Chapter 12 Biosynthesis of Nanoparticles by Microorganisms and Applications in Plant Stress Control



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Abbreviations

AgNPs Silver nanoparticles AuNPs Gold nanoparticles

BacMPs Bacterial magnetic particles
BMs Bacterial magnetosomes
BRECs Bovine retinal endothelial cells

CdS NPs CdS nanoparticles CSE Cell-soluble extract **GTPase** Guanosine triphosphatase HRP Horseradish peroxidase mAhs Monoclonal antibodies Magnetic resonance imaging **MRI MTB** Magnetotactic bacteria PHB Polyhydroxybutyrate

TEM Transmission electron microscope

1 Introduction

Nanotechnology's future applications and advantages in agriculture are immense. This involves the treatment of insect pests by new nanomaterial insecticide formulations (Ragaei and Sabry 2014). One nanometer is understood to be a milliard of a micrometer or a million of a micron. That is around 1/80,000 of human hair diameter or ten times hydrogen atom diameter. American scientists assert that "There is plenty of space at the bottom," which was also held as a way of paying attention to the nanotechnological field. Feynman (1960) discovered technique through which it is possible to manipulate single atoms and molecules, utilizing series with specialized instruments to construct and manage a limited range of necessary scales, etc. In this context, Feynman suggested that the shift in magnitude would lead to scaling problems in various physical phenomena: gravity became less relevant, and surface tension and the attraction of van der Waals might be more relevant. Many experiments on nanoparticles have shown their efficacy toward plant diseases, insects, or other threats. Therefore, such nanoparticles were still only used to repel insects, but also to prepare new products, such as pesticides and insecticides (Prasad et al. 2017a, b). But safety for plants to plants for metal-based nanostructures with far larger volume-to-volume particle size and with specific antimicrobials compared with their bulk materials is one of the latest with the rapid advancement of nanotechnology, and their special properties expand the use of a range of carbon nanomaterials (CNMs). The use of a buckyball molecule fullerene (C60) is, for example, commonly available in computers and aircraft airframes and as drug delivery carriers in the form of biomedicine and carbon nanotubes (CNTs) (Ngan et al. 2015; Liu et al. 2015). These have thoroughly studied interactions between CNMs and plants. In 30-day experiments with hydroponic tension, for instance, graphene concentrations ranging from 250 to 1500 mg/L inhibited wheat growth (Zhang et al. 2016). A great number of physical, electronic, biological, or hybrid methods depend on the fabrication of various classes of nanoparticles. Although organic compounds are most common throughout the production of nanoparticles, the use of dangerous substances severely restricts their medicinal use, especially in medical practice (Liu et al. 2011). Hence, it is of utmost importance that to extend their biomedical applications, healthy, nontoxic, and environmentally friendly approaches are developed for the production of nanomaterials. Synthesizing microorganisms with nanoparticles is one of the choices. The nanoparticles generated by biogenic enzyme process greatly outweigh those generated by chemical processes in many respects. Although the latter is capable of producing large amounts of nanoparticles of given size and shape in a reasonably short period, they become complex, obsolete, expensive, and ineffective and produce dangerous radioactive waste that is dangerous not only to the environment but also to public health. Usage of costly chemicals is avoided via an enzyme solution, and most suitable "green" pathway wasn't as energy-intensive and environmentally friendly as chemical route. A biogenic method is again confirmed by the fact that in varying temperature, pH, and pressure conditions, most bacteria exist. These procedures provide greater catalytic reaction, increased surface area, and enhanced interaction among enzyme and metal ion as a result of the bacterial cell membrane (Bhattacharya and Mukherjee 2008). Nanoparticles are biosynthesized as microorganisms take target ions out of the atmosphere and then transform metal into elemental metal by enzymes formed by cell activity. Depending on where nanoparticles are made, intracellular and extracellular synthesis can be categorized. Throughout the existence of enzymes, the intracellular process is the transport for ions to produce nanoparticles by bacterial cell. Extracellular nanoparticle synthesis includes capturing metal ions on the cell surface and decreasing the amount of ions when enzymes are present (Zhang et al. 2011). To biosynthesize nanoparticles, a number of applications have been used, like selective drug carriers, cancer treatment, gene therapy and DNA sequencing, antiviral activities, biosensors, reaction-enhancing rates, and isolation monitoring.

The objectives of this chapter highlight the extensive properties of inorganic nanoparticles and the synthesis of metal, oxide, sulfide, and other conventional nanoparticles among different species of microorganisms. It will also discuss the proposed pathways for the biosynthesis of inorganic nanoparticles. Size/shape and stabilization of synthesized nanoparticles were affected. Pharmaceutical formulations include such nanoparticles, crop protection, and antibacterial agents. Synthesized biometallic nanoparticles are also investigated by manipulating *Penicillium* species and their uses in pharmaceutical applications (Fig. 12.1).

2 Metallic Nanoparticles

Table 12.1 summarizes several standard nanoparticles made through microorganisms.

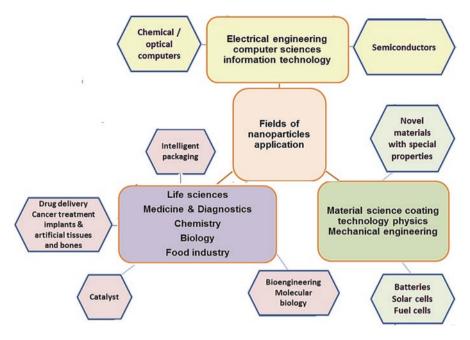


Fig. 12.1 Fields of application of nanoparticles

2.1 Gold Nanoparticles

In chemistry, Au nanoparticles get a long and glorious background to Roman times, wherein they were being used for aesthetic reasons to dye glasses. AuNPs were already used centuries earlier for the treatment of different diseases. Previous study recorded that colloidal gold substances had distinct characteristics than mass gold, which launched the modern era of AuNP synthesis (Hayat 1989). Because of the increasing need to improve environmentally sustainable material synthesis technologies, nanoparticles have received considerable attention as evolving bionanotechnology (overpass of nanotechnology and biotechnology). Extracellular production by Fusarium oxysporum fungus and actinomycete sp. with gold nanoparticles has been documented in previous research. Intracellular synthesis of Verticillium sp. fungal gold nanoparticles has been reported (Ahmad et al. 2003a). Southam and Beveridge (1996) showed nanoscale gold particles could be readily caused inside microbes by cells with Au³⁺ ions. The gold monodisperse nanoparticles were synthesized with Rhodococcus sp. alkalotolerant within extreme biological regulation, like alkaline conditions and environments with marginally greater temperatures (Ahmad et al. 2003b). Lengke et al. (2006a, b) have submitted Au complexes to synthesize filamentous cyanobacteria in various shapes, including spherical, cubic, and octahedral, and to research the mechanisms of nanostructure formation. There have been studies of the development of nanocrystals and nanoalloys using Lactobacillus (Nair and Pradeep 2002). Table 12.1 summarizes some other typical microorganism-formed gold nanoparticles (Konishi et al. 2007a; Singaravelu et al. 2007).

•	•	•				
		Culturing temperature				
Microorganisms	Products	(°C)	Size (nm)	Shape	Location	References
Sargassum wightii	Au	Not available	8–12	Planar	Extracellular	Singaravelu et al. (2007)
Rhodococcus sp.	Au	37	5-15	Spherical	Intracellular	Ahmad et al. (2003a)
Shewanella oneidensis	Au	30	12 ± 5	Spherical	Extracellular	Suresh et al. (2011)
Plectonema boryanum	Au	25–100	<10-25	Cubic	Intracellular	Lengke et al. (2006a)
Plectonema boryanum UTEX 485	Au	25	10 nm-6 µm	Octahedral	Extracellular	Lengke et al. (2006b)
Escherichia coli	Au	37	20–30	Triangles, hexagons	Extracellular	Du et al. (2007)
Yarrowia lipolytica	Au	30	15	Triangles	Extracellular	Agnihotri et al. (2009)
Pseudomonas aeruginosa	Au	37	15–30	Not available	Extracellular	Husseiny et al. (2007)
Pseudomonas rhodesiae	Ag	37	20-100	Spherical	Extracellular	Hossain et al. (2019)
Pseudomonas sp. and Achromobacter Ag	Ag	37	20–50	Spherical		Kaur et al. (2018)
sp.						
Rhodopseudomonas capsulate	Au	30	10–20	Spherical	Extracellular	He et al. (2007)
Shewanella algae	Au	25	10–20	Not available	Intracellular	Konishi et al. (2007a, b)
Brevibacterium casei	Au, Ag	37	10–50	Spherical	Intracellular	Kalishwaralal et al. (2010)
Trichoderma viride	Ag	27	5-40	Spherical	Extracellular	Fayaz et al. (2010)
Bacillus licheniformis	Ag	37	50	Not available	Extracellular	Kalimuthu et al. (2008)
Bacillus siamensis	Ag	37	25–50	Spherical	Extracellular	Ibrahim et al. (2019)
Escherichia coli	Ag	37	50	Not available	Extracellular	Gurunathan et al. (2009)
Shewanella loihica PV-4	Au	30	10–16	Spherical	Extracellular	Lv et al. (2018)
Corynebacterium glutamicum	Ag	30	5-50	Irregular	Extracellular	Sneha et al. (2010)
Trichoderma viride	Ag	10-40	2-4	Not available	Extracellular	Fayaz et al. (2009)
Ureibacillus thermosphaericus	Au	08-09	50-70	Not available	Extracellular	Extracellular Juibari et al. (2011)

