2013a).

Sharma et al. (2015) explored the biological synthesis of AgNPs using the cell-free extract of *Spirulina platensis*. The extracts when interacted with the silver nitrate salt solution form a dark brown solution due to the reduction of the silver ion to AgNPs. The particles (30–50 nm) are spherical in shape and do not create big agglomerates, which indicated the monodispersed nature of NPs stabilised by a capping agent. The study revealed that AgNPs (50 μg/disk) had shown maximum inhibitory effect against *Proteus vulgaris* and *Staphylococcus aureus*, followed by *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus*, and *S. epidermidis* (Sharma et al. 2015).

4.3.2 *Pseudomonas alcaliphila*

Zhang et al. (2011) report a facile economical and green way to synthesize Se nanoparticles (SeNPs) by *Pseudomonas alcaliphila*, which exhibits a high resistance to Se^{2-}. Prior to the synthesis of Se nanomaterials, 1 ml activated P. alcaliphila with or without 5.0 g PVP (50 g/l) was aerobically cultivated under the same method as in the activation test. After 24 h of bacterial growth, 2.63 g sodium selenite pentahydrate (0.1 M) was added into the medium, and then the reaction started. The dimension and morphology of SeNPs collected at different stages of incubation were examined by FESEM. The SeNPs were generated by *P. alcaliphila* and enlarged progressively with increasing reaction time. A few spherical SeNPs appeared among the bacteria at 6 h after adding selenite pentahydrate, while SeNPs with the diameters ranging from 50 to 200 nm were observed after 12 h. Furthermore, with the reaction continuing, a plenty of SeNPs with a diameter of ca. 500 nm were formed at 24 h in the reaction solution. This transformation process of the larger SeNPs grew by consuming small SeNPs was in consistent with the typical Ostwald ripening process (Wang et al. 2010). The EDS spectra derived from a Se nanoparticle indicated that the SeNP was composed entirely of selenium. In the size-controlled experiment, poly(vinyl pyrrolidone) (PVP) was added to the medium to control the size of the SeNPs. SeNPs with distinct and highly regular morphologies and smaller diameters are produced compared to those without PVP. The diameters of the nanospheres also changed throughout the reaction: the diameters of nanospheres ranged from 20 ± 5 nm at the early stages of the reaction to 200 ± 7 nm in the final stage (Zhang et al. 2011). The incubation solution was stable without flocculation in the presence of PVP at the later stages of the reaction. The SeNPs capped with PVP are stable. In the experiment without PVP, SeNPs aged at room temperature for 10–20 days aggregated together to form sphere clusters. Anisotropic growth is induced and it

eventually transformed into the flowerlike structure of t-Se. However, in the presence of PVP, SeNPs aged for 1 month were still stable and uniform (Zhang et al. 2011).

4.3.3 *Bacillus licheniformis*

Bacillus licheniformis is a bacterium that is commonly found in soil and bird feathers (Tiquia et al. 2005). Birds that tend to stay on the ground more than the air (i.e. sparrows) and on the water (i.e. ducks) are common carriers of this bacterium; it is mostly found around the bird's chest area and back plumage (Tiquia et al. 2005). *B. licheniformis* has also been found in manure composts (Tiquia et al. 2007; Pomaranski and Tiquia-Arashiro 2016) *B. licheniformis* is an important commercial bacterium because it is used to produce enzymes, mainly alpha-amylases and proteases. The enzymes are manufactured in large quantities through fermentation. They are then used in many different ways. They are added to cleaning detergents to improve their effectiveness. They help break down organic stains that are otherwise hard to remove. *B. licheniformis* is also used to produce the polypeptide antibiotic bacitracin. Bacitracin is mainly active against Gram-positive bacteria. *B. licheniformis* can be used in the synthesis of nanoparticles (Table 4.2).

4.3.3.1 Synthesis of CdS Nanoparticle

The bacterial strain *Bacillus licheniformis* has shown to be efficient in synthesizing cadmium sulfide nanoparticles (Shivashankarappa and Sanjay 2015). The reaction between cadmium chloride and sodium sulfide was reduced to cadmium sulfide nanoparticles under the influence of enzyme sulfate reductase (Tiquia et al. 2006; Tiquia 2008). The formation of coalescent orange-yellow clusters at the bottom of the tube indicated the formation of nanoparticles The precipitation was highest in the ratio of 1:1 and was found to be least in the ratio of 4:1 of cadmium chloride and sodium sulfide. The formation of CdS precipitate is said to be inversely proportional to the amount nanocrystal formation and the maximum synthesis of nanoparticles been reported to form at stationary phase of cell cycle (Bai et al. 2009; Mousavi et al. 2012). The results reported by Sweeney et al. (2004) also showed that the cells obtained at stationary phase showed little precipitation when compared with cells of late logarithmic phase which had bulk CdS precipitation. Shivashankarappa and Sanjay' results correlate with the above findings showing highest nanoparticles formation and least precipitation at the ratio of 4:1 (Shivashankarappa and Sanjay 2015). The results showed that the CdS nanoparticles were crystalline in nature with size varying from 20 to 40 nm. The stability of nanoparticles was due to protein interaction which may have played an important role as capping agents. The resultant CdS nanoparticles was tested for antimicrobial activity against a range of food borne bacteria *E. coli, B. licheniformis,*

Pseudomonas aeruginosa, Bacillus cereus and *Staphylococcus aureus* and fungi *Fusarium oxysporum, Aspergillus flavus* and *Penicillium expansum*. The antimicrobial activity showed that the CdS nanoparticles of ratio 4:1 of cadmium chloride and sodium sulfide at a concentration of 40 mg/ml showed highest zone of inhibition in *Pseudomonas aeruginosa* and *Aspergillus flavus* (Shivashankarappa and Sanjay 2015).

4.3.3.2 Synthesis of Gold Nanoparticles

Singh et al. (2014a) illustrated a simple green synthesis of AuNPs in vitro using cell lysate supernatant (CLS) of *Bacillus licheniformis*. The process of biosynthesis was extracellular and the gold ions were reduced to stable spherical-shaped AuNPs of average size of 38 nm. The bioprocess was simple and less time consuming as compared to other methods as the need for harvesting AuNPs from within the microbial cells via downstream process is eliminated. Nanoparticles exhibited good quality even in the absence of stabilizing agents. The synthesized AuNPs showed good antimicrobial activity against several Gram-positive and Gram-negative pathogenic bacteria. The extracellular biosynthesis from CLS may serve as suitable alternative to large scale synthesis of nanoparticles in vitro (Singh et al. 2014a).

4.3.3.3 Synthesis of Selenium Nanoparticles

SeNPs have been shown synthesized by the intracellular conversion of toxic selenite ions (Se^{+4}) into nontoxic elemental SeNPs (Se^{0}) under aerobic conditions by the bacterium *B. licheniformis* JS2 (Dhanjal and Cameotra 2011). A method has also been developed for extraction and purification of intracellular nanoparticles from *B. licheniformis* JS2 (Sonkusre et al. 2014). The cell lysis procedure and recovered intracellular SeNPs by bacterial cell lysis using lysozyme and French press, cleaned by successive washes with Tris-HCl buffer and finally separated from insoluble debris by two-phase water-octanol extraction. Spreading of purified and cleaned SeNPs on TSA plate showed no bacterial growth indicating the cell lysis process is highly efficient. The SeNPs ranged from 40 to 180 nm. The particles were found to be stable at physiological temperature and pH. When kept at 37 °C for 5 h in various concentration of bicarbonate buffer, the particles with no charge on the surface did not form agglomerates, whereas the negatively charged particles (−29 mV) agglomerated and settled to the bottom of the tube (Sonkusre et al. 2014). SDS- PAGE and silver staining results showed that the particles were associated with some protein, although the quantity of protein was very low. The FTIR analysis showed that the SeNPs have some functional groups attached to the surface. These findings indicate that the SeNPs have a polymer and/or protein coating on their surface which provides steric stability to them. The neutral charged, non-agglomerating SeNPs at a concentration as low as 2 μg Se/mL were effective in inhibiting proliferation and inducing caspase independent necrosis to human

prostate adenocarcinoma cells (PC3) without causing any significant toxicity to human peripheral blood mononuclear cells. The use of lysozyme and a French press for bacterial cell lysis followed by an organic-aqueous extraction system have proven to be more successful methods for the recovery of intracellular NPs than previously used techniques. By using this extraction procedure, pure and clean, sterically stabilized SeNPs from *B. licheniformis* JS2 (Sonkusre et al. 2014).

