Chapter 3
Biological Synthesis of Nanoparticles by Different Groups of Bacteria

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Contents

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3.1 Introduction

Nanoscience is a rapidly developing field that covers a wide range of application in a large variety of areas of science and technology. The Greek prefix "nano" used in nanoscience, nanomaterials, or nanoparticles means "1 billionth," while 1 nanometer (1 nm) is 1/109 m. An accepted definition of nanoscale materials is materials that are in the 1–100 nm size range in at least one dimension. The dimension factor is important because it allows materials such as carbon nanotubes, which are several micrometers long by few nanometers wide, to be included in the definition (Dobias et al. 2011).

The increasing demand for nanomaterials should be accompanied by "green" synthesis methods in an effort to reduce generated hazardous waste from this industry. Green chemistry would help minimize the use of unsafe products and maximize the efficiency of chemical processes (Sharma et al. 2009). An advantage of biogenic synthesis, over conventional chemical synthesis, is the safer and easier handling of microbial cultures and the simpler downstream processing of biomass as compared to synthetic methods (Rai et al. 2011). Hence, biogenic NP synthesis represents a very interesting greener and more environmentally friendly manufacturing alternative, due to the use of chemicals of lower toxicity and to the use of lower ambient temperatures and lower pressures in the synthesis (Dobias et al. 2011; Prasad 2014, Prasad et al. 2016; Abdel-Aziz et al. 2018). Microbial synthesis is one of such processes, a green chemistry approach that interlinks nanotechnology and microbial biotechnology (Li et al. 2011).

In order to overcome the limitations posed by these conventional methods, there has been a growing demand to develop eco-friendly and rapid synthesis of nanomaterials with the desired size and shape. Consequently, researchers have developed biogenic principles to synthesize nanomaterials by using biological resources such as plants and microorganisms or their products (Schröfel et al. 2011; Prasad 2016, 2017, Prasad et al. 2018).

Microbial synthesis of metal nanoparticles can take place either intracellularly or extracellularly (Jain et al. 2011). Intracellular synthesis of nanoparticles requires additional steps such as ultrasound treatment or reactions with suitable detergents to release the synthesized nanoparticles (Kalimuthu et al. 2008). At the same time extracellular biosynthesis is cheap, and it requires simpler downstream processing. This favors large-scale production of silver nanoparticles to explore its potential applications. Because of this, many studies were focused on extracellular methods for the synthesis of metal nanoparticles (Durán et al. 2005; Prasad et al. 2016).

In natural environment also, microbes produce nanomaterials as part of their metabolism and, hence, can be utilized for various applications discussed in this chapter. The microbes reproduce fast; therefore this characteristic can be well exploited for their use in various aspects. Their use in various applications is well known to everyone in the field of biological sciences. Biotechnology has joined hands and has emerged as an initiative for the study of microbes and its various characteristics in the form of "microbiology." Microorganisms are of size 10–6 nm, and they are referred to as nanofactories, meaning generators of nanoparticles. Since they are present in nature, they are also called as biofactories (Deepak et al. 2011).

Biosynthesis of nanoparticles by microorganisms is a green and eco-friendly technology. Diverse microorganisms, both prokaryotes and eukaryotes, are used for synthesis of metallic nanoparticles, viz., silver, gold, platinum, zirconium, palladium, iron, cadmium, and metal oxides such as titanium oxide, zinc oxide, etc. (Hasan 2015). These microorganisms include bacteria, actinomycetes, fungi, and algae. The synthesis of nanoparticles may be intracellular or extracellular according to the location of nanoparticles (Mann 2001; Hulkoti and Taranath 2014; Prasad et al. 2016).

Biosynthesis of metal nanoparticles by bacteria is due to their defense mechanism (resistance mechanism), the resistance caused by the bacterial cell on metal ions in the environment is responsible for its nanoparticle synthesis (Saklani et al. 2012), and the cell wall being negatively charged interacts electrostatically with the positively charged metal ions. The enzymes present within the cell wall bioreduce the metal ions to nanoparticles, and finally the smaller-sized nanoparticles get diffused of through the cell wall, and the nanoparticles are produced (Mukherjee et al. 2001).