(continued)

Table 12.1 (continued)

		Culturing temperature				
Microorganisms	Products	(°C)	Size (nm)	Shape	Location	References
Bacillus cereus	Ag	25	18–391	Spherical	Extracellular	Ahmed et al. (2020)
Aspergillus fumigatus	Ag	25	5-25	Spherical	Extracellular	Bhainsa et al. (2006)
Aspergillus niger	Ag	25	10–100	Spherical	Extracellular	Al-Zubaidi et al. (2019)
Verticillium sp.	Ag	25	25 ± 8	Spherical	Extracellular	Senapati et al. (2005)
Fusarium graminearum	Ag	25	20–45	Spherical	Extracellular	Ibrahim et al. (2020)
Fusarium oxysporum	Ag	25	5-50	Spherical	Extracellular	Senapati et al. (2005)
Trichoderma harzianum	Ag	25	11–13	Spherical	Extracellular	El-Moslamy et al. (2017)
Trichoderma hamatum	Au	25	5–30	Spherical, pentagonal, and Hexagonal	Extracellular	Abdel-Kareem and Zohri (2018)
Streptomyces griseus	Cu	25	5-50	Spherical	Extracellular	Ponmurugan et al. (2016)
Neurospora crassa	Au, Au/Ag	28	32, 20–50	Spherical	Intracellular Extracellular	Castro-Longoria et al. (2011)
Shewanella algae	Pt	25	5	Not available	Intracellular	Konishi et al. (2007a, b)
Enterobacter sp.	Hg	30	2–5	Spherical	Intracellular	Sinha and Khare (2011)
Shewanella sp.	Se	30	181 ± 40	Spherical	Extracellular	Lee et al. (2007)
Escherichia coli	CdTe	37	2.0–3.2	Spherical	Extracellular	Bao et al. (2010)
Yeast	Au/Ag	30	9–25	Irregular polygonal	Extracellular	Zheng et al. (2010)
Fusarium oxysporum	Au-Ag alloy	25	8–14	Spherical	Extracellular	Senapati et al. (2005)
Penicillium duclauxii	Ag	25	3–32	Spherical	Extracellular	Almaary et al. (2020)
Setosphaeria rostrata	Ag	25	2–50	Spherical	Extracellular	Akther and Hemalatha (2019)
Pyrobaculum islandicum	U(VI), Tc(VII), Cr(VI), Co(III), Mn(IV)	100	N/A	Spherical	Extracellular	Kashefi and Lovley (2000)
Desulfovibrio desulfuricans	Pd	25	50	Spherical	Extracellular	Lloyd et al. (1998)

2.2 Silver Nanoparticles

Ag nanoparticles exhibit Gram-positive bacteria with effective antimicrobial activity, particularly multiresistant strains such as Staphylococcus aureus which is resistant to methicillin, as its bulk counterpart (Panacek et al. 2006). The secrets of nature have contributed to the production of advanced nanoparticles through biomimetic approaches. Researchers have long made efforts to use microorganisms to manufacture as many silver nanoparticles as possible to create eco-friendly nanofactories. Various microbes are recognized as reducing Ag⁺ ions in silver nanoparticles, and most are spherical particles (Fayaz et al. 2010). Klaus et al. (1999) showed that when *Pseudomonas* bacterium is extracted from silver mine, while put within a solution containing aqueous silver nitrate, stutzeri AG259 played a significant function throughout the decrease of Ag+ ions as well as in production with well-defined silver nanoparticles and separate topography of bacteria within periplasmic space. AgNPs were produced as a film or formed in liquid or collected onto their cell surface when fungi Verticillium or Fusarium oxysporum were used (Jain et al. 2011). Table 12.1 lists some other microorganism-developed silver nanoparticles (Kalimuthu et al. 2008; Gurunathan et al. 2009; Sneha et al. 2010; Fayaz et al. 2009; Kalishwaralal et al. 2010; Castro-Longoria et al. 2011; Juibari et al. 2011). Synthesized AgNPs by Hamouda et al. (2019) demonstrated good antibacterial activity toward multidrug-resistant bacteria (Bacillus cereus, Escherichia coli) and anticancer activity toward cell lines of human (breast, colon, liver). Low concentrations of hemolytic activity of AgNPs have been studied and reported as nontoxic to human RBCs. Furthermore, the dynamics of absorption and cytotoxicity of these AgNPs have been studied in the cell lines of breast cancer, enabling them to be shown to be good antibacterial agents, with further proof of the different behavior of AgNPs to cause toxicity in cells and bacteria when collected at pH 7 or 8. Moreover, the theoretically unlimited source of the reducing agent (i.e., leaf extract obtained from agricultural processing waste) and its negligible environmental impact constitute another strength of this method (De Matteis et al. 2019; Tanase et al. 2019). It has been shown that the combination of AgNP_{bio} and simvastatin may be a great future option for bacterial infection control, where lower doses of AgNP_{bio} with the same antibacterial activity are needed when combined with simvastatin (Figueiredo et al. 2019). Also, the synthesized silver nanoparticles had a strong antibiofilm property and were also found to be biocompatible with the red blood cell lysis assay and their association with peripheral mononuclear blood cells and 293 cells of the human embryonic kidney. Mesoflavibacter zeaxanthinifaciens is therefore found to be an excellent source of exopolysaccharide synthesis that assists in production of silver nanoparticles (Oves et al. 2019).

2.3 Alloy Nanoparticles

Using alloy nanoparticles in catalytic reactions, electronics, and optical substances and coatings is of great interest. *Fusarium oxysporum* production of bimetallic Au-Ag alloy and argued that secreted NADH cofactor is a significant determinant of

the composition of Au-Ag nanoparticles (Senapati et al. 2005). Au-Ag metal nanoparticles, biosynthesized by yeast cells, have been studied (Zheng et al. 2010). Nanoparticles of the Au-Ag alloy were commonly produced by extracellular phase, microscopically characterized by fluorescence and electron microscopic transmission, or generally existed as irregular polygonal nanoparticles. Electrochemical research has shown vanillin sensors have been able to enhance electrochemical reaction of vanillin at least five times by changing glass carbon electrodes based on Au-Ag metal nanoparticles. Au-Ag alloy nanoparticles from fungal strains have been used in *Fusarium semitectum* core-shell synthesis of nanoparticles and been very stable for several weeks (Sawle et al. 2008).

2.4 Other Metallic Nanoparticles

It is understood that heavy metals are life-threatening to microorganisms. Microbial tolerance to many other toxic metals is in nature due to its chemical detoxification or even cell-dependent ion excretion by protein complexes acting as ATPase, chemical cations, or anti-transporter protons. Solubility changes play a crucial role as well in resistant bacteria. Konishi et al. (2007b) studied the use of Shewanella algae, a metal ion-reducing bacterium, to obtain platinum nanoparticles. In most cells of Shewanella by time lactate was delivered as an electron donor, aqueous PtC₁₆b₂ ions in elemental platinum were reduced to room temperature and neutral pH within 60 min. Platinum nanoparticles of about 5 nm were found in periplasm. Sinha and Khare have shown that Enterobacter sp. can synthesize mercury nanoparticles (Sinha and Khare 2011). Cultivation conditions (pH 8.0 and lower mercury concentrations) facilitate the synthesis of uniformly sized, spherical, and monodispersed 2–5 nm intracellular mercury nanoparticles. Many of heavy metals with hydrogen as an electron donor of the anaerobic hyperthermophilic microorganism Pyrobaculum islandicum, like U(VI), Tc(VII), Cr(VI), Co(III), and Mn(IV), have been reported to be reduced (Kashefi and Lovley 2000). In palladium nanoparticles, sulfate-reducing bacteria, Desulfovibrio desulfuricans, or metal ion-reducing bacteria sulfur can be synthesized. Table 12.1 also lists some other nanoparticles formed by microorganisms (DeWindt et al. 2005; Lee et al. 2007; Bao et al. 2010).