4.3.3.4 Synthesis of Silver Nanoparticles

Bacillus licheniformis is known to secrete the cofactor NADH and NADH-dependent enzymes, especially nitrate reductase, which might be responsible for the bioreduction of silver ions to silver nanoparticles (Kalimuthu et al. 2008). Kalimuthu et al. (2008) also reported the optimization of production of nitrate reductase from *B. licheniformis*. The particles synthesized using the optimized enzyme activity ranged from 10 to 80 nm. Silver nanoparticles synthesized by *B. licheniformis* have the potential to be anti-angiogenic (Bhattacharya and Mukherjee 2008). Bovine retinal endothelial cells were treated with different concentrations of silver nanoparticles for 24 h in the presence and absence of vascular endothelial growth factor, and 500 nM (IC50) silver nanoparticle solution was able to block the proliferation and migration of bovine retinal endothelial cells. The cells showed a clear enhancement in caspase-3 activity and formation of DNA ladders, evidence of induction of apoptosis. The results showed that silver nanoparticles inhibit cell survival. It is anticipated that nanoparticle-mediated targeted delivery of drugs might significantly reduce the dosage of anticancer drugs with better specificity, enhanced efficacy, and low toxicities (Bhattacharya and Mukherjee 2008).

4.4 Future Directions

Microbial synthesis of nanoparticles has emerged as an important branch of nanobiotechnology. However the developments of a wide variety of synthetic technologies in this review are based on a large number of preliminary experiments, which consume massive human and material resources as well as tediously long time. Different designs of technical routes are required in order to achieve various morphologies and crystallites for different materials, and even for the same material. This results in numerous, complicated and bewildering synthesis methods with no general rule to follow. Due to the lack of unity and established theorems and laws, it becomes unpredictable to accurately build a controllable nanoscale world. The establishment of predictable synthesis methods is necessary (Duan et al. 2015). To improve the rate of synthesis and monodispersity of nanoparticles, factors such as microbial cultivation methods and downstream processing techniques have to be improved and the combinatorial approach such as photobiological methods may be used. The delineation of specific genes and characterization of enzymes that involve

in the biosynthesis of nanoparticles is also required. Thus, the complete knowledge on the underlying molecular mechanisms that mediate the microbial synthesis of nanoparticles is mandate to control the size and shape as well as crystallinity of nanoparticles. At present, laboratory syntheses of nanomaterials are able to deliver only small amounts of products and are plagued by batch-to-batch deviations. This not only limits the scaled-up production of nanomaterials, but also results in inconsistency in the essential characteristics such as size and shape. Therefore, a key challenge facing the practical application and industrialization of nanomaterials is the design of scalable synthesis schemes, with a pressing need for continuous, automatic and scaled-up synthesis schemes. Industrial scale synthesis of metal nanoparticles using biomass needs some processes, including seed culture, inoculation of the seed into the biomass, harvesting the cells, synthesis of nanoparticles by adding metal ions to the cells, separation of cells by filtration, homogenization of the cells to isolate the produced nanoparticles, stabilization of the nanoparticles, product formulation, and quality control (Korbekandi et al. 2009, 2012, 2013; Korbekandi and Iravani 2013; Iravani et al. 2014).

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Chapter 5
Application of Nanoparticles

Abstract In this chapter we discuss the applications of bionanoparticles (BNP) in biomedical and environmental fields. In the biomedical field, these nanoparticles have been investigated for antimicrobial applications, biosensing, imaging, and drug delivery. In the environmental field, nanoparticles have been investigated for applications in bioremediation of diverse contaminants, water treatment, and production of clean energy. Overall, the BNP have attracted the attention of diverse researchers because their syntheses are more environmentally friendly, produces more homogeneously distributed nanoparticles and some of them can be easily biodegradable. Although there are several studies investigating the application of BNP, these nanomaterials are still way less studied than synthetic nanoparticles, since researchers are still identifying the microbiological synthetic pathways of these BNP. It is expected that with the advancement of the understanding of BNP synthesis pathways, the application of BNP will expand to many more fields than biomedical and environmental and will be potentially applied in diverse nanotechnological industries.

5.1 Introduction

The application of nanostructures and nanoparticles of biological nature is an emerging field. These nanoparticles of biological nature or produced by biological systems are typically classified as bionanoparticles (BNP) (Thakkar et al. 2010). Several researchers also classify hybrid nanomaterials composed of both bionanoparticles and synthetic nanoparticles as bionanoparticles (van Rijn and Böker 2011; Yang et al. 2011). In the latter, these bionanoparticles are used together with non-biological nanoparticles to enhance their properties. The interest for BNPs has increased in the past years because they present very different properties and functions than synthetic nanoparticles and they tend to be more biocompatible than their inorganic non-biological counterparts. There are, however, advantages and disadvantages about using BNP instead of synthetic nanoparticles in different fields.

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The most obvious disadvantages of BNP are that they frequently do not withstand high or low temperatures, extreme pH values, presence of harsh chemicals and potential environmental conditions that could lead to their hydrolysis (van Rijn and Böker 2011). It is possible, however, that BNPs from extremophiles might overcome these issues. Research to address these disadvantages is still in its infancy. In the meanwhile, the synthetic inorganic nanomaterial counterparts are preferred, whenever these conditions are present in a given system, since they have higher stability under these conditions than BNPs. Despite these disadvantages, these BNPs have advantages that are essential to produce new exciting materials.

The more obvious advantage of BNPs is that nature has perfected the synthesis and reproducibility of these nanostructures over the last billions of years (van Rijn and Böker 2011). Although the reproducible synthesis of synthetic monodispersed nanoparticles have improved tremendously over the years, it is far from being as perfect as the BNP synthesized by biological systems (Hussain et al. 2016). For instance, in the case of a protein-based nanoparticle, the size and shape are directly dependent on the sequence, composition, and number of amino acids in the protein chain. Typically, any variation in amino acid sequence and length will affect the structure, folding and overall function of the protein. In the case of synthetic nanoparticles, although they can possess catalytic activities, they are not as efficient and operate as well as proteins under physiological conditions.

Other great advantages of BNPs are their very diverse sizes and shapes, as well as their capability to form very complex structures (van Rijn and Böker 2011). For instance, BNPs can have spherical, ring, rod, and even banana shapes. In addition to the shapes, BNPs can form different structures, such as solid, hollow, present pores, or not, within the range of nano- to several micrometers. They can also be formed by one single structure or by self-assembled structures. Some of the BNPs are formed by several non-covalently connected subunits, hence they can easily disassemble and reassemble. This dynamic property is unique to BNPs and can be very useful for different applications, especially in drug delivery.

BNPs are also advantageous over synthetic nanoparticles since they are typically biodegradable (van Rijn and Böker 2011). In many cases, the biodegradability property of BNPs is an advantage over inorganic nanoparticles since synthetic nanoparticles are more difficult to remove without using physical or chemical processes that can be very costly and not very environmentally friendly. This advantage also applies to the biodegradation of BNPs when they are used in vivo and need to be released or disposed in a natural fashion (Lin and Dufresne 2014).

These advantages and unique properties of BNPs make them very attractive for different applications. The field of technological application of nanoparticles is called nanotechnology. Nanotechnology involves not only nanometer sized materials, but also devices and systems at the nano-size level. Recently, these nanoparticles have entered a commercial exploration period. The application of BNPs is still underdeveloped, but several studies have suggested that they can be effectively used in biomedical and environmental applications. In the biomedical applications, nanoparticles are used for example for antimicrobial applications, biosensing, imaging, and drug delivery and; while for environmental applications,

nanoparticles are used for bioremediation of diverse contaminants, water treatment, and production of clean energy. More details on some of these applications are described below.

5.2 Biomedical Applications of BNPs

5.2.1 Bionanoparticles as Anti-microbial Agents

In the U.S. alone, the fourth leading cause of death are hospital acquired infections (i.e. nosocomial infections) with more than 2 million cases reported annually leading to more than $5 billion in added medical costs per year (Wenzel 2007). The majority of these nosocomial infections, about 60–70 %, are associated with bacterial contamination of implanted medical devices (Donlan 2001; Bryers 2008). This number has remained high over the years, especially because of the emergence of antibiotic-resistant pathogenic strains and pathogens displaying multiple drug resistance. Hence, the need of new anti-microbial agents has increased tremendously. Nanoparticles (NPs) are currently being viewed as a powerful nanotechnology to control hazardous microorganisms due to their intrinsic antimicrobial properties. A large number of synthetic NPs have been explored for their antimicrobial properties. These include NPs of silica/iron oxide, graphene, graphene oxide, bifunctional Fe_3O_4-Ag NPs, titanium, copper, zinc, silver and gold, just to name a few (Kang et al. 2008; Rodrigues and Elimelech 2010; Narayanan and Sakthivel 2011; Santos et al. 2012; Mejias Carpio et al. 2014; Musico et al. 2014; Rodrigues et al. 2015).

More recently, BNPs have emerged as an alternative to the NP synthetic process (Narayanan and Sakthivel 2011; Hussain et al. 2016). The synthesis of NPs using biological systems is more attractive, since it is less labor-intensive and does not require expensive toxic chemicals for their production. Hence, the synthesis of BNPs is considered to be a greener process than the current physical and chemical methods of NP synthesis (Gericke and Pinches 2006). Although, researchers have investigated the biological synthesis of BNPs, very few investigations have explored their antimicrobial properties. The most common BNPs investigated as anti-microbial agents are silver, gold, zinc, TiO_2 and biocellulose (Thakkar et al. 2010; Narayanan and Sakthivel 2011; Sharma et al. 2012). These nanoparticles are typically synthesized by either bacteria, fungi, algae, and plants. In this review, we will focus on the BNPs synthesized mainly by bacteria and fungi.

