Chapter 7
Microbial Production of Nanoparticles: Mechanisms and Applications

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Contents

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7.1 Introduction

Microbial bioprocessing for the sustainable production of nanomaterials holds great promise. It is a clean, nontoxic, eco-friendly and cost-effective process that is emerging as a safe biogenic route in the field of nanobiotechnology. It is pertinent that physico-chemical methods for nanomaterial production are capital-intensive, inefficient with regard to materials and energy balance, and may require/produce toxic chemicals. Microbial production of nanomaterials is a type of bottom-up approach where formations of nanomaterials occur by the oxidation or reduction of a metal, and the agents mainly responsible for such processes are the different enzymes secreted by microbial systems. Versatile nanoparticles (NPs) possess unique and biocompatible properties that have encouraged scientists to explore biogenic routes of NP production from different types of microbes, in particular bacteria and fungi (Prasad et al. 2016; Aziz et al. 2016, 2019; Elgorban et al. 2016; Vago et al. 2015; Neveen-Mohamed 2014), and algae (Ebrahiminezhad et al. 2016; Kumar et al. 2014; Aziz et al. 2014, 2015).

Nanoparticles have unique physico-chemical features such as a definite size, a surface area to mass ratio, chemical stability with high reactivity and functionalized structure with desired biocompatibilities (Kong et al. 2017). Microbial NPs have gained attention because of the simplicity of its mode of action, ease of surface modifications, a plethora of applications, such as data storage, antimicrobial, sensing, sustainable agriculture, environment, especially in biotechnology as a nanocarrier for enzyme immobilizations and for drug delivery (Golchin et al. 2018; Prasad 2016, 2017; Prasad et al. 2016, 2018a, b; Verma 2018; Verma et al. 2016; Verma and Barrow 2015; Dykman and Khlebtsov 2017; Verma 2017a, b, c; Kumar et al. 2014; Verma et al. 2013a, b, c, d; Verma et al. 2012). Functionalized microbial NPs can effectively penetrate across obstacles through small capillaries into individual cells, thereby presenting huge probabilities for specific drug delivery to the disease site. Microbial NPs have tremendous potential to deliver multiple drug molecules, recombinant proteins, vaccines, or nucleotides to their target sites effectively (Pelaz et al. 2017).

This chapter provides an overview of microorganism-mediated biogenic synthesis of extracellular/intracellular NPs under ambient conditions. Recent applications of computational techniques to understand the role of capping agents binding to microbial NPs are discussed. The possibility of scaling up the study with respect to fungal gold (Au) NPs is discussed in particular, to envision the scope of large-scale production in an industrial setting. Various applications of microbial NPs, including antimicrobial, specific delivery (bioactives, drugs), and sensing, are also critically discussed.

7.2 Biosynthesis of Extracellular/Intracellular Nanoparticles from Microbes: Mechanism and Capping Agents

Recently, many research studies have been published on the synthesis of NPs through various microorganisms. Amongst various microorganisms, the most notable microorganisms employed for the synthesis of various NPs such as Au,

silver (Ag), and iron (Fe), etc., are the bacteria and fungi. Microorganisms are considered cost-effective and eco-friendly nanofactories for the production of NPs. Because of their intrinsic potential, they produce NPs, which are intra- and/or extracellular in nature (Asmathunisha and Kathiresan 2013; Prasad et al. 2016).

7.2.1 Mechanism

Formation of gold nanoparticles (AuNPs) can occur in either the intracellular or the extracellular space. Extracellular AuNP formation is commonly reported for fungi when Au^{3+} ions are trapped and reduced by proteins in the cell wall. The average size of synthesized AuNPs is approximately 15 nm. The reduction of Au^{3+} ions occurs through the cell membrane and cytoplasmic region (Das et al. 2012a). It is suggested that these regions might be responsible for reduction of Au^{3+} to Au^{0} because of the presence of electron dense particles in these regions, i.e., cell wall and cytoplasmic regions (Das et al. 2012b).