3.2 Nanoparticles and Their Applications

Nanotechnology has become one of the most important technologies in allareas of science. It relies on the synthesis and modulation of nanoparticles, which requires significant modifications of the properties of metals (Visweswara Rao and Hua Gan 2015). Nanomaterials have in fact been used unknowingly for thousands of years; for example, gold nanoparticles that were used to stain drinking glasses also cured certain diseases. Scientists have been progressively able to observe the shape- and size-dependent physiochemical properties of nanoparticles by using advanced techniques. Recently, the diverse applications of metal nanoparticles have been explored in biomedical, agricultural, environmental, and physiochemical areas (Visweswara Rao and Hua Gan 2015; Rai et al. 2016; Abbasi et al. 2016; Giljohann et al. 2010; Pereira et al. 2015; Prasad et al. 2014, 2017). For instance, gold nanoparticles have been applied for the specific delivery of drugs, such as paclitaxel, methotrexate, and doxorubicin (Rai et al. 2016). Gold nanoparticles have been also used for tumor detection, angiogenesis, genetic disease and genetic disorder diagnosis, photo imaging, and photo thermal therapy. Iron oxide nanoparticles have been applied for cancer therapy, hyperthermia, drug delivery, tissue repair, cell labeling, targeting and immunoassays, detoxification of biological fluids, magnetic resonance imaging, and magnetically responsive drug delivery therapy (Khlebtsov and Dykman 2011; Huang et al. 2007; Iv et al. 2015). Silver nanoparticles have been used for many antimicrobial purposes, as well as in anticancer, anti-inflammatory, and wound treatment applications (Ahamed et al. 2010). Due to their biocompatible, nontoxic, self-cleansing, skin-compatible, antimicrobial, and dermatological behaviors, zinc and titanium nanoparticles have been used in biomedical, cosmetic, ultraviolet (UV)-blocking agents, and various cutting-edge processing applications (Ambika and Sundrarajan 2015;

Zahir et al. 2015; Bhuyan et al. 2015). Copper and palladium nanoparticles have been applied in batteries, polymers, plastic plasmonic wave guides, and optical limiting devices (Momeni and Nabipour 2015; Nasrollahzadeh and Sajadi 2015). Moreover, they were found to be antimicrobial in nature against many pathogenic microorganisms. Additionally, metal nanoparticles have been used in the spatial analysis of various biomolecules, including several metabolites, peptides, nucleic acids, lipids, fatty acids, glycosphingolipids, and drug molecules, to visualize these molecules with higher sensitivity and spatial resolution (Waki et al. 2015). In addition, the unique properties of nanoparticles make them well suited for designing electrochemical sensors and biosensors (Peng and Miller 2011). For example, nanosensors have been developed for the detection of algal toxins, mycobacteria, and mercury present in drinking water (Selid et al. 2009). Researchers also developed nanosensors by utilizing nanomaterials for hormonal regulation and for detecting crop pests, viruses, soil nutrient levels, and stress factors. For instance, nanosensors for sensing auxin and oxygen distribution have been developed (Koren et al. 2015).

3.3 Factors Affecting Synthesis of Nanoparticles

Shape and size of nanoparticles depend on the physical and chemical factors. The optimum metal ion concentration, pH, and temperature of reaction mixture play key role in nanoparticle synthesis.

3.3.1 Concentration of Metal Ion

Increasing the concentration of silver ions 1–5 mM in reaction mixture revealed that in 1 mM concentration, the nanoparticle synthesis and size reduction started quickly due to more availability of functional groups in the extract. While increasing the substrate concentration, the large size and aggregation of nanoparticles occurred due to the occurrence of silver ions and functional group (Vanaja et al. 2013).

3.3.2 pH

pH plays an important role in the nanoparticle synthesis of extract with silver ions. Alkaline pH 8.2 showed a sharp peak at 460 nm with maximum production of silver nanoparticles. The sharp peak indicated formation of spherical shape of silver nanoparticles, thus indicating alkaline pH is more suitable for synthesis of nanoparticles. pH plays a role in shape and size control in nanoparticle synthesis. Another report suggests increase in absorption was seen with a decrease in pH and also indicated the production of bigger particles with decrease in pH (Prakash and Soni 2011).

3.3.3 Temperature

Temperature is one of the important physical parameters for synthesis of nanoparticles. Synthesis of nanoparticles while increasing the reaction temperature. The higher rate of reduction occurs at higher temperature due to the consumption of metal ions in the formation of nuclei, whereas the secondary reduction stops on the surface preformed nuclei. The broadening peak obtained at low temperature shows the formation of large-sized nanoparticles, and the narrow peak obtained at high temperature indicates the nanoparticles synthesized are smaller in size. It can be stabilized that higher temperature is optimum for nanoparticle synthesis (Vanaja et al. 2013).

3.3.4 Time

In a study, synthesis of nanoparticles at various time intervals was studied after reaction for 1 h, the AgNPs obtained showed a UV-visible spectroscopy absorption peak, and the intensity of the peak increases as the reaction time is increased, which indicated the continued reduction of the silver ions. The increase of the absorbance with the reaction time indicates that the concentration of AgNPs increases. When the reaction time reached 3 hours, the absorbance increased, and the wavelength value was slightly shifted. This phenomenon continued for reaction times of 6–24 h, indicating that the size of particles was decreased. At the end of the reaction, i.e., 48 h, the absorbance was considerably increased, and there was no significant change in wavelength (430 nm), compared with the 24 h reaction time. The transmission electron microscopy (TEM) results indicate that the samples obtained over a longer time period retained a narrower particle size distribution; the average size of all prepared AgNPs was 20 nm (Darroudi et al. 2011).