5.2.1.1 Silver BNPs

Today, silver nanoparticles are already being commercially used as antimicrobial agents. For example, silver NPs are currently found in surgically implanted catheters in order to reduce the infections caused during surgery, in toys, personal

care products, and silverware. The reason for using silver for anti-microbial applications is because silver possess antifungal, anti-bacterial, anti-inflammatory, and anticancer effects (Kalishwaralal et al. 2009; Sheikpranbabu et al. 2009). Silver NPs have been described to be synthesized by both Gram+ and Gram− bacteria (Nanda and Saravanan 2009; Thakkar et al. 2010). Microbial synthesis of silver nanoparticles, however, is restricted to certain groups of microorganisms since most microbes tend to be sensitive to silver ions. Similarly, reduction of silver ions by microorganisms involve specific biomolecules, such as enzymes, vitamins, and polysaccharides through complex pathways involving electron transfer and conversion of NADPH/NADH to $NADP^+/NAD^+$ (Matsumura et al. 2003; Gholami-Shabani et al. 2014).

In most studies, the silver BNPs were shown to have antimicrobial properties against different microorganisms. For instance, the silver nanoparticles produced by Gram-negative bacteria, e.g. *Klebsiella pneumoniae* and *Shewanella oniedensis* MR-1, were shown to have antimicrobial properties against both Gram+ and Gram− bacteria, such as *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Shahverdi et al. 2007; Suresh et al. 2010). In another study, *Pseudomonas aeruginosa* strain BS-161R produced monodispersed spherical particles with a size range of 13 nm (Kumar and Mamidyala 2011). These NPs exerted antimicrobial activity against a large array of microorganisms in a concentration as low as 8 μg/ml. The microorganisms inhibited by the presence of these NPs were *S. aureus*, *Micrococcus luteus*, *Candida albicans*, and *Candida krusei*.

In the case of Gram-positive bacteria, AgNPs were described to be produced by *Streptomyces hygroscopicus* and *Bacillus licheniformis* (Zinjarde 2012). Typically, the extracellular components of these microorganisms led to the production of AgNPs. In the case of *S. hygroscopicus*, AgNPs in the size of 20–30 nm were produced (Sadhasivam et al. 2010). These BNPS significantly inhibited the growth of medically relevant Gram-negative bacteria (*E. coli* and *Salmonella typhimurium*), Gram-positive bacteria (*B. subtilis* and *Enterococcus faecalis*), and the yeast *C. albicans*. In the case of *B. licheniformis*, the BNPs were able to inhibit biofilm formation of *P. aeruginosa* and *Staphylococcus epidermidis* in a study aiming to prevent growth inhibition of contact lenses (Kalishwaralal et al. 2010).

Fungi is another group of microorganisms also able to produce silver NPs. In most fungus genera, the production of AgNPs has been described to involve the enzyme nitrate reductase, which reduces the metal ions (Duran et al. 2005; Kumar et al. 2007). Studies of production of AgNPs with different fungi species, such as *Cladosporium cladosporioides*, *Fusarium semitectum*, *Fusarium solani*, *Fusarium acuminatum*, and *Trichoderma asperellum*, showed that these microorganisms are able to produce extracellularly AgNPs of 4–100 nm in solutions containing silver salts. These nanoparticles were shown to inactivate not only Gram-negative, but also Gram-positive bacteria and some species of fungi. The AgNPs produced by *Amylomyces rouxii* showed antimicrobial activity against *Shigella dysenteriae* type I, *E. coli*, *S. aureus*, *P. aeruginosa*, *Citrobacter* sp., *B. subtilis*, *C. albicans* and *Fusarium oxysporum* (Sharma et al. 2009). These results suggest that the AgNPs produced by microorganisms has a broad microbial spectrum.

5.2.1.2 Gold BNPs

A few microorganisms from the group of fungi, yeasts, and bacteria have been described to synthesize antimicrobial gold nanoparticles (AuNPs) (Das et al. 2009). Some of them were also investigated for anti-microbial properties. For instance, *Rhizopus oryzae* was able to produce AuNPs with an average diameter of 10 nm through an in situ reduction of $HAuCl_4$ (Das et al. 2009). These AuNPs showed strong antimicrobial property against several Gram-negative and Gram-positive bacteria, as well as against yeasts (*Saccharomyces cerevisiae* and *C. albicans*). *Shewanella oneidensis* could reduce tetrachloroaurate (III) ions to produce homogenous extracellular gold spheres with an average size of 12 ± 5 nm (Suresh et al. 2011a, b). The antimicrobial activity of these AuNPs were shown to be negligible towards *E. coli*, *S. oneidensis,* and *B. subtilis*. This finding was interesting, since it showed that not all gold BNPs have antimicrobial properties. In addition to bacteria, the yeast *Candida guilliermondii* was also shown to produce extracellular AuNPs in the size range of 50–70 nm (Mishra et al. 2011). The AuNPs displayed antimicrobial activity against five pathogenic bacterial strains. These particles were most toxic to *S. aureus*. The comparison of these studies showed that the size and the toxicity levels of the AuNPs to different microorganisms were dependent on the type of microorganisms synthesizing them. It is possible that different capping proteins would be responsible for affecting the antimicrobial properties of AuNPs.

5.2.1.3 Metal Oxides BNPs

Among the metal oxides produced by microorganisms, zinc oxides and titanium dioxide are the most described in the literature (Jones et al. 2008; Jha and Kulkarni 2009). Like silver, zinc oxide BNPs (ZnO NPs) have also received a tremendous amount of attention since the synthesis of synthetic ZnO NPs requires the use of toxic organic solvents, expensive and labor intensive reaction conditions, such as high temperature and pressure, and long refluxing time for its synthesis. The interest for ZnO has grown over the years since it has been shown to have unique antibacterial, antifungal, superior catalytic, UV filtering properties and photochemical activity (Jones et al. 2008). More importantly, ZnO nanoparticles presents antibacterial and antifungal activities at lower concentrations and can be easily used for coating surfaces to serve as antimicrobial surfaces. Additionally, the antimicrobial property of ZnO has been described to be very specific to pathogenic microorganisms. A study in soil, showed that the addition of ZnO nanoparticles acted as antifungal and did not affect the soil fertility like some other antifungal agents (Jayaseelan et al. 2012).

The mechanisms of toxicity of these ZnO NPs towards microorganisms was determined to be related to the disruption of the membrane lipid bilayer followed by release of cytoplasmic contents. Further studies showed that exposure time to the NPs would enhance the cell membrane contact with the nanoparticles and lead to cell

disruption (Sharma et al. 2010). Most studies aiming at producing ZnO BNPs were investigated with plants, a few studies, however, investigated the role of microbes in ZnO BNP production. A study with *Aeromonas hydrophila* demonstrated that this microorganisms could synthesize ZnO NPs (57.7 nm, spherical) that exhibited antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis, Streptococcus pyogenes*, *C. albicans, Aspergillus flavus*, and *Aspergillus niger* (Jayaseelan et al. 2012). Another study with *Pichia fermentans* JA2, showed that the ZnO NPs produced an effective zone of inhibition in the presence of *Enterococcus* sp., *S. aureus*, and *Proteus mirabilis* (Chauhan et al. 2015). To date, only two studies investigated the production of ZnO NPs by microorganisms. Hence, the production of ZnO NPs by microorganisms is still in its infancy, however, these preliminary results with *A. hydrophila* suggests that ZnO NPs produced by microorganisms can be of great potential for antimicrobial applications.

In the case of TiO_2 NPs, the biological synthesis of TiO_2 NPs tends to be preferred over synthetic methods because the biosynthesis provides synthesis rates faster or comparable to the chemical processes. More importantly, the biosynthesis of TiO_2 can be potentially used in various fields that requires human contact with these particles, such as foods, cosmetics, foods and sunscreens (Quadros and Marr 2010). Contrary to ZnO NPs that has just one biosynthesis report, the TiO_2 NPs can be synthesized by different species of bacteria and fungi. For instance, the bacteria *Lactobacillus* sp, *A. hydrophila*, *B. subtilis* and the fungi *F. oxysporum*, *Sachharomyces cerevisae* and *Aspergillus tubingensis* were described to be able to synthesize TiO_2 NPs (Bansal et al. 2005; Jha and Kulkarni 2009; Kirthi et al. 2011; Jayaseelan et al. 2013). Typically, TiO_2 presents antimicrobial activity only when activated with UV light. Very few studies describe the ability of TiO_2 to present antimicrobial activity under visible light (Cheng et al. 2009). However, a more recent study with *A. flavus*, showed that it is possible to synthesize TiO_2 that is active under visible light (Rajakumar et al. 2012). The authors showed that both Gram-positive and Gram-negative microorganisms could be inactivated by these nanoparticles. Inactivation experiments performed by the authors showed that *S. aureus*, *Shigella flexneri* and *Acinetobacter baumannii* were inactivated by the new visible-light-activated TiO_2 BNPs (Cheng et al. 2009). The authors attributed the antimicrobial effect to production of reactive oxygen species, such as hydroxyl radicals, which led to phospholipid peroxidation, followed by cell death. Although, TiO_2 can be used as antimicrobials, researchers tend to prefer to use these nanoparticles for other applications, such as photocatalysts or electrical insulators, as discussed later in this chapter.

