

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 Δ pH, 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

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 cyclomalto-dextrin 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 alkaliphilic 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 Fe₃O₄ nanoparticles formed by *A. ferrooxidans* as a bioactive substance delivery carrier. The Fe₃O₄ 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 Fe₃O₄ nanoparticles showed no cytotoxic, genotoxic and hemolytic effects up to 4.0 mg/ml indicating good biocompatibility of these biological nanoparticles. The Fe₃O₄ 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		
<i>Acidithiobacillus ferrooxidans</i>	Fe ₃ O ₄	Yan et al. (2012)
<i>Lactobacillus acidophilus</i>	Ag	Rajesh et al. (2015)
<i>Lactobacillus acidophilus 01</i>	Ag	Namasivayam et al. (2010)
<i>Lactobacillus acidophilus</i>	Se	Rajasree and Gayathri (2015)
<i>Lactobacillus acidophilus</i> DSMZ 20079T	CdS	El-Raheem et al. (2012)
<i>Pilimelia columelifera</i> subsp. <i>pallida</i> SL19	Ag	Golinska et al. (2015)
<i>Pilimelia columelifera</i> subsp. <i>pallida</i> SL24	Ag	Golinska et al. (2015)
<i>Thiobacillus thioeparus</i>	Fe ₃ O ₄	Elcey et al. (2014)
<i>Actinobacteria</i> C9	Ag	Anasane et al. (2016)
<i>Actinobacteria</i> SF23	Ag	Anasane et al. (2016)
<i>Streptacidiphilus</i> sp. strain CGG11n	Ag	Railean-Plugaru et al. (2016)
<i>Arthrobacter nitroguajacolicus</i>	Au	Dehnad et al. (2015)
Archaea		
<i>Sulfolobus islandicus</i> *	Au	Kalabegishvili et al. (2014)
<i>Sulfolobus islandicus</i> *	Ag	Kalabegishvili et al. (2015)
<i>Sulfolobus acidocaldarius</i> *	Ag	Bartolome et al. (2012), Selenska-Pobell et al. (2011)
Fungi		
<i>Verticillium luteoalbum</i>	Au	Mukherjee et al. (2001a)
<i>Verticillium</i> sp.	Ag	Sastry et al. (2003), Mukherjee et al. (2001b)
<i>Verticillium</i> sp.	Fe ₃ O ₄	Bharde et al. (2006)
<i>Bipolaris nodulosa</i>	Ag	Saha et al. (2010)
<i>Pichia jadinii</i> (formerly <i>Candida utilis</i>)	Au	Gericke and Pinches (2006)
<i>Fusarium oxysporum</i>	Au	Mukherjee et al. (2002)
<i>Fusarium oxysporum</i>	Ag	Senapati et al. (2004), Dhandhukia et al. (2012), Birla et al. (2013), Korbekandi et al. (2013), Husseiny et al. (2015)
<i>Fusarium oxysporum</i>	Au-Ag alloy	Senapati et al. (2005)
<i>Fusarium oxysporum</i>	SrCO ₃	Rautaray et al. (2004)

(continued)

Table 4.1 (continued)