3 Oxide Nanoparticles

Oxide nanoparticles are an essential type of microbial compound nanoparticles. The biosynthesized oxide nanoparticles from both sides have been investigated in this section: magnetic oxide nanoparticles or nonmagnetic oxide nanoparticles. In Table 12.2, many examples of magnetotactic bacteria (MTB) shown in development of nanoparticles of magnetic oxide and biological systems for the production of nanoparticles of nonmagnetic oxide are summarized.

3.1 Magnetic Nanoparticles

Owing to its peculiar microstructure and properties, such as magnetic nanoparticles, strong forces, and its potential to widespread implementation in fields of biological isolation and biomedicine, superparamagnetic nanoparticles become new materials discovered. It is known that magnetic nanoparticles are Fe₃O₄ (magnetite) and Fe₂O₃ (maghemite). Targeted treatment of cancer (magnetic hyperthermia), stem cell filtering and manipulation, drug delivery guidance, gene therapy, DNA sequencing, and magnetic resonance imaging (MRI) have been actively investigated (Fan et al. 2009). Magnetotactic bacteria produce intracellular magnetic particles containing iron oxide, iron sulfides, or either. To differentiate between them and artificially synthesized magnetic particles (AMPs), these particles were pointed as bacterial magnetic particles (BacMPs) (Arakaki et al. 2008). Its associations with bacterial links are presumed to function like biological compass points that allow bacteria to move to oxygen gradients in aquatic environments under geomagnetic field of Earth (Blakemore 1975). BacMPs, as they can be surrounded through biological membranes composed primarily of lipids and proteins, could be quickly spread into aqueous media. In addition, individual BacMPs with better magnetic characteristics involve individual magnetic field or magnetite (Thornhill et al. 1995). Since the first magnetotactic bacteria study in 1975, numerous morphological forms have been described and observed in numerous aquatic environments, including cocci, spirals, vibrants, ovoid bacteria, and multicellular bacteria, with specific characteristics (Spring and Schleifer 1995). For example, magnetotactic cocci showed a high diversity and distribution and were often found on aquatic sediment surfaces. Identification of such type of bacteria shows that it is microaerophilic, including the coccus strain cultivated by magnetic MC-1. In the case of Vibrio bacteria, three optional anaerobic marine vibrating forms were extracted from freshwater salt marshes. As part of Alphaproteobacteria, these bacteria are known to belong to Rhodospirillaceae family, and truncated hexoctahedron-type BacMPs have been synthesized to evolve heterotrophically and organically with chemo. On the other side, parts of the Magnetospirillaceae family are present in sediments containing fresh water. In this family, significant amounts of previously isolated magnetotactic bacteria have been detected by utilizing culture medium and magnetic isolation methods. The first family member was isolated from strain MS-1 of Magnetospirillum magnetotacticum, while the physiological and genetic features of strain MSR-1 of Magnetospirillum gryphiswaldense were also well studied. AMB-1 was discretionary magnetotactic anaerobic spirilla, separated by Arakaki et al. (2008). After 2000, several new magnetotactic bacteria were discovered in different ecological settings. Several of freshly described magnetotactic bacteria were recorded in Table 12.2. Uncultured magnetotactic bacteria were found in distinct environments (Lefevre et al. 2010a). Mesophilic bacteria are the most common cultivated magnetotactic bacteria, which appear to grow less than 30 °C. The majority of uncultivated magnetotactic bacteria is 30 °C and below. Thermophilic magnetotactic bacteria are described in only few studies. Each of magnetotactic bacteria known as HSMV-1 is identified in samples

Table 12.2 Oxide nanoparticles synthesized by microorganisms

		Culturing				
Microorganisms	Products	Products temperature (°C)	Size (nm)	Shape	Location	References
Shewanella oneidensis	Fe ₃ O ₄	28	40–50	Rectangular, rhombic, hexagonal	Extracellular	Extracellular Perez-Gonzalez et al. (2010)
QH-2	Fe ₃ O ₄	22–26	$81 \pm 23 \times 58 \pm 20$	Rectangular	Intracellular	Intracellular Zhu et al. (2010)
Recombinant AMB-1	Fe ₃ O ₄	28	20	Cuboctahedral	Intracellular	Intracellular Amemiya et al. (2007)
Yeast cells	Fe ₃ O ₄	36	Not available	Wormhole-like	Extracellular	Extracellular Zhou et al. (2009a)
Yeast cells	FePO ₄	36	Not available	Nanopowders	Extracellular	Extracellular Zhou et al. (2009b)
WM-1	Fe ₃ O ₄	28	$54 \pm 12.3 \times 43 \pm 10.9$	Cuboidal	Intracellular	Intracellular Li et al. (2007)
Shewanella oneidensis MR-1	Fe ₂ O ₃	25	30-43	Pseudohexagonal/irregular or rhombohedral	Intracellular	Intracellular Bose et al. (2009)
HSMV-1	Fe ₃ O ₄	63	$113 \pm 34 \times 40 \pm 5$	Bullet-shaped	Intracellular	Intracellular Lefevre et al. (2010a)
Saccharomyces cerevisiae	$\mathrm{Sb}_2\mathrm{O}_3$	25–60	2–10	Spherical	Intracellular	Intracellular Jha et al. (2009)
Lactobacillus sp.	BaTiO ₃	25	20–80	Tetragonal	Extracellular	Extracellular Jha et al. (2010a)
Lactobacillus sp.	TiO ₂	25	8–35	Spherical	Extracellular	Extracellular Jha et al. (2010b)
Fusarium oxysporum	TiO ₂	300	6–13	Spherical	Extracellular	Extracellular Bansal et al. (2005)
Fusarium oxysporum	BaTiO ₃	25	4-5	Spherical	Extracellular	Extracellular Bansal et al. (2006)
Fusarium oxysporum	ZrO_2	25	3–11	Spherical	Extracellular	Extracellular Bansal et al. (2004)
Streptomyces spp.	CnO	25	78–80	Spherical	Extracellular	Extracellular Hassan et al. (2019)

of springs in which temperatures varied between 32 and 63 °C (Lefevre et al. 2010b). TEM images of the untouched HSMV-1 cell discovered single polar flagel-lum and single bullet-shaped magnetosome string. The average number per cell of magnetosome crystals is 12 ± 6 and 113 ± 34 nm by 40 ± 5 nm. Report's findings indicate that certain magnetotactic bacteria may at least indicate mild thermophilicity. Under conditions where magnetotactic bacteria are present and are expected to develop as high as 63 °C and where *Magnetosome magnetitis* (Magnetosomes are membranous structures present in magnetotactic bacteria) is deposited, maximum temperature level has been extended (Lefevre et al. 2010b). The use of yeast cells as a template has been reported to synthesize magnetic Fe₃O₄ materials with a mesoporous structure (Zhou et al. 2009a, b). Table 12.2 (Amemiya et al. 2007; Li et al. 2007; Bose et al. 2009; Perez-Gonzalez et al. 2010; Zhu et al. 2010;) mentions several other magnetic oxide nanoparticles.

3.2 Nonmagnetic Oxide Nanoparticles

Many oxide nanoparticles, including TiO₂, Sb₂O₃, SiO₂, BaTiO₃, and ZrO₂ nanoparticles, were also investigated in addition to magnetic oxide nanoparticles (Jha et al. 2009). A green, cheap-cost, repeatable biosynthesis induced by Sb₂O₃ nanoparticles of *Saccharomyces cerevisiae* has been described (Jha and Prasad 2010). The synthesis was carried out in compliance with room temperature. Analysis has shown that the Sb₂O₃ device is a 2–10 nm spherical aggregate (Jha et al. 2009). For processing of SiO₂ and TiO₂ nanoparticles of soluble SiF62- and TiF62-anionic complexes, *Fusarium oxysporum* (Fungus) is used. *F. oxysporum* 4–5 and 3–11 nm were also prepared from tetragonal BaTiO₃ and quasispheric ZrO₂ nanoparticles in size (Bansal et al. 2004, 2005, 2006).