3.4 Common Methodologies for Synthesis of Metal Nanoparticles Using Microbes

3.4.1 Extracellular Mechanism

The test strain (culture) is grown in suitable media and incubated on orbital shaker at 150 rpm at 37 °C. After incubation the broth is centrifuged, and the supernatant is used for synthesis of nanoparticles. The supernatant is added to separate reaction vessels containing the metal ions in suitable concentrations and incubated for a period of 72 h. The color change of the reaction mixture suggests the presence of nanoparticles in the solution, and bioreduction of silver ions in the solution is monitored by sampling the aqueous solution and measuring the absorption spectrum using a UV-visible spectrophotometer. The morphology and uniformity of silver

nanoparticles are investigated by X-ray diffraction (XRD) and scanning electron microscopy (SEM), while the interaction between protein and AgNPs is analyzed using Fourier transform infrared spectroscopy (FTIR) (Jeevan et al. 2012).

3.4.2 Intracellular Mechanism

The culture is grown in suitable liquid media incubated on shaker at optimal temperature. After incubation the flask is kept at static condition to allow the biomass to settle following which the supernatant is discarded and sterile distilled water is added for washing the cells. The flask iskept steady for 30 min to settle the biomass post which the supernatant is again discarded. This step is repeated for three times. The biomass is then separated from the sterile distilled water by centrifugation for 10 min. The wet biomass is exposed to 50 ml of sterilized aqueous solution of metals at various dilutions and incubated on shaker at suitable temperature till visual color change is observed. The change in color from pale yellow to brownish color indicates the formation of silver nanoparticles, pale yellow to pinkish color indicates the formation of gold nanoparticles, and the formation of whitish yellow to yellow color indicates the formation of manganese and zinc nanoparticles (Waghmare et al. 2011).

In summary, the extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing them in the presence of enzymes, while in intracellular synthesis ions are transported into the microbial cell to form nanoparticles in the presence of enzymes (Kalabegishvili et al. 2012). The biosynthesized nanoparticles have been used in a variety of applications including drug carriers for targeted delivery, cancer treatment, gene therapy and DNA analysis, antibacterial agents, biosensors, separation science, and magnetic resonance imaging (Li et al. 2011).

3.5 Nanoparticle Synthesis by Bacteria

The most important challenge in nanotechnology today is to cost-effectively tailor the optical, electric, and electronic property of NPs by controlling the configuration as well as monodispersity. This goal could be achieved using bacterial organisms in an organized manner (Gericke and Pinches 2006). In the last few years, fabrication of AgNPs has increased extensively owing to its immense applications (Morones et al. 2005). Bacteria possess remarkable ability to reduce heavy metal ions and are one of the best candidates for nanoparticle synthesis. For instance, some bacterial species have developed the ability to resort to specify defense mechanisms to quell stresses like toxicity of heavy metal ions or metals. It was observed that some of them could survive and grow even at high metal ion concentrations (e.g., *Pseudomonas stutzeri* and *P. aeruginosa*) (Bridges et al. 1979; Haefeli et al. 1984).

Among the milieu of natural resources, prokaryotic bacteria have been most extensively researched for synthesis of metallic nanoparticles. One of the reasons for "bacterial preference" for nanoparticle synthesis is their relative ease of manipulation (Slawson et al. 1992).

In nature, bacteria are frequently exposed to diverse and sometimes extreme environmental situations. Survival in these harsh conditions ultimately depends on their ability to resist the effects of environmental stresses. Natural defense mechanisms exist in bacteria to deal with a variety of stresses such as toxicity arising from high concentrations of metallic ions in the environment. The major bacterial species used for the synthesis of metallic nanoparticles include *Acinetobacter* sp., *Escherichia coli*, *Klebsiella pneumoniae*, *Lactobacillus* spp., *Bacillus cereus*, *Corynebacterium* sp., and *Pseudomonas* sp. (Mohanpuria et al. 2008; Iravani 2014; NVKV Prasad et al. 2011). Bacteria are known to synthesize metallic nanoparticles by either intracellular or extracellular mechanisms.

The first synthesis of Ag nanoparticles by bacteria was reported in 2000. Joerger et al. (2000) used *Pseudomonas stutzeri* AG259 to synthesize Ag nanoparticles with size less than 200 nm. Bacteria were grown on Lennox L (LB) agar substrate, containing 50 mmol/L $AgNO_3$, at 30 °C for 48 h in the dark (Lengke et al. 2006). In 2008, biosynthesis of silver nanocrystals by *B. licheniformis* was studied. Aqueous silver ions were reduced to silver nanoparticles when added to the biomass of *B. licheniformis*. This was indicated by the change in color from whitish yellow to brown. The probable mechanism for the formation of silver nanoparticles involves the enzyme nitrate reductase (Kalimuthu et al. 2008).