Acidophile	Nanoparticle	References
<i>Fusarium oxysporum</i>	CdS	Ahmad et al. (2002)
<i>Fusarium oxysporum</i>	Fe ₃ O ₄	Bharde et al. (2006)
<i>Fusarium oxysporum</i>	ZrO ₂	Bansal et al. (2004)
<i>Fusarium oxysporum</i>	SiO ₂	Bansal et al. (2005), Kannan et al. (2015)
<i>Fusarium oxysporum</i>	TiO ₂	Bansal et al. (2005)
<i>Fusarium oxysporum</i>	BaTiO ₃	Bansal et al. (2006)
<i>Fusarium oxysporum</i>	CdSe	Kumar et al. (2007)
<i>Aspergillus niger</i>	Ag	Kumar et al. (2008)
<i>Aspergillus fumigatus</i>	Ag	Bhainsa and D'Souza (2006), Navazi et al. (2010)
<i>Aspergillus flavus</i>	Ag	Moharrer et al. (2012), Vigneshwaran et al. (2006)
<i>Aspergillus clavatus</i>	Ag	Saravanan and Nanda (2010), Verma et al. (2010)
<i>Aspergillus temeri</i>	Ag	Kumar et al. (2012)
<i>Aspergillus terreus</i>	Au	Baskar et al. (2014)
<i>Aspergillus terreus</i>	Ag	Li et al. (2012)
<i>Aspergillus niger</i>	CuO	Etefagh et al. (2013)
<i>Aspergillus niger</i>	Zn	Noorbatcha and Salleh (2014)
<i>Aspergillus oryzae</i>	Ag	Phanjom and Ahmed (2015)
<i>Aspergillus</i> spp.	Zn	Pavani et al. (2011)
<i>Aspergillus</i> spp.	Pb	Pavani et al. (2012)
<i>Aspergillus</i> spp.	Cu	Pavani et al. (2013)
<i>Aspergillus tubingensis</i>	MgO	Raliya et al. (2014)
<i>Penicillium</i> sp.	Ag	Zhang et al. (2009), Hemath et al. (2010)
<i>Penicillium fellutanum</i>	Ag	Kathiresan et al. (2009)
<i>Penicillium purpurogenum</i>	Ag	Nayak et al. (2010)
<i>Penicillium nalgiovense</i>	Ag	Maliszewska et al. (2014)
<i>Penicillium atramentosum</i> KM	Ag	Sarsar et al. (2015)
<i>Penicillium citrinum</i>	Ag	Honary et al. (2013)
<i>Penicillium chrysogenum</i>	Au	Sheikhloo and Salouti (2011)
<i>Penicillium purpurogenum</i> NPMF	Ag	Nayak et al. (2011)
<i>Penicillium nalgiovense</i> AJ12	Ag	Maliszewska et al. (2014)
<i>Penicillium aurantiogriseum</i>	CuO	Honary et al. (2012)
<i>Penicillium citrinum</i>	CuO	Honary et al. (2013)
<i>Penicillium waksmanii</i>	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 *Staphylococcus 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. acidophilus* 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 AgNO₃ 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. 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 AgNO₃ 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 ($\lambda = 250\text{--}700$ nm) has been observed at $\lambda = 420 \pm 5$ 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 $\lambda = 2$ 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 thioiparus*

The ability of the iron-reducing bacterium, *Thiobacillus thioiporus* 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. thioiporus* was enriched in 9 K medium with graded doses of FeSO₄. Higher concentration of FeSO₄ (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

Sulfolobus 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 (HAuCl₄) 1–3 mM aqueous solution at pH ~ 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 AgNO₃ 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 $\mu\text{g/ml}$ 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 $\text{K}_3[\text{Fe}(\text{CN})_6]$ and $\text{K}_4[\text{Fe}(\text{CN})_6]$ 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 $\sim 6\text{--}7$ 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 dark. 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 fluorescens* 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 *Aspergillus* 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 platensis*

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, pharmaceuticals, 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		
<i>Spirulina platensis</i>	Ag, Au	Kalabegishvili et al. (2013a)
<i>Spirulina platensis</i>	Ag	Sharma et al. (2015), Ahmed et al. (2015), Tsibakhashvili et al. (2010)
<i>Bacillus</i> sp.	Ag	Tayde (2012)
<i>Nostoc</i> sp.	Ag	Ahmed et al. (2015)
<i>Thermomonospora</i> sp.*	Au	Ahmad et al. (2003)
<i>Thermomonospora</i> sp. 67 Th*	Au	Kalabegishvili et al. (2013b)
<i>Streptomyces</i> sp. 211A	Ag	Tsibakhashvili et al. (2010)
<i>Pseudomonas alcaliphila</i>	Se	Zhang et al. (2011)
<i>Bacillus licheniformis</i>	Au	Singh et al. (2014a)
<i>Bacillus licheniformis</i>	CdS	Shivashankarappa and Sanjay (2015)
<i>Bacillus licheniformis</i> JS2	Se	Dhanjal and Cameotra (2011)
<i>Bacillus licheniformis</i>	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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