4 Sulfide Nanoparticles

As quantum dot fluorescent biomarker and cell marking agent, sulfide nanoparticles have been strongly bounded to fundamental and technological research for its fascinating, innovative, optical, and electronic characteristics, in addition to oxide nanoparticles (Yang et al. 2005). Microorganisms have nanocrystal CdS synthesized, and it constitutes one typical form of sulfide nanoparticle. It was found that *Clostridium thermoaceticum* would aggregate CdS both on cell surface and in CdCl₂ media in existence of cysteine hydrochloride in raising environment, most likely serving as a sulfide source (Cunningham and Lundie Jr 1993). *Klebsiella pneumoniae* was reported to create CdS (20–200) nm of on cell surface, exposing growth environment to Cd²⁺ ions. Intercellular nanocrystals, consisting of rootite chrystal phase were formed, while *E. coli* incubates CdCl₂ and Na₂SO₄ (Sweeney et al. 2004). Depending on cell growth process, nanocrystal formation differs greatly and

increases by approximately 20 Escherichia coli cultivated in stationary stage relative to that produced in retard logarithmic period. S. pombe, C. pombe, and S. glabrata (yeasts) were used in the production of CdS nanoparticles with intracellular cadmium mixture. PbS and ZnS nanoparticles have been designed and synthesized using biological systems. ZnS with 2-5 and 8 nm mean diameter intracellular nanoparticles were used with Desulfobacter and R. sphaeroides (Bai et al. 2006). The use of Rhodobacter sphaeroides, whose diameters are regulated by culture time, was also used to synthesize PbS nanoparticles (Bai and Zhang 2009). For extracellular development of sulfide metal nanoparticles, eukaryotic organisms like fungi have been reported for being ideal candidates (Ahmad et al. 2002). Certain stabilized metal-metal sulfide nanoparticles like CdS, ZnS, PbS, and MoS₂ may be formed extracellularly by fungus Fusarium oxysporum when exposed to aqueous metal sulfate solution. Quantum dots were produced from Cd²⁺ ion interaction to sulfide ions supplied via reduction of sulfide ions. Other types of sulfide nanoparticles were magnetic Fe₃S₄ or FeS nanoparticles. Uncultured magnetotactic bacteria have documented the development of Fe₃S₄ (Bazylinski et al. 1995). A sediment sample of magnetotactic bacteria was analyzed, and about 105 cells are collected the following purification by racetrack treatment. In uncultured cells, magnetosomes showed extended rectangular shapes. The overall amount of magnetosomes in each cell was around 40, and they have been usually observed with big groups of cells. Magnetosomes forming a chain-like structure were detected alongside major clusters. Sulfate reduction bacteria may generate magnetic FeS nanoparticles (Watson et al. 1999). Table 12.3 shows many sulfide nanoparticles formed via microorganisms.

5 Other Nanoparticles

A broad range of species from organic/inorganic composites in biological systems, are utilizing biopolymers, like microbial cells and protein, with organized structures. In addition to the above mentioned nanoparticles, microbe synthesis has been reported as SrCO₃, PbCO₃, CdCO₃, PHB, CdSe, and Zn₃(PO₄)₂ (Table 12.4). SrCO₃ crystals were produced with ionic Sr²⁺ ions while incubating demanding fungi (Rautaray et al. 2004). Researchers assume even through fungal development of *Fusarium oxysporum* in higher cognitive superstructures, protein excretion modulated the morphology and hierarchical assembly of strontianite crystals. Through yeast biotemplates, zinc phosphate nanopowder was produced (Pandian et al. 2009). Production of Zn₃(PO₄)₂ particles with a butterfly-like microstructure between 10–80 nm diameter and 80–200 nm in length was shown. It has been demonstrated that *Fusarium oxysporum* in extremely luminescent room temperature would synthesize CdSe quantum dots (Yan et al. 2009).

Table 12.3 Sulfide nanoparticles synthesized by microorganisms

Microorganisms Produ Multicellular Fe ₃ S ₄		Culturing temperature				
	Products	(D _o)	Size (nm)	Shape	Location	References
Prokaryotes	S ₄	25	Not	Not available	Intracellular	Intracellular Lefevre et al. (2010b)
			available			
Uncultured Prob	Probably	Not available	Not	Rectangular	Extracellular	Extracellular Arakaki et al. (2010a, b)
Magnetotactic poly/	polyphosphate		available			
Daccolain						
Rhodopseudomonas CdS palustris	· ·	30	8	Cubic	Intracellular	Bai et al. (2009)
Coriolus versicolor CdS	10	25	100–200	Spherical	Extracellular	Extracellular Sanghi and Verma (2009)
Lactobacillus	100	25-60	4.9 ± 0.2	Spherical	Intracellular	Prasad et al. (2010)
Yeast 1 CdS	10	25-60	3.6 ± 0.2	Spherical	Intracellular	Intracellular Sweeney et al. (2004)
E. coli	10	25	2–5	Wurtzite	Intracellular	Intracellular Sweeney et al. (2004)
				crystal		
Rhodobacter sphaeroides ZnS		Not available	10.5 ± 0.15 Spherical	Spherical	Extracellular	Extracellular Bai et al. (2009)
Sulfate-reducing bacteria FeS		Not available	2	Spherical	Extracellular	Extracellular Watson et al. (1999)

Table 12.4 Other miscellaneous nanoparticles synthesized by microorganisms

	•	,				
Microorganisms	Products	Culturing temperature (°C) Size (nm)	Size (nm)	Shape	Location	References
Fusarium oxysporum	PbCO ₃ , CdCO ₃	27	120-200	Spherical	Extracellular	Extracellular Sanyal et al. (2005)
Fusarium oxysporum	SrCO ₃	27	10–50	Needlelike	Extracellular	Extracellular Rautaray et al. (2004)
Brevibacterium casei	PHB	37	100-125	Not available	Intracellular	Not available Intracellular Pandian et al. (2009)
Yeasts	$Zn_3(PO_4)_2$	25	$10-80 \times 80-200$ Rectangular	Rectangular	Extracellular	Extracellular Yan et al. (2009)
Fusarium oxysporum	CdSe	10	9–15	Spherical	Extracellular	Extracellular Kumar et al. (2007)

6 Mechanism of Nanoparticle Synthesis by Microbes

Different microorganisms have numerous pathways of nanoparticle creation. Nanoparticles, though, are usually shaped as follows: metal ions first were trapped in microbial cells or on the surface. Then, trapping metal ions in existence of enzymes was limited to nanoparticles. In fact, in two distinct ways, microorganisms affect mineral formation. At any point, you can change a solution's composition to oversaturate it or undersaturate it. Another way for microorganisms to affect mineral formation is through organic polymers that could affect nucleation by encouraging (or preventing) stabilization of first mineral seeds (Benzerara et al. 2010). Potential mechanisms for the production of some common nanoparticles were discussed in this section: gold and silver, heavy metals, and magnetic and sulfide nanoparticles. The basic process for intracellular creation of silver and gold nanoparticles from Verticillium sp. or algal biomass has not been entirely known. However, the observation in which nanoparticles have grown on mycelium surface rather than in the solution supports the following hypothesis: first electrostatic interactions of ions with the overlooked cell wall of carboxylated groups of enzymes have captured fungal cells on the surface. The metal ions were then reduced to nuclei of gold or silver, which were then produced further by reduction and aggregation (Sneha et al. 2010). It was suggested that nitrate reductase enzyme can synthesize nanoparticles of B silver (Kalishwaralal et al. 2008). Nitrate ions activate this enzyme and silver ions are reduced into silver. Reducing enzyme metals in electron shuttles is a potential way of minimizing silver ions. Nitrate reductase enzymes based on NADH and NADH-reliant enzymes are the essential factors for metal nanoparticle formation. NADH and NADH-reliant enzymes, especially nitrate reductase, are considered to be secrets for Bacillus licheniformis, which may be essential for biosynthesis of Ag+ to Ag⁰ or continued development of silver nanoparticles (Husseiny et al. 2007). Molecular and proteomic response to hazardous conditions in metalloplastic microorganisms can lead to the development of heavy metal nanoparticles (Reith et al. 2007). Toxic effect of the microorganisms on its survival is caused by strong metal ions like Ag+, Cd2+, Co2+, CrO42+, Cu2+, Hg2+, Pb2, Ni2+, and Zn2+. To counteract certain impact or precisely control metal metabolism, microorganisms develop molecular and proteomic reactions (Nies 1999). Microbes have many essential genes of metal tolerance that allow cell removal through a range of techniques, including complexity, excretion, or limitation of precipitation. In conditions that require large amounts for moving ions of heavy metal, as mine waste dumps and metalworking plant flows including natural sedimentary areas, metallophilic microbes thus flourish (Tang et al. 2005). A multistage method is thought to be a molecular mechanism of BacMP biomineralization. First sage is cytoplasmic membrane invagination, which is a predecessor to BacMP membrane (Arakaki et al. 2008). The mechanism for envelope formation remains unknown. Vesicular pathways for magnetotactic bacteria were more likely similar to other eukaryotes, or precipitation is controlled by particular GTPase. In a linear cytoskeletal filament chain, vesicles which were formed were then assembled. Aggregation of iron ions in vesicles is the second