There are two main precursors of AuNPs in the biosynthetic process: $HAuCl_4$, which dissociates to Au^{3+} ions (Khan et al. 2013), and AuCl, which dissociates to Au^{+} (Zeng et al. 2010). However, it is not clear whether the diffusion of the Au^{3+} ions through the membrane occurs via active bioaccumulation or passive biosorption. It may be due to the toxicity of Au^{3+} ions, which increases the porosity of the cellular membrane. The enzymatic reduction mechanism of Au^{3+} is the same for intracellular and extracellular AuNPs (Gupta and Bector 2013). It has been observed that NADH-dependent reductases are involved in the bioreduction process while working on AuNP biosynthesis by the soluble protein extract of the fungus *Fusarium oxysporum* (Mukherjee et al. 2002). However, the role of the specific protein(s) involved in Au reduction has not yet been identified.

Some fungi, namely *Candida albicans*, produce phytochelatins, an oligopeptide chain of glutathione, cysteine, and glycine that is involved in the biosynthesis of AuNPs. Phytochelatins are formed in the pathogenic fungus with the aid of the transpeptidation reaction of dipeptides from a glutathione molecule.

7.2.2 Capping Agents

Microorganisms use extracellular proteins as capping agents to minimize AuNP aggregation and thus stabilize the nanocrystal because small NPs are unstable. As fungi secrete a variety of enzymes and proteins, there are specific organic molecules that act as capping agents. Three capping proteins with a molecular weight of about 100, 25, and 19 kDa from AuNPs synthesized by *Fusarium oxysporum* were identified as plasma membrane ATPase, 3-glucan binding protein, and glyceraldehyde-3-phosphate dehydrogenase (Zhang et al. 2011).

Capping agents are used to minimize NP aggregation, thus stabilizing the nanocrystal, and resulting in the production of NPs with a narrow size and shape

distribution that may further be applicable for biomedical and industrial purposes. Many surfactants have also been reported and used as capping agents to alter the desired shape and size of the NPs, but these are difficult to remove and do not easily degrade. Thus, the commercial surfactants are hazardous to the environment (Liu et al. 2005; Gittins et al. 2000). Keeping in mind the limitation possessed by these chemicals, there is an urgent need to use environmentally friendly capping agents and design green biochemicals on a commercial scale for NP synthesis. There are various molecules that could act as capping agents, but there are some green capping agents with their potential role.

7.2.2.1 Biomolecules

Microbes secrete various biomolecules; these molecules of microbial origin improve the homogeneous preparation of NPs by adhering to green chemistry rules. Amino acids act as efficient reducing and capping agents to synthesize NPs. Different types of amino acids were used as capping agents and the same were employed for the synthesis of AuNPs (4–7 nm in size) using tetra auric acid. Of the 20 amino acids, L-histidine was adopted, which was found to reduce tetra auric acid ($AuCl_4$) to AuNPs. The concentration of L-histidine was found to affect the size of the NPs and their aggregates. Moreover, the amino and carboxy groups present in the amino acids caused the reduction of $AuCl_4$ and coating of the NP surface (Maruyama et al. 2015).

7.2.2.2 Polysaccharides

Polysaccharides act as capping agents in NP synthesis as they are low-cost, hydrophilic, stable, safe, biodegradable, and nontoxic. In NP synthesis, water is used as a solvent, in place of toxic solvents (Duan et al. 2015; Akhlaghi et al. 2013). A polysaccharide such as dextran, a polymer of glucose molecules, is hydrophilic, biocompatible, nontoxic, and used for coating many metal NPs (Virkutyte and Varma 2011). The components of natural honey act as a source of both reducing and protecting agents to synthesize spherical AuNPs of 15 nm in size in water. Fructose present in the honey is supposed to act as a reducing agent, whereas proteins are responsible for the stabilization of the NPs (Philip 2009). AgNPs are synthesized within the size range of 2–14 nm using aminocellulose as a reducing and capping agent. The amino cellulose stabilizes aqueous colloidal solutions of NPs and shows significant antibacterial action against all bacterial isolates (Cheng et al. 2013).

7.3 Understanding the Role of Capping Agents or Biomolecules Binding to Nanoparticles via Computational Techniques

With the upcoming new field parameters such as supercomputing and computational fields, we can compile the interaction of molecules. Different techniques are being employed to study interactions, namely, density functional theory (DFT), molecular dynamics simulations, docking, etc. The interaction of biomolecules using a phase display approach identifies the physical link between peptide and substrate interactions. These interactions provide the controlled placement and assembly of molecules, thereby broadening the scope of NP synthesis (Whaley et al. 2000).