In 2008, silver nanoparticles in the range of 50 nm were synthesized by the supernatant of *B. licheniformis* when silver nitrate was added to it. The synthesized silver nanoparticles were highly stable. Also, the time required for reaction completion was 24 h (Kalimuthu et al. 2008). Biosynthesis of silver nanoparticles using microorganisms is rather slow. However, finding microorganisms to synthesize Ag nanoparticles is an important aspect. Shahverdi et al. (2007b) reported on the rapid synthesis of metallic nanoparticles of silver using the reduction of aqueous Ag^+ ion using the culture supernatants of *Klebsiella pneumoniae*, *Escherichia coli* (Lee 1996), and *Enterobacter cloacae* (*Enterobacteriaceae*). The synthetic process was quite fast, and silver nanoparticles were formed within 5 min of the silver ion coming into contact with the cell filtrate (Shahverdi et al. 2007a). However, the culture supernatants of different bacteria from *Enterobacteriaceae* are potential candidates for the rapid synthesis of silver nanoparticles. In 2009, investigated was the effect of different visible-light irradiation on the formation of silver nanoparticles from silver nitrate using the culture supernatant of *Klebsiella pneumoniae*. In addition, the study experimentally investigated the liquid mixing process effect on silver nanoparticle synthesis by visible-light irradiation. That study successfully synthesized evenly dispersed silver nanoparticles of uniform size and shape in the range of 1–6 nm and average size of 3 nm (Mokhtari et al. 2009). Another report focused on the synthesis of metallic bio-nanoparticles of silver using a reduction of aqueous Ag^+ ion with the culture supernatants of *Staphylococcus aureus*. The observation indicated that the reduction of the Ag^+ ions took place extracellularly. Also, the reaction

between this supernatant and Ag^+ ions was carried out in bright conditions for 5 min (Nanda and Saravanan 2009).

Moreover, Brock and Gustafson (1976) reported that *Thiobacillus ferrooxidans*, *T. thiooxidans*, and *Sulfolobus acidocaldarius* were able to reduce ferric ion to the ferrous state when growing on elemental sulfur as an energy source. *T. thiooxidans* was able to reduce ferric iron at low pH medium aerobically. The ferrous iron formed was stable to autoxidation and *T. thiooxidans* was unable to oxidize ferrous iron, but the bioreduction of ferric iron using *T. ferrooxidans* was not aerobic because of the rapid bacterial reoxidation of the ferrous iron in the presence of oxygen (Brock and Gustafson 1976). Other biomineralization phenomena, such as the formation of tellurium (Te) in *Escherichia coli* K12 (Taylor 1999), the direct enzymatic reduction of Tc (VII) by resting cells of *Shewanella* (previously *Alteromonas*) *putrefaciens* and *Geobacter metallireducens* (previously known as strain GS-15) (Lloyd et al. 1999), and the reduction of selenite to selenium by *Enterobacter cloacae*, *Desulfovibrio desulfuricans*, and *Rhodospirillum rubrum* (Kessi et al. 1999), have been reported, as well. Mullen et al. (1989) examined the ability of *Bacillus cereus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* for removing Ag^+, Cd^{2+}, Cu^{2+}, and La^{3+} from solution. They found that bacterial cells were capable of binding large quantities of metallic cations. Moreover, some of these bacteria are able to synthesize inorganic materials like the magnetotactic bacteria, which synthesize intracellular magnetite NPs (Lovley et al. 1987).

Pseudomonas stutzeri AG259 has been reported to fabricate Ag particles (Joerger et al. 2000), which are accumulated within the periplasmic space of bacterial cell of 200 nm. *Lactobacillus*, a common bacterial strain present in the buttermilk, synthesizes both Au and Ag NPs under standard conditions (Nair and Pradeep 2002). Rapid synthesis of metallic NPs of Ag using the reduction of aqueous Ag^+ has been achieved in the cultural supernatants of *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae* (Shahverdi et al. 2007b). Recently detailed studies confirmed that synthesis of Ag can be triggered through the liquid mixing process developed in the visible-light spectrum by *Klebsiella pneumoniae* (Mokhtari et al. 2009). Extracellular biosynthesis of 40 nm Ag NPs by the culture supernatant of *Bacillus licheniformis* has been customized as an easy way to work out the process (Kalimuthu et al. 2008). Varshney et al. (2011) have reported a rapid biological synthesis technique for the synthesis of spherical Cu nanoparticles in the size range of 8–15 nm using nonpathogenic *Pseudomonas stutzeri*. Recently, an innovative approach has been used for the synthesis of copper nanoparticles where *P. stutzeri* bacterial strain was used for copper nanoparticle synthesis from electroplating wastewater. The bacterial strain was isolated from soil and found that it produced 50–150 nm-sized cubical copper nanoparticles (Varshney et al. 2011). Prakash et al. (2011) reported extracellular synthesis of silver nanoparticles by bacteria *Bacillus cereus* collected from the riverine belt of Gangetic Plain of India. Synthesized nanoparticles were spherical in shape and in the range of 10–30 nm in size. Antibacterial effect of the synthesized AgNPs was tested with gram-negative and gram-positive bacteria *E. coli* and *Streptococcus* in varying strength of nanoparticles, and it was observed that the lowest concentration up to 50 ppm was sufficient

to inhibit bacterial growth. *Bacillus* species has depicted to synthesize metal nanoparticles; researchers showed the ability of bacteria to decrease silver and fabrication of extracellularly, consistently circulated nanoparticles, ranging from 10 to 20 nm in size (Sunkar and Nachiyar 2012). The silver-producing bacteria isolated from the silver mines exhibit the silver nanoparticles accumulated in the periplasmic space of *Pseudomonas stutzeri* AG259 (Slawson et al. 1994). Bacteria are also used to synthesize gold nanoparticles. Sharma et al. (2012) reported that whole cells of a novel strain of *Marinobacter pelagius are* applicable for stable, monodisperse gold nanoparticle formation. Prasad et al. (2007) had reported the use of *Lactobacillus* strains to synthesize the titanium nanoparticles. The understanding of natural processes will apparently help in the discovery of entirely new and unexplored methodology of metal nanoparticle synthesis.