stage in BacMP biomineralization. The movement of foreign iron is internalized by proteins and siderophores. An oxidation-reduction mechanism strictly controls internal iron. Closely bound BacMP proteins activate and/or regulate magnetized nucleation of crystal in the final step. Magnetite generation functional roles can be performed by different membrane proteins of BacMP. This requires iron supersaturation deposition, preservation of conditions of reduction, and iron oxidation to reduce or dehydrate ferrihydrate to magnetite (Arakaki et al. 2008). This implies mineralization. Perez-Gonzalez and the staff recently suggested a new possible Magnetitis synthesization method that uses both passive and active Shewanella oneidensis (Spring and Schleifer 1995). Secondly, Fe²⁺ activity occurs as a terminal electron admitter, as bacteria use ferrihydrite, and the cell pH value may be increased by the amino acid bacterial metabolism. Localized accumulations of Fe²⁺ and Fe³⁺ on a network, bacterial surface wall, cell compositions, or cell particles allow a passive mechanism to be precipitated by magnetite system to supersaturate magnetite process. It was proposed that the production of CdS NP was due to disulfide (cystine) bridges that could be related to slashing of S-H bonds or creation of new nanoparticle surface bonds, namely, Cd-thiolate (Cd-S-CH₂COOH) S-Cd-bond complex (Sanghi and Verma 2009). Cadmium thiolate group CoOH interacts with hydrogen bond, not with NH₂ protein. CdS-capped nanoparticles also bind to hydrogen bond groups of NH₂ (Tang et al. 2005). A coordinated link between oxygen Cd²⁺ ion atom was created by one of the carboxylic oxygen group atoms, COOH, thus competing with the thiol group to construct surfaces with CdS nanoparticles (Lover et al. 1997). In general, microbes synthesize nanoparticles by implanting metal ions, followed by enzyme reduction, on cell surfaces (extracellular) or in cells (intracellular). Using fungal cellular structure and cell membrane sugars, these metal ions can be absorbed and reduced. With different microorganisms, mechanisms of synthesis of nanoparticles differ. Three options, for example, consist of an extracellular synthesis of nanoparticles, i.e., action by both electron shuttle quinones or nitrate reductase. Penicillium and many other fungal species have initiated the synthesis of nitrate reductase (Deepa and Panda 2014). Nitrate reductase activity was conducted using 2,3-diaminophthalene nitrites (Kumar et al. 2007). Oxysporum is associated with quinone extracellular shuttle, NADPH-dependent reductases, and nitrate reductase. Studies have shown AgNP production is generated earlier with 33 kDa protein and then with protein capping agent (free amine groups and cysteine) that maintains NPs of Aspergillus flavus (Soni and Prakash 2011). Metal ions were trapped firstly in the cell surface of fungi by electrostatic activity by intracellular synthesis and later reduced with enzymes inside the cell wall, contributing to NP construction and production (Singh et al. 2014). Silver nanoparticles involved in nitrate reductase enzyme Bacillus licheniformis are synthesized. NADH and NADH-based enzymes essential for Ag+ bioreduction and subsequent production of AgNPs secrete Bacillus licheniformis (Husseiny et al. 2007). Reduction of Ag + requires a process of reducing electron shuttle enzyme to metallic silver by convincing nitrate ions and silver ions. Strong metal nanoparticles (Co²⁺, CrO₄²⁺, Pb²⁺, Zn²⁺, Hg²⁺, Cd²⁺) synthesize genetic and proteomic reactions that specifically control metal homeostasis and fight harmful effects (Reith et al. 2007). Shewanella oneidensis synthesis, moreover, involves active and passive pathways. Owing to

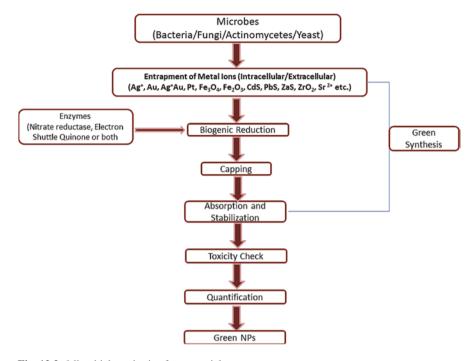


Fig. 12.2 Microbial synthesis of nanoparticles

amino acid metabolism and efficient Fe²⁺ growth, pH value rises, accompanied with active Fe²⁺ or Fe³⁺ levels that enable magnetite process to aggregate, *if* ferrihydrite is used by bacteria. The research was conducted on the production of disulfide (cysteine) cross-section CdS NPs that cause S–H bond divide and the new model nanoparticle complex (Cd–S–CH₂COOH) (Sanghi and Verma 2009). Acid carboxylic COOH groups with a hydrogen bond resulted in CdS nanoparticle capping bonds with NH₂ groups (Tang et al. 2005), cadmium-thiolate complex reaction. A coordination connection between Cd²⁺ and oxygen atoms has been generated by one carboxylic atom (–COOH) that competes to thiol for building nanoparticles on CdS surfaces (Li et al. 2007). Covalent binding to nanoparticles of carboxylic acids while still inhibiting the growth of surface oxides that minimize the magnetic characteristic of cobalt can induce biocompatibility. For the rational design of such entities, recognizing the origin of acid-metal interaction is important, but possibly most experimentally a difficult stage (Farkas et al. 2020) (Fig. 12.2).

7 Regulation of Nanoparticle Size and Morphology

It's so well established that electronic and optical characteristics of nanoparticles depend enormously on their size and shape. Significant attention was paid to monitoring the scale, shape, and media support for nanoparticles. Special emphasis has