Amino acid residues present in the biomolecules show facet-specific binding for the formation of Au nanoplates and helps in the fabrication of nanostructures (Shao et al. 2004). This mechanism has been further explored using molecular dynamics simulation with the application of an intermolecular potential CHARMM-METAL. The adsorption strength correlates with the degree of coordination of polarizable atoms, i.e., O, N, and C to different epitaxial sites. It has been observed that the size and geometry of NPs determine the adsorption energy and show significant attraction to the metal surface (Feng et al. 2011). Facet-specific interaction of biomolecules with inorganic materials was also investigated using the DFT method. It is further demonstrated that the specific surface recognition of an amino acid side chain occurs because of the combination of various processes such as electron exchange, dispersion, and partial charge transfer showed great binding affinity (Ramakrishnan et al. 2015). The results showed that electrostatic interactions are responsible for the binding of biomolecules, i.e., amino acid residues. The short-term elevation of reaction temperature provides fast and high adsorption affinity of oligonucleotides on the surface of AuNPs and it was observed that the binding ability depends on the length of the oligonucleotide and its nucleotide composition (Epanchintseva et al. 2017).

7.4 Case Study of Scaling Up with Respect to Fungal Biosynthesis of Gold Nanoparticles

Microbes are found to be small nanofactories, and microorganism synthesis of NPs has united biotechnology, biological science, and technology in a brand-new field of nanobiotechnology (Fariq et al. 2017; Abdel-Aziz et al. 2018). Microbes are used everywhere in the world for the biological synthesis of NPs because they grow fast, are simple to cultivate, and can grow at numerous temperatures, pH values, and pressure (Rai and Duran 2011). Microbes use their intrinsic potential to synthesize NPs of inorganic material by an intracellular and extracellular reduction

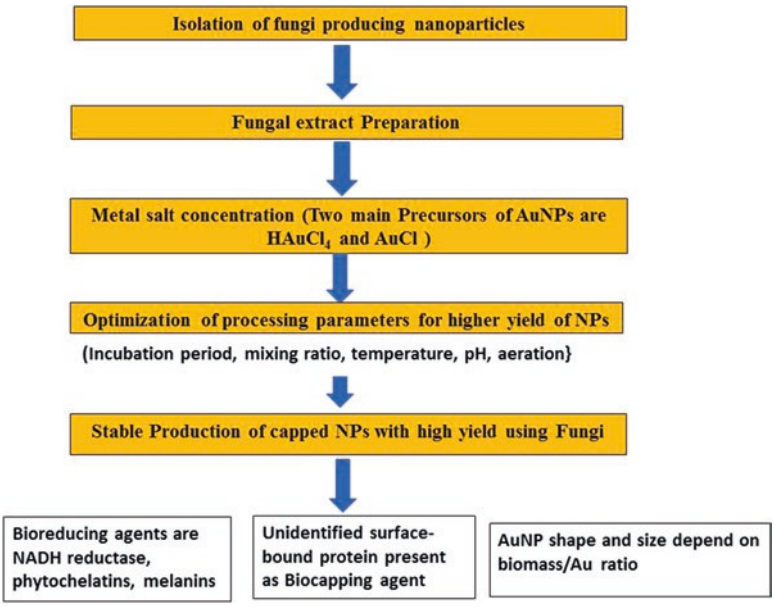

Fig. 7.1 Biosynthesis of gold nanoparticles (NPs) using fungi

mechanism (Fig. 7.1). Some enzymatic activities of microbes turn metal ions, which are trapped by microorganisms, into the elemental form (Li et al. 2011). Heavy metal ions can be reduced by bacteria to produce NPs. Large-scale sustainable production and less frequent use of toxic chemicals are some of the advantages of bacteria-based NP synthesis, but less control over size, shape, and distribution of NPs and laborious culturing processes are some of the disadvantages. Fungi also possess various intracellular and extracellular enzymes capable of producing monodispersed NPs with well-defined geometries and sizes (Fig. 7.2). Stable and easy biological NP synthesis can be achieved by mycosynthesis. Because of the relatively larger biomass, the yield of NPs is high in fungi compared with bacteria. Fungi exhibit a great capacity to bind metal salts to their cell wall, which leads to a higher uptake of metal and provides greater tolerance of metals, eventually resulting in massive NP productions (Fig. 7.3). Three possible mechanisms have been proposed to explain the mycosynthesis of metal NPs: nitrate reductase action; electron shuttle quinones; or both. Fungi have been reported to produce NPs with diverse sizes and shapes. The studies on fungi can be easily extrapolated to others. Fungal production of NPs, is achieved at the extracellular and at the intracellular level.