5.2.1.4 Nanocellulose BNPs

Bionanocellulose is not considered to have antimicrobial microbial properties by itself, however, this nanomaterial can serve as a scaffold for antimicrobials due to its unique properties. For instance, nanocellulose can provide a porous network structure, which is essential for the transfer of antibiotics or other antimicrobial

agents into a wound, and at the same time can serve as an efficient physical barrier against external infectious agents (Andresen et al. 2007). Another advantage of nanocellulose is that this nanomaterial is compatible with biological tissues and presents significant bioavailability and biodegradability (Maneerung et al. 2008; Luan et al. 2012). In order to obtain an antimicrobial nanocellulose material, researchers have investigated many physical or chemical process to couple antimicrobial agents with nanocellulose. Typically, nanocellulose-based antimicrobial biomaterials can be produced in two different ways. The first, involves incorporation of other anti-microbial nanoparticles, such as silver, titanium, gold, etc. The second, involves the incorporation of organic antimicrobial agents, such as antibiotics or enzymes (e.g. lysozymes) to confer antimicrobial properties to nanocellulose.

The most common anti-microbial nanoparticle used to impregnate nanocellulose is silver. Silver has been the most extensively studied and used anti-microbial nanoparticle since ancient times to fight infections and prevent spoilage. The silver nanoparticles have effective antibacterial, antifungal, as discussed earlier on this chapter. Simple impregnation of silver nanoparticles is a common approach to introduce a silver antimicrobial agent into nanocellulose-based nanomaterials. This impregnation confers to the nanocellulase similar antimicrobial property to silver nanoparticles. In this case, the unique property of nanocellulose, as a porous network structure, facilitates the transfer of the antimicrobial silver ions into the wound, meanwhile serving as an efficient physical barrier against any external infection (Andresen et al. 2007). In addition to silver nanoparticles, recent studies reported that zinc-oxide nanoparticles could also be used for impregnation on nanocellulase. These composite were able to yield antibacterial properties to the nanocellulose composite (Azizi et al. 2013; Martins et al. 2013).

Besides nanoparticles, other antimicrobial agents were also easily incorporated in nanocellulose. Examples of such compounds are: porphyrin, octadecyldimethyl (3-trimethoxysilylpropyl) ammonium chloride (Andresen et al. 2007), allicin and lysozyme (Jebali et al. 2013). The incorporation of such compounds led to a nanocellulose material with excellent antimicrobial properties against diverse microorganisms. Overall these antimicrobial nanomaterials from nanocellulose exhibited compatibility with biological tissues as well as significant bioavailability and biodegradability (Luan et al. 2012). There were, however, issues raised in these studies about the balance among the improvements of antimicrobial activity, cytotoxicity to human cells, and duration effect of antimicrobial properties, which still needs to be investigated for all these nanocellulose impregnated materials before real biomedical applications.

5.3 BNPs for Biosensing Applications

The sensors described in the literature that are BNP-based are typically composed by gold NPs or magnetic NPs. These biosensors BNP-based are frequently described for biomedical diagnosis or forensic analysis to detect biological agents,

as well as toxic compounds or diseases (Diamond 1998). Sensors are composed typically of two main components: a recognition element (i.e. bioreceptor) and a signal transduction element (i.e. transducer). The recognition element typically binds to a specific compound, called analyte. In the case of gold nanoparticles, these NPs can be used as biosensors since they exhibit unique electronic and optical properties. These properties are directly related to the size and shape of these nanoparticles. For instance, AuNPs possess an intense absorption peak in the 500–550 nm range (Jain et al. 2006), due to surface plasmon resonance (SPR) (Eustis and El-Sayed 2006).

Surface plasmon resonance occurs when a photon from an incident light hits the gold NP surface (Fig. 5.1). The incident photons lead to resonant excitation of the conductive electrons of the gold nanoparticles. This movement of the electrons are called plasmon and they can generate an electric field and generate changes in the refractive index in the vicinity of the surface (Homola et al. 1999). Detection of the sample analyte can be obtained by measuring the changes in the reflected light after immobilization of the analytes on the nanoparticle surface. Typically, the amount of surface concentration can be quantified by monitoring the reflected light intensity or tracking the resonance angle shifts. A SPR biosensor has a detection limit in the order of 10 pg/mL and has been described to be one of the most powerful biosensing technologies. Typically, the interparticle plasmon coupling can lead to a red-shift (650 nm) and broadening of the plasmon band, which can be detected colorimetrically.

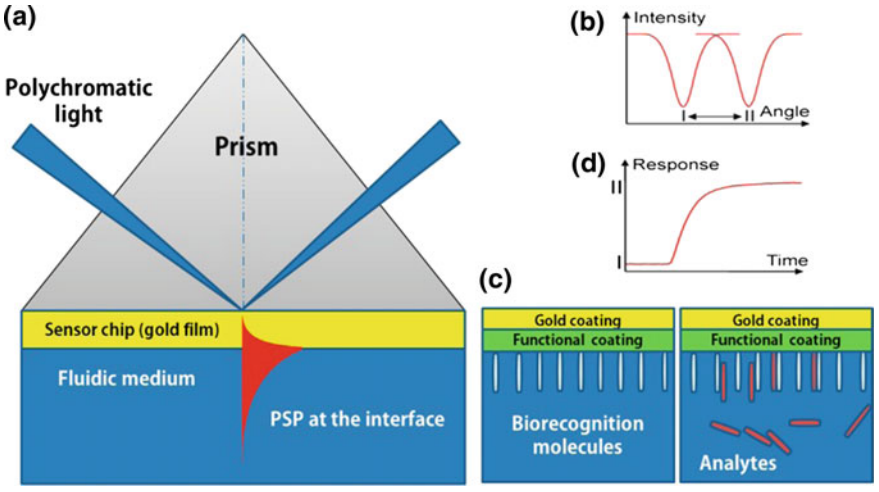

Fig. 5.1 Concept of a surface plasmon resonance (SPR) biosensor: **a** Kretschmann geometry of the ATR method; **b** spectrum of reflected light before and after refractive index change; **c** analyte-biorecognition elements binding on SPR sensor surface and **d** refractive index changes caused by the molecular interactions in the reaction medium. *Source* Nguyen et al. (2015). Copyright © 2015, MDPI. Reproduced with permission

Besides the plasmon property of gold nanoparticles, these particles possess conductivity properties that can be applied to electroanalytical biosensing (Yu et al. 2003). In the literature, there are several systems describing the use of nanoparticle-enzyme hybrids that can be used as electrochemical sensors of diverse health related molecules, proteins and even DNA. For instance, a bioelectrocatalytic sensor for glucose measurements (for diabetes) was developed by binding the AuNPs to the glucose oxidase enzyme (apo-GOx). After binding the enzyme-AuNP hybrid system to an electrode, the sensor could effectively detect the electrical changes in the presence of different concentrations of glucose under real physiological conditions (Xiao et al. 2003). An analogous electron transfer sensor was developed to monitor hydrogen evolution from a zinc-substituted cytochrome c immobilized on TiO_2 NPs (Astuti et al. 2005).

In the case of protein sensors, Velev and Kaler developed an antigen–antibody based protein sensor that was designed to detect human IgG at 0.2 pM (Velev and Kaler 1999). The antibody was immobilized in AuNPs. Besides antibodies, DNA is another biomolecule relevant for biomedical fields that can be detected with AuNP-based sensors. Mirkin et al. developed a selective DNA sequence based on oligonucleotide-functionalized Au-NPs (Cao et al. 2002; Park et al. 2002). In this sensor, short-chain oligonucleotides were deposited onto the electrode surfaces containing gold nanoparticles. In the presence of the target DNA sequence, the DNA could hybridize to the oligonucleotides. To enhance the sensitivity of the sensor down to 50 fM and to increase the mutation selectivity factor to about 1:100,000, the authors deposited silver on the surface of the gold nanoparticles. The coating of the AuNP with silver enhanced significantly the conductivity, and therefore, the sensitivity of the sensor.

Another biosensor method using AuNP is the Surface enhanced Raman scattering (SERS) method (Fig. 5.2) (Aroca et al. 2005; Doering et al. 2007). These AuNP sensors were developed by labelling AuNP with Raman-active dyes and oligonucleotides. These biosensors are able to detect simultaneously different target DNA molecules (Cao et al. 2002). This SERS method is also described to be able to detect the interactions of protein-small molecules and protein-protein, as long as the NPs are coupled with specific proteins and Raman dyes to detect these interactions (Cao et al. 2003). In addition to biosensors using AuNPs, researchers have also used magnetic nanoparticles, with or without coupling with AuNPs.