recently been put in the form regulation, as it also allows properties to be optimized to the highest degree of versatility, which gives particles their distinctive character. Although physical and chemical techniques are capable of generating, over a short time, significant quantities of nanoparticles of certain size and shape, these techniques are complex and present certain disadvantages, such as the development of radioactive waste that is hazardous not just to the environment but even to public health. Microbes that are considered to have been efficient green nanofabrics can regulate the size and shape of biological nanoparticles. Two fungal cultures of gold nanoparticles of different morphologies and sizes, Verticillium luteoalbum and one labeled isolate 3–6 (Gericke and Pinches 2006), were found to have an intracellular synthesis. Particle formation rate and particle size may be manipulated to a certain degree by manipulating parameters such as exposure times to pH, temperature, gold, and AuCl₄. As demonstrated by electron microscopy scans, numerous morphologies of particles were present, including circular, triangular, hexagonal, and other shapes. Shape and size of particles ranged dramatically from several nanometers to around 100 nm. Their observations often found that particles of spheres seemed to be lower than particles of triangles and hexagons. During the study, screened bacterial cultures appeared to intracellularly synthesize thin, nearly homogenous gold nanoparticles. Particles were mainly noticed in the cell cytoplasm, with most spherically shaped particles. Gurunathan et al. (2009) investigated optimal process requirements to complete AgNP production and particle size reduction. In a synthesis of AgNPs, process temperatures and pH values have been used to detect optimum conditions, various mediums, and media of varying AgNO₃ concentrations. A nitrate medium with a 5 mM AgNO₃, a reaction temperature of 60 °C, and a pH of 10 was described as the maximum synthesis subject. It took only 30 min to achieve more than 95% conversion using Escherichia coli supernatant culture under these optimum conditions. The rate of synthesis of identical particles obtained using chemical methods is comparable or faster. Average particle size can be tuned by varying the AgNO₃ concentration, temperature of reactions and pH from 10–90 nm. During the synthesis of the Pt nanoparticles, the cell-soluble extract (CSE) might decrease the Pt(IV) into nanoparticles that were stable by means of binding protein and exhibit both g in solution. Strong initial Pt(IV) levels seemed to have led to more regular and geometric particles. More hydrochloride (pH to 4) was produced inside the system at high initial amounts of Pt(IV), leading to precipitation of biocomposites of nanoparticle proteins and consequently a reduction in the level of soluble particle size in colloids. Besides, without cellular restrictions, high size and type variations of protein-stabilized biogenic Pt(0) nanoparticles can be synthesized. Magnetotactic bacteria create uniform size and morphological iron oxide magnetic particles. Magnetite shaped by magnetotactic bacteria takes different forms such as cuboid, rhombic, and rectangular shape of a bullet. A high degree of biological regulation has been observed in various species-dependent crystal morphologies and structures (Amemiya et al. 2007). It is discovered that Mms6 is a big protein closely linked to Magnetospirillum magneticum AMB-1, the surface of bacterial magnetites (Arakaki et al. 2010a). With a uniform cuboctahedral morphology, protein was shown to intercede the creation of magnetite crystals. Formation of magnetite with synthetic peptides imitating Mms6 protein was examined. A spherical structure of 0.70-0.90, similar to one of the bacterial magnetites and particulate matter formed by the Mms6 protein, was demonstrated by particles synthesized with short peptides comprising the Mms6 C-terminal acid region. Also, if other peptides are added in production, rectangular morphology was observed with circularities of 0.60-0.85 (Arakaki et al. 2010b). The same group developed an additional method for highly controlled synthesis of magnetite crystals using the recombinant magnetotactic bacterial protein Mms6 in aqueous solutions at reduced temperatures. Crystallographic study of magnetite crystals reveals that Mms6 mediates the development of a peculiar crystal shape of magnetite particles with narrowscale distribution close to that seen in magnetic bacteria. Mms6 aggregates have a high affinity for iron ions in aqueous solution and have motif sequence in many biomineralization scaffold proteins, close to other organisms. If compared to Mms6, crystals have identical sizes (20 nm) and morphologies (cuboctahedral). This means that Mms6 has a direct impact through the synthesis process on size and shape of nanoparticles (Amemiya et al. 2007). Particle size control for other nanoparticles has also been seen. For instance, Yan et al. (2009) find that yeast induction is an efficient way of achieving a small diameter distribution of zinc phosphate powders. To prevent the large accumulation of Zn₃(PO₄)₂ particles to completely control particle size and shape, their method used the yeast feature in reaction mechanism.

8 Nanoparticle Applications

Nanomedicine is a booming scientific area with a vast potential to improve human disease diagnosis and care (Fadeel and Garcia-Bennett 2010). The most widely used nanomedicine nanoparticles are fluorescent biologic labeling, drug/molecular delivery agents, as well as tissue engineering (Tian et al. 2008), heat tumor destruction (hyperthermia), MRI contrast enhancement, and phagokinetic analysis (Parak et al. 2002). Many reviews and research articles have been published that analyze nanoparticles' applications in biomedicine (Piao et al. 2011). Though biosynthesized nanoparticles are relatively new, research has been initiated on applications in drug delivery, cancer care, genetic modification and DNA sequencing, antimicrobials, biomaterials, and response enhancement.

8.1 Antibacterial Agent

Silver-based antiseptics were stressed in recent times due to proliferation and rise of microorganism resistance to various antibiotics. The use of *Trichoderma viride* fungus in silver nanoparticles was biosynthesized (Fayaz et al. 2010). Aqueous silver (Ag+) ions were found to be decreased in solution when exposed to *Trichoderma viride* filtrate, resulting in production of pretty stabilized AgNPs. Nanoparticles

have also been tested with multiple antibiotics for increased antimicrobial activity toward Gram (positive and negative) bacteria. With the existence of AgNPs, antibacterial efficacy of erythromycin, chloramphenicol, ampicillin, and kanamycin toward test strains has been improved. Strongest enhancement effect of ampicillin against test strains was detected. Results showed greater antimicrobial effects in combination with antibiotics with AgNPs and offered valuable insight into the production of new antibacterial agents. Duran et al. (2007) have demonstrated that extracellularly generated silver nanoparticles utilizing F. oxysporum could be integrated through woven materials in an effort to avoid or decrease contamination with infective bacteria like S. aureus. Silver nanoparticles of Acalypha wilkesiana (AW-AgNPs) demonstrated substantial repression toward dominant Gram-negative and Gram-positive selected bacteria. Therefore, AW-AgNPs may be suggested as a potential antimicrobial and therapeutic agent against multidrug-resistant pathogens (Dada et al. 2019). The key components of AgNPs, CuONPs, AuNPs, and ZnONPs have been updated and commonly used for therapeutic and medicinal purposes (e.g., as antibacterial, antifungal, antiviral, anti-amebial, anticancer, anti-angiogenic, anti-inflammatory factors). These particles were suggested as alternatives to standard antibiotics to overcome bacterial resistance due to their excellently described antibacterial activity toward Gram (positive and negative) bacteria. Nanoparticles utilize mechanisms involved that differ from traditional therapies, with the benefit of becoming effective toward antibiotic resistance bacteria which have already formed, as well as by attacking several biomolecules that compromise resistant strain growth (Sánchez-López et al. 2020).

9 BM-NPs: Synthesized as Antimicrobial, Antiviral, and Scolicidal Potential from *Penicillium* Species

There have been studies of silver nanoparticle (AgNPs) biosynthesis caused by *Penicillium citrinum* (Yassin et al. 2017). Biogenic AgNPs toward aflatoxinic *A. flavus* were also tested. Biogenic AgNPs toward aflatoxinic A. flavus var. columnaris isolated from sorghum seeds were also tested for antifungal activity (Fig. 12.3). They showed that action of AgNPs toward *Aspergillus flavus* varied from 20.28 to 50.00%, and 224.5 to 4001.8 ppm were calculated at ED50 and ED95, respectively. Such antifungal activity was linked to the cell membrane and cytoplasm modification, membrane permeability, and DNA energy depletion. In extracellular biomimetic synthesis, AgNPs induced by *Penicillium chrysogenum* strain FGCC/BLS1 have been reported (Saxena et al. 2017). Their analysis showed potent antibacterial activity of AgNP at 100 ppm and antifungal activity at 100 ppm toward *E. coli*, *K. pneumoniae*, and *S. aureus* against phytopathogenic fungi *sclerotiorum*. In hemolytic test with a dose of 10 ppm in red blood cells, no cytotoxicity was observed. Exceptionally, biogenic synthesis of gold nanoparticles in an extracellular approach with *P. funicular* BL1 in 18–28 nm range has been documented

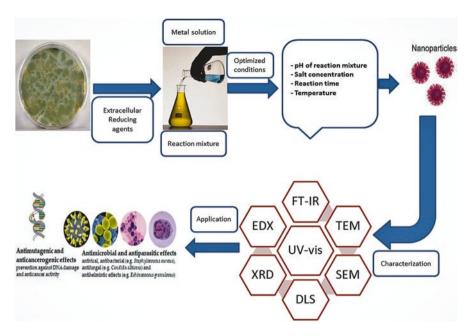


Fig. 12.3 A modern version of pharmaceutical nanobiotechnology and the interface of nanotechnology, bacteria, and pharmaceutical ability

(Maliszewska et al. 2017). They demonstrated a photodynamic inactivation of Candida albicans planktonic and biofilm cells in combination with synthesized biogenic AuNP exposure to rose bengal (RB). AuNPs showed no unusual murder of Xe lamp glare exposure to Candida albicans. However, killing was shown to be a fair efficiency of Candida albicans when RB and biogenic NPs are administered together like photosensitizing agent. Combination of RB and AuNP showed that 4.7 log10 and 4.89 log10 had decreased CFUs, which were 99.91 and 99.99%, while 98.21 and 99.37% were killed by RB alone after the same time. Furthermore, by using Penicillium spp. biosynthesized AgNPs. in an extracellular way (Verma et al. 2013). Maximum antibacterial activity in AgNPs was observed in Bacillus and Pseudomonas spp., accompanied by E. coli and Salmonella spp. at concentrations of 1 mg/mL if used in conjunction with tetracycline, and maximum inhibition was observed in Salmonella, Pseudomonas, and Escherichia coli. A research was performed using a disc diversion approach for Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Candida albicans to determine antimicrobial activities of biofabricated AgNPs of Penicillium aculeatum Su1. In either study, 200 µg/mL AgNPs had strongest antibacterial effect on all listed strains compared to 100 µg/mL AgNPs with a big variation relative to 50 and 200 µg/mL AgNO₃ (Osman et al. 2015). Notably, Solanki et al. (2016) extracellularly synthesized AgNPs using Penicillium brevicompactum between 6.28 and 15.12 nm. All through research, antimicrobial activity of biofabricated AgNPs has been evaluated utilizing disc-diffusion methods for clinically isolated pathogenic bacteria such as