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 and group 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). Klaus et al. (1999) 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. 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, we have investigated the molecular basis of biochemical synthesis of AgNPs from *Morganella* sp. RP-42, an insect midgut isolate (Parikh et al. 2008). We 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 (silE, silP, and silS) were identified in silver-resistant *Morganella* sp. The homologue of silE from *Morganella* sp. showed 99% nucleotide sequence similarity with the previously reported gene, silE, 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 an elegant study by Nair and Pradeep (2002), common *Lactobacillus strains* present in buttermilk were exposed 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. 2006). 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 to 100 °C (Lengke et al. 2006). Among the first reports of intracellular semiconductor nanoparticle synthesis, Sweeney et al. (2004) demonstrated that *E. coli*, when incubated with cadmium chloride ($CdCl_2$) and sodium sulfide (Na_2S), spontaneously formed cadmium sulfide

(CdS) semiconductor nanocrystals. They showed that the formation of nanocrystals was markedly affected by physiologic parameters. Indeed, the entry into stationary phase increased the yield by 20-fold (Sweeney et al. 2004). In line with these observations, Cunningham and Lundie (1993) found that *Clostridium thermoaceticum* precipitates CdS at the cell surface as well as in the medium when exposed to $CdCl_2$ in the presence of cysteine hydrochloride as a source of sulfide in the growth medium. In a separate report of bacterial synthesis of nanoparticles, Watson et al. (1999) demonstrated that sulfate-reducing bacteria synthesize strongly magnetic iron sulfide (FeS) nanoparticles on their surfaces. The magnetic nanoparticles (about 20 nm in size) were separated from the solution by a high gradient field of 1 Tesla. Of note, bacterially produced FeS is an adsorbent for a wide range of heavy metals and some anions. Furthermore, magnetite is a common product of bacterial iron reduction and could be a potential physical indicator of biological activity in geological settings (Watson et al. 1999). Interestingly, Bharde et al. (2005) have demonstrated magnetite nanoparticle synthesis by Acinetobacter, a nonmagnetotactic bacteria. In prior studies, biosynthesis of magnetite was found to be extremely slow (often requiring 1 week) under strictly anaerobic conditions. However, this study reported that *Acinetobacter* spp. were capable of magnetite synthesis by reaction with suitable aqueous iron precursors under fully aerobic conditions (Bharde et al. 2005). Importantly, the extracellular magnetite nanoparticles showed excellent magnetic properties (Bharde et al. 2005). Bacteria have also been used to synthesize gold nanoparticles. For example, microbial synthesis of gold nanoparticles was achieved by Konishi et al. (2004) 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. 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. (2007) incubated *R. capsulata* biomass and aqueous chloroauric acid (HAuCl4) solution at pH values ranging from 7 to 4. 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. In both 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) (Du et al. 2007). Ahmad et al. (2003c) have performed a series of studies on bacterial synthesis of gold nanoparticles. In one such study, they used extremophilic actinomycetes like *Thermomonospora* sp. to efficiently synthesize monodisperse gold nanoparticles. By comparing this with their earlier work on gold nanoparticle synthesis from a fungus, *Fusarium oxysporum*, they postulated that reduction of metal ions and stabilization of the gold nanoparticles occur 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, c). In a separate study, these authors used alkalotolerant *Rhodococcus* sp. (Ahmad et al. 2003b) for intracellular synthesis of good-quality monodisperse gold nanoparticles. Notably, the metal ions were not toxic to the cells as evidenced by the continued growth of the cells even after the biosynthesis of gold nanoparticles.

Bacteria among microorganisms and prokaryotes have received the most attention in the area of AuNPs synthesis (Whiteley et al. 2011). For the first time microbial synthesis of AuNPs was reported in *Bacillus subtilis* 168 which revealed the presence of 5–25 nm octahedral NPs inside the cell wall (Beveridge and Murray 1980). In *Rhodopseudomonas capsulata*, spherical AuNPs with 10–20 nm range have been observed (He et al. 2007) at lower concentration and nanowires with network at higher concentration (He et al. 2008). Six cyanobacteria have been reported for production of AuNPs. *Plectonema* sp. (Lengke et al. 2006; Brayner et al. 2007), *Anabaena* sp., *Calothrix* sp., and *Leptolyngbya* sp. have been exploited for the AuNPs synthesis (Lengke et al. 2007). Single-cell protein of *Spirulina platensis* was also shown to produce AuNPs and Au core-Ag shell NPs (Govindaraju et al. 2008). An overview on bacterial synthesis of AuNPs is given in Table 3.1. If one tries to group the AuNPs-producing bacteria according to the 9th edition of *Bergey's Manual of Systematic Bacteriology* (2005), the members belonging to groups glidobacteria and *Beta-*, *Epsilon-*, and *Zetaproteobacteria* have not been reported so far (Shedbalkar et al. 2014).