For instance, Mirkin's group developed a "bio-barcode" type of biosensor for nucleic acids or proteins involving both AuNPs and magnetic NPs. In the case of nucleic acid detection, magnetic nanoparticles carrying a partial complementary sequence to the target DNA is hybridized to a "bio-barcoded" AuNPs in the presence of the target DNA. After magnetic separation of the hybridized nucleic acids and denaturation of the double stranded DNA, the bio-barcode is released for analysis through PCR. This method is comparable to many PCR-based approaches (Nam et al. 2004). In the case of protein detection, the magnetic NPs contain specific antibodies to the target protein. After interaction of the target protein with

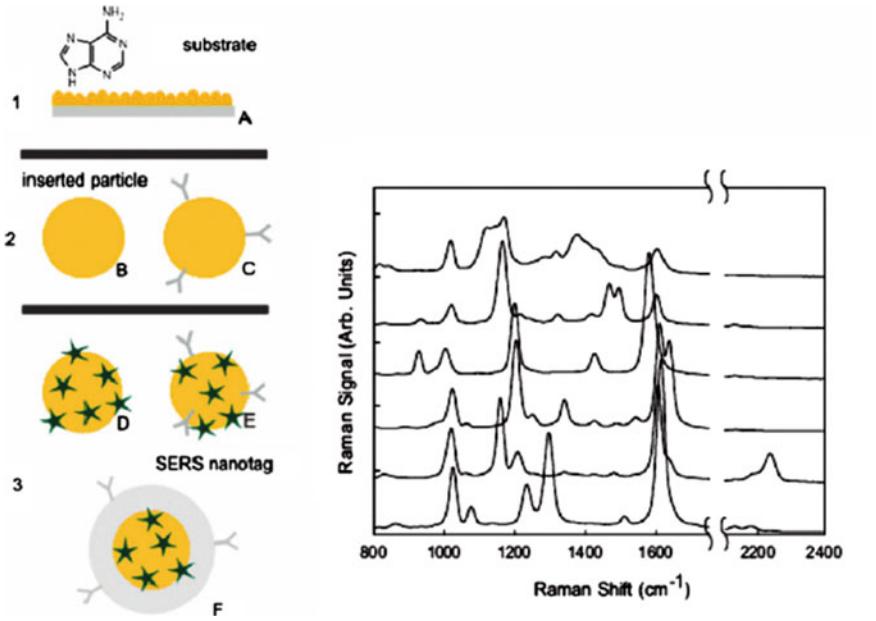

Fig. 5.2 Architectures used in SERS experiments. *A* 2D substrate with adenine on the surface. *B* Bare particle. *C* Antibody-targeted particle. *D* Reporter labeled particle. *E* Targeted and labeled particle. *F* Targeted particle with encapsulated Raman label. *A* Represents the substrate approach, *B* and *C* are examples of the inserted particle approach while *D*, *E*, and *F* can all be thought of as SERS nanotags/Raman spectra of six different Nanoplex biotags. From *top* to *bottom*, the label molecules used were 4-[4-hydroxyphenylazo]pyridine, 4,4′-azopyridine, d8-4,4′-dipyridyl, bis (4-pyridyl)ethylene, bis(4-pyridyl)acetylene, 4,4′-dipyridyl. *Source* Doering et al. (2007). Copyright © 2007, Wiley. Reproduced with permission

the antibody, the AuNPs is exposed to obtain and antigen-antibody interaction. This approach was described for detection of mixtures of DNA and proteins by using a mixture of different bio-barcoded AuNPs probes (Stoeva et al. 2006).

Another biosensor using magnetic nanoparticles was developed by Peng et al. In this study, a multi-functional core–shell glucose oxidase–Au–polydopamine–Fe_3O_4 magnetic bionanoparticle (GOx–Au–PDA–Fe_3O_4 MBNPs) was fabricated to detect glucose (Peng et al. 2013). In another study with magnetic bionanoparticles only, the authors developed a high-performance amperometric fructosyl valine (FV) biosensor (Chawla and Pundir 2011). This study aimed to immobilize the enzyme fructosyl amino-acid oxidase (FAO), as a model enzyme, on a core–shell magnetic bionanoparticles modified gold electrode. Chitosan was used to introduce amino groups onto the surface of the magnetic BNPs. The sensor showed outstanding sensitivity, response time, and long term shelf-life. These studies show that BNPs, especially AuNPs and Magnetite NPs can be used to develop biosensors for different medical applications.

5.4 Bionanoparticles for Imaging

As of today, there are several imaging tools available for the medical field. The most common ones are magnetic resonance imaging (MRI), optical (OI) and ultrasound (USI) imaging (Sharma et al. 2006). Among these imaging tools, magnetic and luminescent/fluorescent NPs have contributed significantly to the advancement of bioimaging tools (De et al. 2008). Fluorescent NPs, such as AuNPs, are frequently used for OI, while magnetic NPs are typically used for MRI. In addition to inorganic nanoparticles, such as Au and iron oxides, virus particles have also been described to serve for imaging purposes (Manchester and Singh 2006). In this section we will discuss how these NPs can be applied for MRI or OI and present a few examples of inorganic and organic NPs used in MRI and OI applications.

MRI is one of the most powerful and noninvasive imaging technology possessing exceptional soft tissue contrast and resolution. MRI relies on magnetic fields and radio frequencies. The signal intensity of MRI is related to the relaxation times of protons from free molecules, such as lipids, water, and proteins that are present in organs (Lam et al. 2013). Because of the development of highly specialized and efficient contrast agents, MRI has become one of the most powerful noninvasive imaging tools in medicine. In MRI, to increase contrast, various inorganic nanoparticles and contrast agents are administered prior to the scanning. The contrast agents used can be composed by either ferrites, magnetite, or iron oxide NPs. These contrast agents are responsible for providing negative contrast in the image.

The main BNP currently investigated for MRI application, as contrast agent, is ferritin. This protein stores a ferrihydrite/magnetite core/shell structure and is synthesized by microorganisms. When the ferritin iron content is removed, magnetite predominates. Furthermore, the exterior shell of ferritin can be easily functionalized, which facilitates incorporation of specialized binding sites or other dyes (Uchida et al. 2007). There are descriptions in the literature, where ferritin can be conjugated with different Alexa Fluor dyes. This conjugation leads to fluorescence resonance energy transfer (FRET) in a single nanoparticle (Fernandez et al. 2008). The incorporation of optical functionality in magnetic NPs is of growing interest since it would allow simultaneous target labeling, optical and magnetic imaging. This simultaneous imaging would facilitate cell sorting and even cell separation for diagnostics and treatment (Wang et al. 2005; Wetz et al. 2007).

In addition to using magnetic NPs to enhance MRI signals, researchers have also embedded iron oxide in AuNP shells. In this case, the iron oxide provides magnetism, whereas the Au shell incorporates the optical properties of AuNPs (as described later in this section) (Lim et al. 2007; Boisselier and Astruc 2009). The application of AuNPs for the development of new MRI contrast agents has also been investigated. In these studies, AuNPs were used as templating carriers of gadolinium chelates, which is currently used in MRI imaging. The results showed that the sensitivity of magnetic resonance imaging improved significantly with this composite. Besides using AuNPs for MRI, these NPS can also be used for OI.

The OI involves fluorescent labelling of a target macromolecule, organelle, cells or even tissues for their location and diagnosis. This tool relies on specific binding of the fluorescent nanoparticle to a target to reveal its location under a fluorescence microscope. The most used BNP for OI is AuNP, which includes AuNPs with nanorods and nanospheres shapes. AuNPs can be used for OI by producing antibody-conjugated AuNPs. These conjugated nanoparticles are able to bind to antigens either in cells or tissues. The main characteristic of AuNPs that makes this NP attractive over other labeling dyes is because they are extremely stable under continuous illumination and do not bleach. Furthermore, the AuNPs fluorescence spans over an entire spectrum of light; these NPs exhibit plasmonic effects and fluorescence in both one-photon (Eustis and El-Sayed 2005; Li et al. 2005) and two-photon (Imura et al. 2005) modes with high efficiencies in contrast to other organic fluorophores. These unique properties of AuNPs make them perfectly suited for OI.

In addition to inorganic BNPs, more recently, virus NPs were recognized as part of nanotechnological applications involving BNPs. Viruses have been considered as BNPs since they are of biological nature and are typically found in the nanosize range like most NPs (Steinmetz 2010). Plant and certain animal viruses are particularly of interest since they typically do not cause diseases in humans. More importantly, viral NPs can be engineered to target a specific cell or tissue type and to contain fluorescent or contrast agents necessary for imaging. The proteinaceous composition of viral NPs allows the incorporation of specific ligands and imaging agents, on a single platform (Young et al. 2008), which may lead to potential applications in targeted imaging and therapy. One example of application of viral NPs for MRI is the study performed with the Cowpea chlorotic mottle virus (CCMV). This BNPs was engineered and expressed in the yeast Pichia pastoris. The authors in this study successfully produced and purified the CCMV coat protein, which possess a metal binding domain. The resulting modification of CCMV with Gd^{3+} was able to generate a paramagnetic NPs for MRI application (Allen et al. 2005). This report is just one of many reports using viral NPs from different origins for the development of image-contrast agents. Other studies have also investigated the viral NPs for OI applications.