Industrial production of homologous and heterologous proteins is achieved using a high concentration of the fungal secretome. For example, the entomopathogenic fungus *Beauveria bassiana* has been reported for the expression of a functionally active class I fungal hydrophobin (Kirkland and Keyhani 2011). The tripeptide glutathione, which is a well-known reducing agent, is involved in metal reduction, and in yeasts and fungi it participates in cadmium sulfide (CdS) biosynthesis. Recombinant

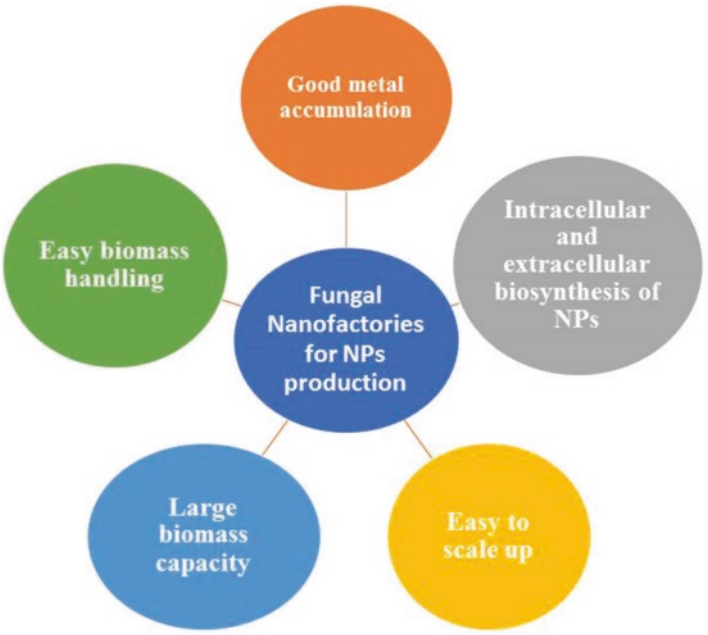

Fig. 7.2 Fungal are the ideal nanofactories for NP production

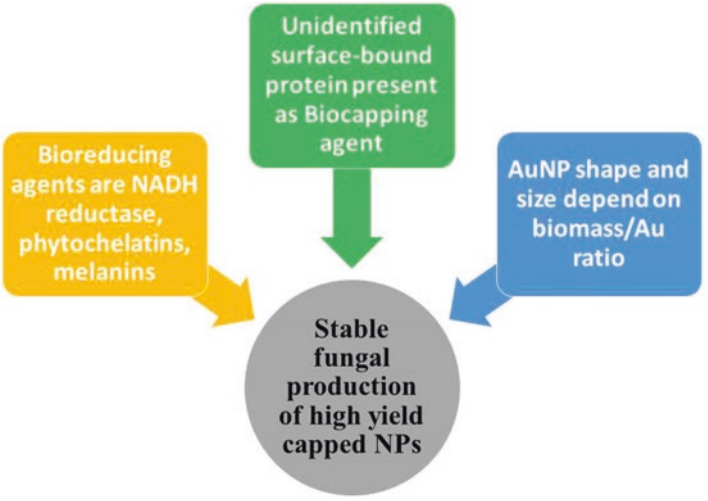

Fig. 7.3 Factors responsible for the stable fungal production of capped NPs

expression of glutathione in *E. coli* for CdSNP production has been reported by Chen et al. (2009). Use of *Verticillium* sp. for the synthesis of AuNPs was the first case where eukaryotic organisms were used for NP synthesis (Mukherjee et al. 2001). Extracellular and intracellular synthesis of NPs was reported on the cytoplasmic