For example, Ag nanoparticles have been synthesized using *Pseudomonas stutzeri* AG259 bacterium via a mechanism involving the NADH-dependent reductase enzyme that donates an electron and oxidizes to NAD. The electron transfer results in the biological reduction of Ag ions to Ag nanoparticles (Ahmad et al. 2003a). In a similar study, Husseiny et al. (2007) were able to reduce Au ions using *Pseudomonas aeruginosa* that resulted in the extracellular synthesis of Au nanoparticles. However, some other researchers have also shown the noninvolvement of biological enzymes. For example, Liu et al. (2009) were able to produce Au nanoparticles from dried cells of *Bacillus megaterium*. A similar study by Sneha et al. (2010) using a *Corynebacterium* sp. also revealed that a nonenzymatic reduction mechanism was involved in nanoparticle formation. The reduction of nanoparticles is believed to be the result of a combination of several factors. The first factor is the presence of some organic functional groups at the cell wall that induce reduction, and the second depends on the appropriate environmental parameters such as pH and temperature being present (Lin et al. 2001). For example, the dried biomass of *Lactobacillus* sp. A09 and *Bacillus megaterium* D01 can reduce Ag ions via the interaction of functional groups present on the cell wall to produce silver nanoparticles (Jin-Zhou et al. 2000). Size, shape, and composition of a nanoparticle can be significantly influenced by pH and temperature (Hulkoti and Taranath 2014). For example, particle size is an important factor since novel and unique physicochemical properties are more pronounced at smaller sizes. Therefore, there is a need to optimize synthesis parameters during nanoparticle formation to enhance the overall particle properties. In particular, selecting the appropriate culture media for a specific

Table 3.1 The major bacterial species that have been used to synthesize a variety of nanoparticles along with extracellular or intracellular methods

Bacteria	Nanoparticles	Synthesis method	References
Bacillus flexus	Ag	Extracellular	Priyadarshini et al. (2013)
Lactobacillus spp.	Ag	Extracellular	Ranganath et al. (2012)
Klebsiella pneumoniae	Se	Intracellular	Fesharaki et al. (2010)
Streptomyces sp.	Mn and Zn	Intracellular	Waghmare et al. (2011)
Streptomyces sp.	Ag	Extracellular	Chauhan et al. (2013)
Pseudomonas aeruginosa	Ag	Extracellular	Shivakrishna et al. (2013)
Thermomonospora sp.	Gold	Extracellular	Ahmad et al. (2003c)
Rhodococcus sp.	Gold	Intracellular	Ahmad et al. (2003b)
Pseudomonas aeruginosa	Gold	Extracellular	Husseiny et al. (2007)
Pseudomonas fluorescens	Cu	Extracellular	Shantkriti and Rani (2014)
Bacillus subtilis	Au	Intracellular	Castro et al. (2014)
Pseudomonas stutzeri	Ag triangles and hexagons	Intracellular	Castro et al. (2014)
Lactobacillus sp.	Au, Ag, Au-Ag alloys	Intracellular	Castro et al. (2014)
Desulfovibrio desulfuricans	Pd	Intracellular	Castro et al. (2014)
S. oneidensis	Pd	Intracellular	Castro et al. (2014)
Rhodopseudomonas capsulata	Au	Extracellular	Castro et al. (2014)
Cupriavidus necator	Pd	Intracellular	Castro et al. (2014)
Pseudomonas putida	Pd	Intracellular	Castro et al. (2014)
Paracoccus denitrificans	Pd	Intracellular	Castro et al. (2014)
Pseudomonas aeruginosa	Au, Ag, Pd, Fe, Rh, Ni, Ru, Pt	Extracellular	Castro et al. (2014)
Pyrobaculum islandicum	Au	Extracellular	Castro et al. (2014)
G. sulfurreducens	Au	Extracellular	Castro et al. (2014)
Pyrococcus furiosus	Au	Extracellular	Castro et al. (2014)
Morganella sp.	Ag	Intracellular	Castro et al. (2014)
Bacillus licheniformis	Ag	Intracellular	Castro et al. (2014)
Pseudomonas deceptionensis	Ag	Extracellular	Singh et al. (2016)
Weissella oryzae	Ag	Intracellular	Singh et al. (2016)
Bacillus methylotrophicus	Ag	Extracellular	Singh et al. (2016)
Brevibacterium frigoritolerans	Ag	Extracellular	Singh et al. (2016)
Bhargavaea indica	Ag and Au	Extracellular	Singh et al. (2016)
Bacillus amyloliquefaciens	CdS	Extracellular	Singh et al. (2016)
Bacillus pumilus	Ag	Extracellular	Singh et al. (2016)
Bacillus persicus	Ag	Extracellular	Singh et al. (2016)
Bacillus licheniformis	Ag	Extracellular	Singh et al. (2016)
Lysinibacillus sphaericus	Ag	–	Gou et al. (2015)
Lactobacillus mindensis	Ag_2O	–	Dhoondia and Chakraborty (2012)