The reason viruses are also attractive BNPs for optical imaging is because most viruses have multivalent capacities that allow incorporation of fluorochromes compatible with different microscopes for imaging and diagnosis. As a rule of thumb, the application of viral BNPs in the imaging and diagnosis of alterations in cells or tissues, which are related to diseases, depends on the use of fluorochromes that are compatible with the imaging technology. In the case of fluorochromes, imaging has been achieved with the development of two-photon laser scanning microscopy (TPLSM) (Denk et al. 1990) and development of fluorophores optimized for two-photon absorption (TPA) (Massin et al. 2013). The TPLSM has been very promising since it produces background-free images with reduced photobleaching and photodamage (Niehl et al. 2015). This system was investigated with the Tobacco mosaic virus (TMV). In a recent study, researchers synthesized TMV particles carrying a two-photon fluorophore to obtain images of mouse brain

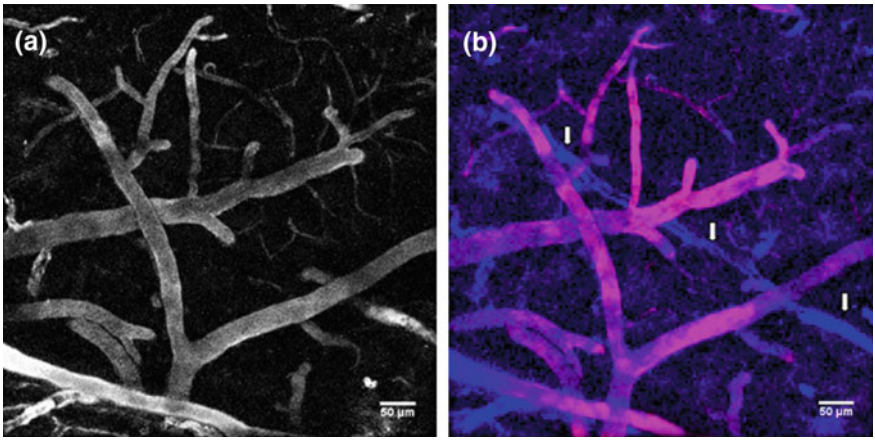

Fig. 5.3 Intravital imaging of the mouse brain vasculature with TMV-BF3 particles. **a** Mouse brain vessels labeled with TMV-BF3 at 1 h after intravenous injection into the tail vein. **b** Same observation window as shown in **a** but after a second injection, this time with sulforhodamine B; *blue*, fluorescence emitted from TMV-BF3; *red*, fluorescence emitted from sulforhodamine B. The 3D projections were performed with Fiji software using the standard deviation projection method. *Source* Niehl et al. (2015). Copyright © 2015, Frontiers. Reproduced with permission

vasculatures (Fig. 5.3). In this study, the authors demonstrated that the fluorescent signal emitted from the BNP was stable and did not leak into surrounding tissues. This study suggested that viral NPs have the potential for visualization of pathological alterations in the brain vasculature in a noninvasive manner. Caution, however, needs to be taken with viral NPs, since it is still not clear whether they present any in vivo toxicity and immunogenicity. However, these studies open the doors for potential future application of theses viral NPs for imaging.

5.5 Bionanoparticles for Drug Delivery

In the past decades, researchers investigating pharmaceuticals realized that drug delivery is a fundamental part of drug development. An ideal drug delivery system should maintain therapeutic concentration and drug stability over time, and permit reproducible and long-term release of the drug at the target site (Kubik et al. 2005). Nanotechnology has emerged as a new approach for drug delivery; and most of the studies to date with nanotechnology focus in cancer research. These studies demonstrated that both inorganic and organic nanoparticles can be employed successfully for drug delivery. Among the BNPs that have been investigated for drug delivery there are magnetic, cellulose and viral nanoparticles.

The most investigated nanoparticles for drug delivery are magnetic nanoparticles. These nanoparticles are either functionalized with anticancer drugs or encapsulated with biocompatible polymers that contain the drug to be delivered.

For instance, one study convalently functionalized the magnetic nanoparticles with doxorubicin (DOX), an anticancer drug (Kattan et al. 1992). The authors showed that the drug delivery system released the drug in mild acidic conditions. Another study, showed that an antibody-conjugated magnetic Poly-(D,L-lactide-co-glycolide) (PLGA) nanoparticles with doxorubicin could be used for simultaneous anticancer drug release and imaging system, because of the magnetite NPs. A similar study, used the antibody Herceptin1 for targeting breast cancer (Yang et al. 2007). These studies indicated that the system is very efficient for delivery of drugs to different cancerous cells.

Although most of the magnetic-targeted drug carriers uses synthetic Fe_3O_4 or Fe_2O_3 as cores coated with biocompatible polymers for drug delivery, this system presents several issues. The major issues for using these nanoparticles are that they have the propensity to aggregate in aqueous solutions and during their synthesis, it is difficult to control their shape and size, as well as their drug loading, which tends to be low (Sarikaya 1994). More recently, researchers have investigated bacterial magnetosomes, synthesized by certain bacteria, as replacements for synthetic magnetic-targeted drug carriers (Schuler and Frankel 1999; Hopkin 2004). These BNPs have unique features, such as narrow and nano-scale size distribution, paramagnetism and are typically membrane bounded (Bazylinski et al. 1994; Sun et al. 2007). These BNPs have also been previously used as carriers for antibodies, enzymes, and nucleic acids (Sun et al. 2007). More recently, these BNPs were demonstrated to also effectively serve as a drug delivery system for doxorubicin. The results showed superior drug delivery with this BNP.

Another well study drug-delivery system is viral capsid. These particles are typically monodispersed, possess nanometer sizes, can reversibly disassemble and reassemble upon certain stimuli and therefore provides an easy route for loading and release of drugs. In addition to drugs, viral particles have been used to store artificial DNA (Mukherjee et al. 2006), single enzymes (Comellas-Aragones et al. 2007), and DNA micelles with hydrophobic components inside the hydrophobic domain of the micelle (Minten et al. 2009). These properties of viruses offers unique possibilities to introduce DNA as a gene therapy system, as well as specific drugs or bio-catalysts delivery systems into the body.

Biocellulose is another BNPs that has a long history in drug delivery applications. Cellulose matrices are frequently used for oral drug delivery through tablets since it has an exceptional compaction property. Cellulose also can be easily modified to bind different drugs. For instance, Burt and collaborators used this property of cellulose to bind water soluble antibiotics and anticancer agents (Jackson et al. 2011). Nanocellulose-based drug carriers can be typically divided into three forms: microspheres (or microparticles), hydrogels (or gels), and membranes (or films) (Table 5.1).

Lin and collaborators developed a pH-sensitive cellulose-alginate microsphere drug delivery system. This system presented higher encapsulation efficiency, reproducible swelling patterns and more importantly, continuous and sustainable release of the drug (Lin et al. 2011a). Hydrogels have also been produced with nanocellulose. In one investigation, the nanocellulose was grafted with cyclodextrin

Table 5.1 Types of drug carrier systems based on nanocellulose

Carrier form	Material component		Model drug	Release time and medium	Ref
	Nanocellulose	Matrix			
Microsphere or bead	Cellulose nanocrystals (CNC)	EA; MMA; BMA	Propanolol hydrochloride	12 h in pH 6.8 PBS	Jackson et al. (2011)
		Sodium	Theophylline	16 h in pH 7.4, pH 6.8, pH 1.0 PBS	Lin et al. (2011b)
	Cellulose nanofibrils (CNF)	–	Indomethacin; nadolol; atenolol; metropolol tartrate; verapamil; ibuprofen	10–14 d in pH 7.4 PBS	Kolakovic et al. (2012)
		Hydrophobin	Itraconazole	90 min in pH 1.2 NaCl/HCl solution	Valo et al. (2011)
Hydrogel or gel	CNC	Cyclodextrin/Pluronic	DOX	6.5 d in water	Lin and Dufresne (2013)
		Cyclodextrin/Pluronic	Bovine serum albumin	20 h in pH 7.4 PBS	Zhang et al. (2010)
		Regenerated cellulose	Bovine serum albumin	48 h in simulated body fluid	Wang and Chen (2011)
	Bacterial Cellulose (BC)	–	Bovine serum albumin	48 h in pH 7.4 PBS	Muller et al. (2013)
		–	Collagen; hyaluronan; growth factors	36–96 h in PBS	Lin et al. (2011a)
		Acrylic acid	Bovine serum albumin	8 h in simulated intestinal fluid	Amin et al. (2012a)
		Polyacrylamide	Theophylline	24 h in pH 7.4 PBS	Pandey et al. (2013)

(continued)

Table 5.1 (continued)