E. coli, S. aureus, and P. aeruginosa. They found that regardless of whether AgNP concentration improved, a dose-dependent zone of inhibition often increased. The inhibition zone for the 10 µL concentration between 7 and 16 mm was found in depth, while for the 20 µL concentration, the inhibition region was significantly found between 9 and 28 mm. In addition, Khan and Jameel (2016) extracellularly biosynthesized AgNPs with *Penicillium fellutanum* within a domain of 10–100 nm. Antifungal activity was assessed through the use of discharge assays against Candida glabrata, Candida albicans, and Candida tropicalis, though AgNO₃ solution was not found to inhibit the region. Ammar and El-Desouky (2016) have also documented biosynthesis induced by HA₂N Penicillium expansion between 14 and 25 nm. For A. ochraceus and A. niger with disc-diffusion process, researchers even searched for an antifungal role for biogenic AgNPs. In particular, at concentration of 9 µg AgNPs in A, maximum inhibition level was observed in Aspergillus niger. Moreover, AgNPs with culture medium concentration of 220 µg/100 mL were found to cause, with 52.18% decrease percentage, the most important mycotoxin produced by Aspergillus ochratoxin, called Aspergillus ochraceus. Majeed et al. (2016) have documented an extracellular approach of biomimetic synthesis of AgNPs ranging from 30 to 60 nm. Appraised antibacterial activity of AgNPs using Proteus vulgaris, Staphylococcus aureus, Escherichia coli, and Vibrio cholera diffusion methods. For disc-diffusion research, every disc was saturated for 20 µg/mL of AgNPs. Antibiotics such as amoxicillin, carbenicillin, cefixime, ofloxacin, and piperacillin were contrasted with AgNPs. Antimicrobial activity of Ag nanoparticles recorded strong via a zone of inhibition for E. coli, V. cholera, P. vulgaris, and S. aureus. Amusingly, Ag nanoparticles strengthened their antibacterial efficacy in combination with the aforementioned antibiotics. Moreover, Sarsar et al. (2015) recorded biogenic AgNP production utilizing 5-25 nm range of Penicillium atramentosum KM filtrate extract. Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Micrococcus luteus, Staphylococcus aureus, and Salmonella typhimurium disc-diffusion process tested antibacterial activity. Significant antimicrobial activity toward Bacillus cereus has been observed. A considerable surface area was provided as AgNPs, contributing to its connection to the cell wall, increasing the integrity of cell membranes causing apoptosis, and the authors advocate it for stronger bacterial communication. It also showed a substantial increase of antibacterial activity of microgravity-synthesized AgNPs than of usual gravity-synthesized AgNPs (Sheet et al. 2017). A research was carried out by Ali et al. (2014) that otherwise recorded antimicrobial activity for AgNP extracellular/intracellular production using Pseudomonas citreonigrum with micro-dilution technique toward B. subtilis, S. aureus, S. typhimorium, E. coli, and P. aeruginosa and demonstrated antifungal effect toward Aspergillus utilizing micro-dilution technique. In this research, the antiviral effect toward type 2 herpes virus and the cytotoxicity toward three cancer cell lines were also seen. Significant antiviral activity at concentrations of 50 μg/mL, medium antiviral activity at concentrations of 25 μg/mL, or poor performance at concentrations of 12.5 µg/mL has been seen in extracellular environment-generated AgNPs, while far poorer results were found in intracellular AgNPs at concentrations of 50 and 25 µg/mL. Authors proposed throughout viral membrane whether disulfide linking areas in the glycoprotein subunit would interact with AgNPs smaller than 10 nm in size because of their surface plasmon vibration and broad efficient dispersion cross-section including its individual AgNPs. It is important to remember that *P. aculeatum* used a mean diameter of about 60 nm and good scolicidal effect toward *Echinococcus granulosus* protoscolices. Extracellular biosynthesis of AuNPs is documented (Barabadi et al. 2017). Their results show that after 120 min of exposure, the scolicidal behavior of AuNPs was equal to that of AgNP, selenium NPs, 20% AgNO₃ at 20 min, and isotonic saline at 20%.

Synthesis of extracellular AgNP has been recently documented by Sheet et al. (2017) to assess its biological and physicochemical role, using microgravity and ordinary conditions. Findings indicate cytotoxic effects of microgravitysynthesized ANPs on cancer cells are much greater than standard severitysynthesized ANPs. In the range of 4-55 nm of exploited Penicillium aculeatum Su1, extracellular biosynthesis of AgNPs was stated (Ma et al. 2017). This research revealed that biosynthesized AgNPs are far more biocompatible with human bronchial epithelial cells than AgNO3 and were substantially dose-determined toxic to A549 cells via IC₅₀ of 48.73 µg/mL, reflecting a potential impact on human pulmonary adenocarcinoma cell proliferation. Moreover, cytotoxic activity of AgNPs was biosynthesized with the use of *Penicillium* spp. in vitro in a sample. Cell lines with human colon adenocarcinoma (HT-29) ranging from 5 to 100 μg/mL were tested in contrast to normal Vero cell lines. Findings showed that AgNPs of IC50 had a cytotoxic effect of 30 µg/mL to HT-29, while IC50 was anticipated to be far greater than 50 µg/mL for the standard Vero cell line (Verma et al. 2013). Also, a research study found that biogenic AgNPs provided cytotoxic effects on the A549 cancer cell line, whereas their toxicity was significantly lower at the same level as the usual Vero cell line. Expansion of AgNPs by active oxygen species, which causes oxidative damage that induces higher levels of necrosis at higher levels and not just affects critical enzymes, was explained by researchers (Majeed et al. 2016). Ali et al. (2014) also reported intracellular/extracellular AgNP biosynthesis by using P. citreonigrum throughout the order of 10-50 nm. AgNPs were tested for cytotoxicity on (breast, colon, liver) cell lines. In dramatic terms, extracellular AGNPs showed significantly greater inhibition effect of three cancer cell lines than intracellular NPs. For this relation, researchers indicated that interruptions of AgNPs in the mitochondrial breathing chain might contribute to ROS, which interrupts ATP production and leads directly to DNA damage. Furthermore, Vazquez-Muñoz et al. (2019) provide a deeper understanding of the complementary mechanism of AgNPs and antibiotics to effectively fight antimicrobial pathogens to alleviate current crises due to antibiotic resistance, particularly those with multidrug-resistant microorganisms.

10 Microbial-Based Crop Safety Nanoparticle Applications

Through the manufacture of nanomaterials, the distribution of inorganic fertilizers and biopesticides to agriculture or a fully qualified approach to gene transfer, nanobiotechnologies, including detection and control for phytopathogens and food safety against infections, can be widely used (Fig. 12.4). Nanoparticle crop protection applications are considered effective if they stay active in extreme conditions like temperature variations, target pathogen penetration, tolerance to phytopathogens, cheap cost of formulation preferably in advanced mode of action, and social and economic advantages (Smith et al. 2008). In growing effectiveness and stabilization of utilized cells and enzymes, nanoparticles play a pivotal role. Nanomaterials result from biomolecular integration (enzymes, metabolites, etc.) or full cell hybrid systems with different agricultural uses (Bailey et al. 2010). Microbe-integrated nanoparticles gain from improved biological efficacy, fast fixation over the wide surface region, increased bioavailability and versatility, reduced toxicity, and improved mass delivery systems. Next NPs are trapped and nanomaterials are fused, and active ingredient is released in a controlled manner. The use of NP aids would involve a tailored distribution strategy based on the actions and environmental conditions of phytopathogens. For instance, DNA-coated AuNPs have been utilized as a shot to bombard plant and tissue cells to induce gene transfer in gene gun protocol (Vijayakumar et al. 2010). Microbes (bacteria, fungi) and its metabolites (enzymes, inhibitors, antibiotics, toxins) have been able to use biocontrol factors to protect plants or to improve the productivity of plants for years.