(continued)

Table 3.1 (continued)

Bacteria	Nanoparticles	Synthesis method	References
L. acidophilus	Ag	Extracellular	Mohseniazar et al. (2011)
L. casei	Ag	Extracellular	Mohseniazar et al. (2011)
L. reuteri	Ag	Extracellular	Mohseniazar et al. (2011)
Escherichia coli	Cd	Intracellular	Shah et al. (2015)
Pseudomonas aeruginosa	Au	Extracellular	Shah et al. (2015)
Pseudomonas stutzeri	Ag	Intracellular	Shah et al. (2015)
Streptomyces sp.	Ag	Extracellular	Zarina and Nanda (2014)
Bacillus cereus	Ag	Extracellular	Prakash et al. (2011)
Escherichia coli	Ag	–	Kushwaha et al. (2015)
Escherichia coli	Cu	Extracellular	Shobha et al. (2014)
Mycobacterium psychrotolerans	Cu	Extracellular	Shobha et al. (2014)
M. morganii	Cu	Extracellular	Shobha et al. (2014)
Pseudomonas sp.	Cu	Extracellular	Shobha et al. (2014)
Pseudomonas stutzeri	Cu	Extracellular	Shobha et al. (2014)
Streptomyces sp.	Cu	Extracellular	Shobha et al. (2014)
Planomicrobium sp.	TiO_2	Extracellular	Malarkodi et al. (2013)
Bacillus subtilis	Au	Intracellular	Southam and Beveridge (1996)
Bacillus subtilis	Co_3O_4	Extracellular	Shim et al. (2010)
Bacillus subtilis	TiO_2	–	Kirthi et al. (2011)
Bacillus licheniformis	Au	–	Kalishwaralal et al. (2009)
Bacillus licheniformis	Ag	Extracellular	Kalishwaralal et al. (2009)
Escherichia coli	CdS	Intracellular	Sweeney et al. (2004)
Escherichia coli	CdTe	Extracellular	Bao et al. (2010)
Escherichia coli	Au	Intracellular	Du et al. (2007)
Escherichia coli	Pt	–	Attard et al. (2012)
Acinetobacter sp.	Fe_3O_4	Extracellular	Bharde et al. (2005)
Acinetobacter sp.	Si/SiO_2	Extracellular	Singh et al. (2008)
Magnetospirillum gryphiswaldense	Fe_3O_4/Fe_3S_4	Intracellular	Schübbe et al. (2003)
Geobacter sulfurreducens	Pd	Extracellular	Yates et al. (2013)
Klebsiella pneumoniae	Ag	Extracellular	Mokhtari et al. (2009)
Klebsiella pneumoniae	Se	Intracellular	Fesharaki et al. (2010)
Lactobacillus sp.	TiO_2	Extracellular	Jha et al. (2009)
Morganella psychrotolerans	Ag	Extracellular	Ramanathan et al. (2010)
Clostridium thermoaceticum	CdS	Extracellular	Cunningham and Lundie (1993)
Desulfobacteraceae spp.	ZnS	–	Labrenz et al. (2000)
Shewanella oneidensis	UO_2	Extracellular	Marshall et al. (2006)
Shewanella algae	Au	Intracellular	Konishi et al. (2006)
Shewanella algae	Pt	–	Konishi et al. (2007)
Pseudomonas aeruginosa	Au	Extracellular	Husseiny et al. (2007)

(continued)

Table 3.1 (continued)