Carrier form	Material component		Model drug	Release time and medium	Ref
	Nanocellulose	Matrix			
Membrane or coating for tablet	CNF	–	Paracetamol	5–10 min in water	Kolakovic et al. (2011)
		–	Lysozyme	10 h in pure water or water/ethanol solution	Cozzolino et al. (2013)
		–	Caffeine	9 h in water	Lavoine et al. (2014)
		–	Indomethacin	30 d in pH 5.0 phosphate buffer	Kolakovic et al. (2012)
		–	Itraconazole	90 d in pH 1.2 NaCl/HCl solution	
		–	Beclomethasone dipropionate	90 d in water	
	BC	–	Paracetamol	2 h in pH 5.8 PBS	Amin et al. (2012b)
		–	Lidocaine	7 h in pH 7.4 PBS	Trovatti et al. (2011)
		–	Lidocaine; ibuprofen	8 h in pH 7.4 PBS	Trovatti et al. (2012)
		–	Caffeine	15 h in pH 7.4 PBS	Silva et al. (2014)
		–	Berberine hydrochloride, berberine sulfate	24 h in pH 2.1 HCl or H_2SO_4 solution; pH 6.8 PBS; pH 12.0 NaOH solution	Huang et al. (2013)
		–	Glycerin	24 h in in vivo evaluation (skin)	Almeida et al. (2014)
		Poly(vinyl alcohol)	Vanillin	1 h in water	Stroescu et al. (2013)
Aerogel	CNF	–	Beclomethasone dipropionate	700 min in pH 8.0 SDS solution	Valo et al. (2013)

for the encapsulation of the drug doxorubicin. The drug release study revealed that the hydrogels exhibited prolonged release of the drug (Lin and Dufresne 2013). Kolakovic and collaborator demonstrated the formation of nanocellulose films that had long-lasting sustained drug delivery properties (Kolakovic et al. 2011). The study demonstrated that the film allowed sustainable release of certain model drugs over a period of three months. The authors also showed that certain drugs had limited release due to poor diffusion. The authors explained that the difference in the release for the different drugs was due to the drug solubility in the solution medium (Kolakovic et al. 2012). In another study regarding biocellulose membranes, Huang and collaborators investigated the delivery of berberine hydrochloride and berberine sulfate in comparison to commercial tablets. The results showed that the membranes significantly extended the duration of the release of the drugs (Huang et al. 2013).

In addition to the traditional drug trapping strategies, some researchers have also explored the direct attachment of drug molecules to nanocellulose. This process was done through covalent coupling of the drug to the nanocellulose. Researchers employed in this process a series of oxidation, reductive-amination, and esterification reactions (Dash and Ragauskas 2012). Overall, the use of natural nanocellulose as a drug delivery system is a very attractive concept, however the physiological influence in the release of drug release, interactions between the drug molecules and the NPs are still of concern and needs to be further investigated.

5.6 Environmental Applications of BNPs

5.6.1 BNPs for Environmental Remediation

Besides the medical applications of BNPs, researchers have also investigated their application for environmental remediation (Njagi et al. 2011; Hussain et al. 2016). In these studies, microorganisms or BNPs have been shown to clean hazardous waste sites (Elango and Roopan 2015; Kalaiselvi et al. 2015). Current studies show two main ways of cleaning contaminated sites using BNPs, the first one involves adsorption of the pollutant and the other involves degradation or dehalogenation of the contaminant.

Adsorption is a very attractive method to remove contaminants, since is typically highly efficient, minimizes chemical sludge and does not require strong technological knowledge (Aksu 2005). The main drawback of this technique is that it can be mainly used in surface water and groundwater. Removal by adsorption using BNPs were investigated mainly for heavy metals and textile dyes (Dotto et al. 2012a, b). In the case of heavy metals, researchers were able to remove heavy metals using cellulose BNPs and nanoparticles made of microbial biomass.

In the case of bacterial cellulose nanofibers (BCF), researchers have become interested in this BNP because of the unique structure and properties of this bionanomaterial (Carpenter et al. 2015). This BNP possess a nanofibrillar structure,

high mechanical strength, high surface area-to-volume ratio, inherent environmental inertness, and can be easily functionalized by incorporating chemical moieties that will increase the binding efficiency of pollutants. These properties are essential for adsorbents used in the remediation of environmental contaminants (Shah and Brown 2005; Wan et al. 2006).

The more commonly used method to increase adsorption capacity of cellulose is carboxylation. Yu and collaborators investigated the incorporation of succinic acid groups onto cellulose. They demonstrated that this modification significantly increases the binding efficiency of this NP to lead and cadmium (Yu et al. 2013). The removal of these heavy metals was further enhanced by the conversion of the carboxylic acid groups to sodiated carboxylates. Another example of successful carboxylation that led to improved heavy metal removal was demonstrated by Srivastava and collaborators. The incorporation of carboxylic groups into cellulose increased by 3–10 % the removal of Ni^{2+}, Cr^{3+}, Cd^{2+} and Pb^{2+} than unmodified NPs (Srivastava et al. 2012). Besides heavy metals, Ma and collaborators demonstrated that cellulose could remove radioactive uranyl ions (UO_2^{2+}) from an aqueous solution (Ma et al. 2012). The presence of carboxylate groups in the cellulose produced a material able to achieve 2–3 times greater removal of UO_2^{2+} than traditional adsorbents (i.e. silica particles, hydrogels, polymer particles, and montmorillonite). Alternatively, researchers also demonstrated that the incorporation of cysteine, which possess thiol groups, was effective in the removal of Cr(VI) and Pb(II) (Yang et al. 2014).

Studies have also demonstrated that biosorption using non-active (dead) microbial mass, allows the removal of pollutants from aqueous solutions (Gupta and Suhas 2009; Ngah et al. 2011). The advent of nanotechnology and the unique properties of nano-based materials have led researchers to investigate the application of dead microbial biomass conversion to nanoparticles. Dotto's research group has investigated extensively the production of Spirulina platensis bionanoparticles (Dotto et al. 2012a, b; Dotto et al. 2013) from dead microbial biomass for the removal of diverse pollutants, such as textile dyes, heavy metals and phenol. The reason for the good removal of these BNPs was because of the high surface area-to-volume ratio, which led to a faster mass transfer, and a variety of functional groups (carboxyl, hydroxyl, sulfate, phosphate and others) in the nanoparticles that have high affinity for these pollutants.

Besides adsorption, researchers have also investigate the application of BNPs for the catalytic removal of contaminants in water sources, soils and sediments. Bacterial cellulose nanofibers (BCF)-modified with other nanoparticles have been shown to exhibit excellent catalytic hydrogenation performance (Patel and Suresh 2008). For instance, a study demonstrated that it is possible to modify BCF with palladium (Pd) and copper (Cu) to prepare a BCF composite material for the application in catalytic denitrification (Sun et al. 2010). In this study, modified BCF nanocomposite was prepared by immersing BCF in a solution containing $PdCl_2$ and $CuCl_2$, followed by reduction of the absorbed metals into the BCF with potassium borohydride. The Pd-Cu/BCF showed high catalytic activity when used for water denitrification.

In addition to BCF, researches have also extensively investigated the application of BNPs for dehalogenation of organic contaminants. BNPs of Pd have attracted extensive interest for remediation purposes because Pd is one of the most widely applied catalysts in chemistry for dehalogenation, reduction (Mabbett et al. 2004; De Windt et al. 2006), hydrogenation (Creamer et al. 2008; Wood et al. 2010) and C–C bond forming reactions (Sobjerg et al. 2009) under ambient conditions, and currently synthetic production methods of Pd nanoparticles are not very environmentally friendly or sustainable for large scale production. Most research, however, for commercialization of bio-Pb focused on dehalogenation of contaminants in wastewater, groundwater and in soil remediation.

Extensive studies have been done for PCB degradation using bio-Pd-catalyzed dehalogenation (Baxter-Plant et al. 2003; De Windt et al. 2005). These studies showed that PCBs have a fast dehalogenation rate in the presence of bio-Pd (De Windt et al. 2006). Another study, however, pointed out that release of Pd in aquatic environment would be troublesome, hence, the magnetical characteristics of some bio-Pd nanoparticles (Creamer et al. 2011) or the combination of Pd with biogenic magnetite NPs (Coker et al. 2011) have been investigated. This approach would facilitate the recovery of the catalyst after reaction and prevent its release to the environment. The treatment of groundwater contaminated with a mixture of chlorocyclohexanes (HCH) and chlorobenzenes using bio-Pb in a fluidized bed reactor has also been investigated by Hennebel and collaborators (Hennebel et al. 2010). Results showed superior treatment than existing activated carbon filters. In soil, Cr(VI) remediation using bio-Pd produced by C. pasteurianum was shown to be effective (Chidambaram et al. 2010). However, the injection of Pd into soils can be concerning, since leaching of Pd(II) or nanoparticulate Pd(0) into the soil and the groundwater could potentially happen. Hence, strong attachment of Pd to the cells is essential, or alternatively, ex situ treatment should be preferred since Pd can be extracted from the soil after treatment. Although BNPs have been investigated for site remediation, they are still not being commercialized due to potential hazardous effects to the environment. More research needs to be done to investigate their impact to the environment.