Coating of polymeric NPs provided advanced pathways for improving efficiency and stability of biocontrol agents, as gravity preparations for formulations supplied to targeted pathogens with a directed distribution system. Besides, trapped nanomaterial products can support the growth of soil and plants (Peteu et al. 2010). Fungal biological control factors are highly precise and are widely available without ingestion, for mass manufacturing by contact. Many fungal genotypes (Beauveria, Nomuraea, Verticillium) spread infection via conidia, requiring humidity to allow host pathogenesis to germinate (Kulkarni et al. 2008). To stabilize Myrothecium complex enzymes, nanoformulation with chitosan and montmorillonite clay NPs was produced and demonstrated for Fusarium spp. Gossyphilous Phenacoccus and biocontrol, with a sluggish discharge of enzymes (cotton mealybug). Antifungal hydrolases and enhanced chitina and chitosanase enzymes are induced by Chito nanoparticles handled with curcuma plants to protect plant host that have made them resistant to turmeric red Pythium aphanidermatum rhizome (Anusuya and Sathiyabama 2013). Silica-based NPs (60 nm) packed with fluorescent dye and covalently linked with microbe surface antigen-specific antibodies are sensitive. Copper is converted through metal NPs by popular plant species (Phragmites australis and Iris pseudacorus) if produced using endomycorrhizal fungi in polluted soil (Manceau et al. 2008). The inhibition efficacy of Ag₂S nanocrystals and ZnTiO₃ was higher. In corn-treated plants by silica NPs, greater tolerance to F. oxysporum and A. niger has been exhibited (Suriyaprabha et al. 2014). TiO₂ NPs have improved

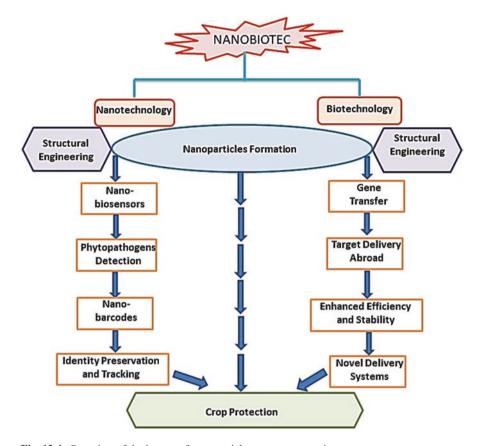


Fig. 12.4 Overview of the impact of nanoparticles on crop protection

and provided defense toward Alternaria brassicae (Bacillus amyloliquefaciens) in Brassica napus rhizosphere (Palmqvist et al. 2015). Zinc oxide NPs showed that conidium and Penicillium expansum conidium were inhibited, resulting in fungal mat absence (He et al. 2010). Magnetic reverse of nanoparticles is an extremely precise and sensitive approach. To detect *Prunus necrotic ringspot virus* promptly, reverse transcription loop-mediated isothermal amplification (RTLAMP) was established (Zong et al. 2014). Incubated under atmospheric conditions with combination of CdCl₂ + SeCl₄ and CdCl₂ + TeCl₂ by electron transmission microscopy (TEM) and electron diffraction under specific conditions, high fluorescence CdSe QDs and CdTe QDs are metabolized by F. oxysporum (Shaligram et al. 2009). Yeast cells have also been used for nanoparticle cadmium telluride (CdTe) biosynthesis QD of tunable fluorescence emission (Nayak et al. 2010). To reduce time to classify unique phytopathogens, the nucleic acid sensor bound to quartz crystal microbiological sensor surface could be coupled with rapid PCR protocols (Maliszewska et al. 2013). Through the use of AgNPs, nanobiotechnology has lately become more effective toward multiple phytopathogens. AgNP interaction with microbes

increases because of a higher surface-to-volume proportion and hence greater permeability (Kim et al. 2008). This reduced solution results in the production of highly stable AgNPs with sizes of 5–40 nm when aqueous silver (Ag⁺) ions are treated with *Trichoderma viride* filtrate (Fayaz et al. 2010). Antibiotic mixture with AgNPs has been tested to have a stronger antimicrobial effect on many types of bacteria (Aziz et al. 2014, 2015, 2016). Infection of *S. aureus* pathogens in textiles for extracellularly formed AgNPs containing *F. oxysporum* was reduced (Duran et al. 2007). Highest inhibition of disease was also found in *Colletotrichum* species (*C. acutatum*, *C. gloeosporioides*, *C. higginsianum*, *C. nigrum*, *C. orbiculare*, *C. dematium*) or cucumber, pumpkin, and powdery mildew. DNA-directed AgNPs can be removed by *Xanthomonas perforans* leaf spot disease (Ocsoy et al. 2013).

In other studies, biogenic silver nanoparticles have impregnated and reported superior antibiotic disc activity (chloramphenicol) with two pathogenic bacteria Abelmoschus esculentus and Citrullus lanatus (Citrobacter freundii and Erwinia cacticida) diseases (Paulkumar et al. 2014). Substantial antifungal effect toward spot blotching disease in wheat induced by Bipolaris sorokiniana has been metabolized and illustrated (Mishra et al. 2014). Xanthomonas axonopodis fluorescent silica nanoparticles (FSNP) were correctly demonstrated in tomatoes and peppers in conjunction with antibody molecules to prevent vesicatoria that cause bacterial spot disease (Mishra et al. 2010). Nanoparticles include antibodies used to detect Xanthomonas axonopodis (Yao et al. 2009). Ag nanoparticles increasingly attracted researchers worldwide for their antimicrobial agents so their production is more cost-effective and competitive for plant disease control. If utilized in consortiums with several other nanocrystals, numerous studies have shown powerful effects on AgNPs. With the use of Ag-SiO₂ NPs, Botrytis cinerea has been reduced by significant antifungal activity (Oh et al. 2006). Ag nanoparticles have been tested toward Phoma glomerata, Phoma herbarum, Fusarium semitectum for antifungal activity with fluconazole spp., Trichoderma, and C. albicans through disc-diffusion method (Gajbhiye et al. 2009). Throughout the type of Colletotrichum gloeosporioides (competence of anthracnosis), B. sorokiniana, M. grisea, and S. cepivorum, sclerotium-forming phytopathogenic fungi, the existence of AgNPs has been significantly inhibited. AgNP fungistatic and fungicidal action against Ambrosian fungus Raffaelea spp. and Fusarium culmorum was examined, as well as some pathogenic yeasts (Candida albicans, Candida parapsilosis, Candida tropicalis) (Kasprowicz et al. 2010). Inhibition effect has shown to be 15 mg of AgNP toward Alternaria alternata, Botrytis cinerea, Curvularia lunata, Macrophomina phaseolina, Sclerotinia sclerotiorum, and Rhizoctonia solani.

11 Conclusion

Nanomedicine is a thriving scientific area with enormous potential for human diseases to be properly diagnosed and treated. Biological synthesis of microbial nanoparticles for "green chemistry" is considered safe, nontoxic, and

environmentally acceptable. Depending on the location of intracellular and extracellular production of nanoparticles, microorganisms, like bacteria, leaves, fungi, and actinomycetes, may be used. Shape and size of nanoparticles in intracellular particle form could be manipulated to a certain degree using control factors like pH, temperature, substrate concentration, and exposure time. The study is presently being performed to monitor molecular and proteomic microorganisms. These techniques and their industrial use in medicine and health care are expected to be applied on a large scale in the next few years, with latest developments and ongoing attempts to increase the efficiency of particulate synthesis and to explore biomedical applications. Over the last decade, there have been huge advances in the field of nanoparticles developed by the microorganism and their applications. However, to improve synthesis and track size and morphology of particles, a lot of work needs to be done. Compared with the physical and chemical process, it is recognized that production of nanoparticles with microbes (several hours, even some days) is a really slow process. Reducing time of production would make this path even more appealing. Particle size and monodisperse particles are two main concerns in the assessment of nanoparticle synthesis. Efficient particle size and monodisperse regulation must therefore be thoroughly examined. Several studies have shown that after a certain period, nanoparticles produced by microorganisms can decompose. The stability of biological nanoparticles therefore needs further research and should be improved. Because particle shape control in the physical and chemical production of nanoparticles is indeed research subject, biological mechanisms with the ability to specifically regulate particle shape would seem to have significant benefits. Adequate control of particle size and monodisperse particle may be given with varying conditions like microorganism type, microbial growth phase, growth medium, synthesis, pH, substratum concentrations, target nanoparticles' origin compound, temperature, process period, and nontarget ion addition. Biosynthesis methods are also beneficial, as nanoparts are mostly covered by lipid molecules, which give biological stability and solubility, which is important for biomedical applications and other synthetic processes for bottling. Research is currently being conducted to control genomic and proteomic cells. Shorter response period and high composition efficiency are being achieved with a deeper understanding of the system of molecular and cellular synthesis, particularly separation and characterization for those molecules responsible for nanoparticle depletion.

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