Bacteria	Nanoparticles	Synthesis method	References
Rhodopseudomonas capsulata	Au	Extracellular	He et al. (2008)
Rhodopseudomonas palustris	CdS	Intracellular	Bai et al. (2009)
Bacillus licheniformis	Ag	Extracellular	Kalimuthu et al. (2008)
Bacillus licheniformis	Ag	Extracellular	Kalishwaralal et al. (2009)
Klebsiella pneumoniae	Ag	–	Shahverdi et al. (2007b)
Escherichia coli	Ag	–	Shahverdi et al. (2007b)
Enterobacter cloacae	Ag	–	Shahverdi et al. (2007b)
Pseudomonas stutzeri	Ag	–	Joerger et al. (2000)
Klebsiella pneumoniae	Ag	–	Mokhtari et al. (2009)
Staphylococcus aureus	Ag	Extracellular	Nanda and Saravanan (2009)
Thermoanaerobacter sp.	Cu	Extracellular	Jang et al. (2015)
Thiobacillus thioparus	Fe_2O_3	–	Elcey et al. (2014)
Escherichia coli	CdS	Intracellular	Sweeney et al. (2004)
Azoarcus sp.	Se	Intracellular	Fernández-Llamosas et al. (2016)
Geobacillus sp.	Au	Intracellular	Correa-Llantén et al. (2013)
Marinobacter pelagius	Au	–	Sharma et al. (2012)
Myxococcus virescens	Ag	Extracellular	Wrótniak-Drzewiecka et al. (2014)
Streptomyces spp.	Ag	Extracellular	Tsibakhashvil et al. (2010)
Bacillus subtilis	Ag	Extracellular	Saifuddin et al. (2009)
Thermomonospora sp.	Au	Extracellular	Ahmad et al. (2003c)
Escherichia coli	Ag	Extracellular	El-Shanshoury et al. (2011)
Bacillus subtilis	Ag	Extracellular	El-Shanshoury et al. (2011)
Arthrobacter sp.	Au	Extracellular	Kalabegishvili et al. (2012)
Arthrobacter globiformis	Au	Extracellular	Kalabegishvili et al. (2012)
Rhodococcus sp.	Au	Intracellular	Ahmad et al. (2003b)
Bacillus sp.	Ag	Extracellular	Das et al. (2014)
Bacillus megaterium	Ag	Extracellular	Saravanan et al. (2011)
Pantoea agglomerans	Se	Intracellular	Torres et al. (2012)
Bacillus licheniformis	Ag	Extracellular	Shivaji et al. (2011)
Proteus mirabilis	Ag	Intra-/ extracellular	Samadi et al. (2009)
Pseudomonas fluorescens	Au	Extracellular	Syed et al. (2016)
Pseudomonas stutzeri	Ag	Intracellular	Klaus et al. (1999)
Morganella sp.	Ag	Extracellular	Parikh et al. (2008)

(continued)

Table 3.1 (continued)

Bacteria	Nanoparticles	Synthesis method	References
Lactobacillus strains	Ag and Au	Intracellular	Nair and Pradeep (2002)
Plectonema boryanum	Ag	Intracellular	Lengke et al. (2007)
Escherichia coli	CdS	Intracellular	Sweeney et al. (2004)
Clostridium thermoaceticum	CdS	Intra-/ extracellular	Cunningham and Lundie (1993)
Acinetobacter spp.	Fe_2O_3	Extracellular	Bharde et al. (2005)
Shewanella algae	Au	Intra-/ extracellular	Thakkar et al. (2010)
Rhodopseudomonas capsulata	Au	Extracellular	He et al. 2007
Escherichia coli	Au	Intracellular	Du et al. (2007)
Thermomonospora sp.	Au	Extracellular	Ahmad et al. (2003c)
Rhodococcus sp.	Au	Intracellular	Ahmad et al. (2003b)
Klebsiella pneumoniae	Ag	Extracellular	Shahverdi et al. (2007b)
Pseudomonas aeruginosa	Au	Extracellular	Husseiny et al. (2007)
Shewanella oneidensis	U (IV)	Extracellular	Marshall et al. (2006)
Lactobacillus acidophilus	Se	–	Visha et al. (2015)
Pseudomonas alcaliphila	Se	–	Zhang et al. (2011)
Morganella sp.	Metallic Cu	Extracellular	Saif Hasan et al. (2008)
Pseudomonas sp.	Ag	Extracellular	Yadav et al. (2015)
Ochrobactrum sp.	Ag	Extracellular	Thomas et al. (2014)

bacteria and the particular metallic salt is important since these two parameters form the basis of nanoparticle synthesis and can influence particle yield (Roh et al. 2001; Nair and Pradeep 2002; Yong et al. 2002). Studies by He et al. (2008) using bacterium *Rhodopseudomonas capsulata* have shown that particle size and morphology can be influenced by both metallic salt concentration and medium pH. At pH 6, dilute concentrations of $AuCl_4$ tended to produce spherical Au nanoparticles ranging in size from 10 to 20 nm. Upon increasing the salt concentration, this reaction tended to produce Au nanowires at pH 6 (He et al. 2008). Also, when the pH was changed to 4, dilute salt concentrations tended to produce both spheres and triangular nanometer scale plates (Husseiny et al. 2007). The studies clearly indicated that controlling medium pH directly influenced nanoparticle morphology during formation. Table 3.1 summarizes the major bacterial species that have been used to synthesize a variety of nanoparticles along with extracellular or intracellular methods.

3.6 Conclusion

Bio-based approaches are still in the development stages, and stability and aggregation of the biosynthesized NPs, control of crystal growth, shape, size, and size distribution are the most important experienced problems. Furthermore, biologically

synthesized NPs in comparison with chemically synthesized ones are more polydisperse. 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).Mechanistic aspects have not been clearly and deeply described and discussed. Thus, more elaborated studies are needed to know the exact mechanisms of reaction and identify the enzymes and proteins which involve nanoparticle biosynthesis. The large-scale synthesis of NPs using bacteria is interesting because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes. It seems that by optimizing their action conditions and selecting the best bacteria, these natural nanofactories can be used in the synthesis of stable NPs with well-defined sizes, morphologies, and compositions.

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