5.6.2 Application of BNPs in Water Treatment

Another environmental application of nanoparticles is in water treatment. Some BNPs, such as cellulose nano- and microfibers have been investigated for the fabrication of membranes for water treatment due to the dimensions and strength of this material. Studies have investigated the fabrication of membranes with pristine cellulose, but also the incorporation of this nanomaterial into different polymer matrices. Among the polymers investigated with BCF, there are poly(vinylidene fluoride) (PVDF), poly(ethylene oxide) (PEO), poly(acrylonitrile) (PAN), cellulose triacetate, poly(vinyl alcohol) (PVA), poly(3-hydroxybutyrate) (PHB), poly(ether sulfone) (PES), and polypyrrole (PPy) (Wang et al. 2013; Kong et al. 2014; Lalia

et al. 2014; Carpenter et al. 2015). These different membranes were investigated for nanofiltration, microfiltration, ultrafiltration, membrane distillation, and hemodialysis. Researchers demonstrated that additions of different amounts of biocellulose within the polymer matrices generated membranes with different properties, such as membranes with different tensile strength, surface hydrophilicity, selectivity, permeability and even resistance to biofouling. Typically an improvement on the performance of the membranes was observed with incorporation of small amounts of BCF.

Most of these bacterial cellulose nanofibers (BCF) membranes have also been modified with other nanoparticles to generate hybrid nanocomposite materials (Cho et al. 2005). For instance, BCF membranes produced by *Acetobacter xylinum* have been modified with titanium dioxide (TiO_2) nanoparticles dopped with nitrogen and fluorine to improve catalytic activity of TiO_2 under visible light (Brauer and Szulczewski 2014; Wei et al. 2014). This nanohybrid membrane was able to inactivate both Gram-negative and Gram-positive bacteria under fluorescent light. The authors also demonstrated that the photocatalytic activity of these membranes against microorganisms was dependent on the type of bacteria, and degree of N–F-co-doped TiO_2. Silver nanoparticles are also very attractive nanomaterials for coating BCF membranes. Silver have been used for centuries to treat potable water, due to the antibacterial properties at trace levels of these metals. Silver nanoparticles, however, possess greater surface area than bulk silver, hence it is more bioactive against microorganisms (Mpenyana-Monyatsi et al. 2012). Silver nanoparticles have been used extensively used in water filtration applications to prevent fouling of membranes (Dankovich and Gray 2011; Carpenter et al. 2015).

Organic and biological fouling reduces the water flux during treatment. Self-cleaning membrane mechanisms is very attractive since it can eliminate many cleaning chemicals used for membrane cleaning. Hence, AgNPs have been used extensively as membrane coatings to prevent biofouling of membranes and other types of filters. For instance, an ultrafiltration membrane made of poly (vinylidene fluoride) (PVDF) was modified with AgNPs to prevent both organic and microbial antifouling (Li et al. 2013). The modification of the membrane with AgNPs improved the hydrophilicity of the membrane surface, which led to a reduced contact angle (81–68°) and increased permeate flux (36.4–108.6 L/m^2 h). The organic antifouling and biofouling performance of the membranes were investigated with bovine serum albumin and *E. coli* as model foulants, respectively. The results confirmed the superior antifouling property of the AgNP coated PVDF membranes.

Besides membranes, silver nanoparticles have also been used to coat beads, paper filters and ceramic filters. In the study with AgNPs with coated resin beads, these beads were used to develop a column filtration system for microbial inactivation (Mthombeni et al. 2012). The authors evaluated the performance of the coated resin beads as a function of bed mass, initial bacterial concentration and flow rate using *E. coli* as model contaminant in water. The *E. coli* survival rate were plotted as breakthrough curves (BTCs). The results were modeled using sigmoidal regression equations to obtain relevant rate parameters. The performance of the

column was determined using the capacity of the bed and the number of bed volumes processed at breakthrough point. Results show that performance increases with a decrease in initial bacterial concentration, an increase in flow rate and an increase in bed mass.

Paper filters were also coated with AgNPs to inactivate microorganisms percolating through the filter instead of by sieving mechanisms during filtration. In this study, the AgNPs were deposited on cellulose fibers of a blotting paper sheet. The authors investigated the leaching of silver ions and their antimicrobial capabilities. The results showed that the nanoparticles released about 0.1 ppm of silver ions that could inactivate 6 and 3 logs of *E. coli* and *E. faecalis*, respectively (Dankovich and Gray 2011). This amount of silver ions, even though it was antimicrobial, was below the current USEPA and WHO limits for silver in drinking water. These results show that the presence of AgNPs could be effective against hazardous microorganisms in drinking water.

Other studies related to point-of-use drinking water purification using ceramic porous media with AgNPs were also investigated (Ren and Smith 2013). The authors investigated several methods to incorporate AgNPs in the ceramic filters, such as paint-on, dipping, and fire-in method. The authors investigated a water sample with complex chemistry, which was moderately hard and contained monovalent and divalent inorganic ions. The ceramic porous medium fabricated with Ag-NPs by the paint-on and dipping methods, presented significant release of AgNPs into the water effluent as opposed to the fire-in method (where the AgNPs were added to the ceramic components during the ceramic porous medium fabrication). These results demonstrated that the fire-in method produces a better quality filter with longer antimicrobial properties than the others method.

The application of silver nanoparticles have been extensively investigated and are now commercially available for home-water purification systems. Among the current water treatment systems already available on the market, there are Aquapure and QSI-Nano (Dhandapani et al. 2012).

5.6.3 Renewable Energy Source

Nanomaterials have also been extensively explored in supercapacitors, batteries and fuel cells to assist in sustainable energy generation. For instance, Ni_3S_2 nanoparticles have been successfully incorporated on bacterial cellulose nanofibers (BCN) to be used in a supercapacitor (Yu et al. 2014). Supercapacitors are considered a new-type of energy store device with higher power density than traditional dielectric capacitors and batteries (El-Kady et al. 2012). They can be used as a power back-up for portable electronic devices as well as in electrical vehicles (Miller and Simon 2008; Simon and Gogotsi 2008). The new nanocomposite of Ni_3S_2/BCN presented high specific capacitance and good cycle stability. The authors used in the setup of the supercapacitor a positive electrode with Ni_3S_2/BCN and just BCN as a negative electrode in 2 M KOH electrolyte. This asymmetric

supercapacitor showed excellent cycling stability with 97 % specific capacitance retained after 2500 cycles.

In addition of supercapacitors, researchers have also explored BCNs in batteries. This bionanomaterial has attracted a lot of attention since it can be fabricated on a large scale via a simple and cost effective method (Wan et al. 2015). More importantly, BCN is environmentally friendly, can be abundantly synthesized by diverse bacterial species, and is highly conductive and flexible. These properties make this bionanomaterial suitable for the fabrication of flexible electrodes. In order to improve electrochemical performance researchers have commonly incorporated metal oxides, i.e. SnO_2, MnO_2, Fe_2O_3 and Fe_3O_4 to these BCNs. Among these oxides, Fe_3O_4 has been considered to be one of the most promising electrode materials since it has high theoretical capacity, low processing costs, abundance and environmental friendliness (Wu et al. 2010; Wan et al. 2015). This nanoparticle, however, aggregates very easily, possess poor electronic conductivity and large volume variation, which limit its performance. Alternatively, the construction of hybrid Fe_3O_4/BCNs have shown to solve these issues and produce working electrodes in lithium-ion batteries without metal current collectors, conducting additives, or binders. Another biomaterial investigated for battery applications as ion insertion materials was siliceous material produced by bacteria and diatoms (Joerger et al. 1999). These studies clearly show that different BNPs can be used for applications in batteries.

Another growing research field applying BNPs for energy generation is proton exchange membranes for fuel cells. These membranes have attracted a lot of attention because of their high power density, high energy conversion efficiency, tensile strength, environmental friendliness, and the hydroxyl groups on its backbone which provide high hydrophilicity. These features are crucial for the operation of polymer electrolyte membrane fuel cells (Wang 2004; Yang et al. 2009). The main material used for these membranes are bacterial cellulose, typically from *A. xylinum.* These membranes are typically modified with other nanoparticles, such as palladium and platinum, to increase the electron current and better catalyze the generation of hydrogen oxidation reaction in microbial fuel cells. More importantly, these biomembranes with other nanoparticles have shown higher thermal stability and lower gas crossover when compared to other synthetic polyelectrolyte membranes. These results indicate that renewable bacterial cellulose membranes are promising prospects for membranes used in the fuel cell field.

5.7 Final Conclusions and Remarks

The production of nanoparticles by microorganisms has been showing to be very promising for different applications in biomedical and environmental fields. These bionanoparticles can be used by themselves in these applications or combined with other bionanoparticles or synthetic nanoparticles. Despite the fact that several researchers have investigated the synthesis of these BNPs and their potential

application, it is still essential to understand what would be the health and environmental impacts of these nanoparticles compared to their synthetic counterparts. Several researchers believe that BNPs are more environmentally friendly, which is true for their synthesis, but it is still not clear whether these particles are really safer for Nanotechnological applications than the synthetic ones.

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Erratum to: Extremophiles: Applications in Nanotechnology

Erratum to:
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In the original version of the book, the incorrect corresponding author name "Debora Frigit Rodrigues" has been changed as "Sonia Tiquia-Arashiro" for Chaps. 1–4 and "Debora Rodrigues" is the corresponding author for Chap. 5.

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