membrane and on the surface of the fungal mycelia. Intracellular generation of AuNPs has been demonstrated by typical purple color formation of the mycelia mass. Transmission electron microscopy (TEM) analysis showed that particles of well-defined geometry such as triangular, hexagonal or spherical shapes, were formed on the cell wall and quasi-hexagonal morphology was formed on the cytoplasmic membrane. *Verticillium* fungi can grow and replicate even after exposure to metal ions; therefore, fungi can be used commercially for the production of NPs. Fungal secretome has been studied very little until now, and knowledge of it is still at an early stage. In the case of fungi, the role that extracellular proteins and enzymes play in Au reduction and AuNP capping is advantageous for the large and relatively unexplored fungal secretome. Fungal biomass has a high concentration of cationic biosorption sites; thus, they have been used to remove metal cations from water (Das 2010). Biosorption on fungal biomass is higher than on bacteria, particularly at low pH. For example, under nonviable conditions, various Gram-negative bacteria can immobilize Au^{3+} at about 0.35 mM g^{-1} dry cells at pH 3 (Tsuruta 2004). At pH 2.5, *Aspergillus* sp. can immobilize about 1 mM g^{-1} dry cells (Kuyucak and Volesky 1988). Various fungal species, such as *Verticillium* sp., *Verticillium luteoalbum*, *Colletotrichum* sp., *Fusarium oxysporum*, *Trichothecium* sp., *Fusarium semitectum*, *Alternata niger*, *Helminthosporium solani*, *Trichoderma viride*, *Rhizopus oryzae*, *Aureobasidium pullulans*, *Penicillium brevicompactum*, *Cylindrocladium floridanum*, *Mucor hiemalis*, *Candida albicans*, etc., have been exploited for the biosynthesis of NPs (Zhang et al. 2011; Kumar et al. 2011; Das et al. 2009; Xie et al. 2007; Gericke and Pinches 2006; Shankar et al. 2003; Mukherjee et al. 2002; Mukherjee et al. 2001; Aziz et al. 2016).

Mukherjee et al. (2002) incubated fungal extract with 10^{-3} M $AuCl_4$ in the dark and were able to produce AuNPs of various morphologies. Bhambure et al. (2009) used *Aspergillus niger* to biosynthesize extracellular AuNPs and treatment of the fungal supernatant with aqueous Au^{+} ions produced NPs with an average particle size of 12.79 nm. Castro-Longoria et al. (2011) also demonstrated that fungus *Neurospora crassa* strains N150 can synthesize Ag, Au, and bimetallic NPs. *N. crassa* was indicated to be a potential nanofactory for metallic NP synthesis. Several advantages of using this fungus are that it is a nonpathogenic organism, has a fast growth rate, induces rapid reduction of metallic ions, stabilizes NPs, and carries out facile and economical biomass handling. In another study, on the assumption that all the Au in a solution is reduced to form NPs, the authors suggested that the approximate mole concentration of the synthesized NPs might be given (Link et al. 1999). Du et al. (2011) estimated the concentration of synthesized AuNPs measuring 45 nm within the range 10^{-9} to10^{-10} M in accordance with TEM analysis and the density of bulk face-centered cubic Au.

Das et al. (2012a, b) employed the protein extract of *Rhizopus oryzae* for the biogenic production of NPs by using reducing chloroauric acid ($HAuCl_4$). A plant pathogenic fungus named *Fusarium oxysporum* produced extracellular AuNPs (Thakker et al. 2013). Another plant pathogenic fungus named *Nigrospora oryzae* produced AuNPs with anthelmintic efficacy (Kar et al. 2014). Vago et al. (2015) demonstrated one-step biosynthesis of AuNPs by mesophilic filamentous fungi.

Magdi and Bhushan (2015) produced extracellular AuNPs by *Penicillium chrysogenum* using Au chloride ion solution ($HAuCl_4 \bullet 3H_2O$). NPs were characterized to determine the composition, shape, structure, and particle size. Dhanasekar et al. (2015) demonstrated the use of cell-free filtrate of filamentous fungus *Alternaria* sp. for the synthesis of isotropic and anisotropic AuNPs for the first time.

Pei et al. (2017) used new fungus *Mariannaea* sp. HJ cells (cell-AuNPs) and cell-free extracts (extract-AuNPs) for biosynthesis of AuNPs. Bioprocessing of AuNP synthesis was optimized for initial Au ion concentrations and pH. The authors reported that initial Au ion concentrations of 2 mM under a neutral pH of 7 were optimized for both cells and their extract. Cell-AuNPs of various shapes, including spherical, hexagonal, and irregular shapes were produced, with an average size of 37.4 nm, whereas the extracts-AuNPs with an average size of 11.7 nm were almost spherical- and pseudo-spherical-shaped.

It can be inferred from the recent studies discussed above that fungi are ideal microorganisms that can have the potential for use in an industrial setting for large-scale production.

7.5 Applications of Microbial Nanoparticles

Microbial NPs are being used in different sectors ranging from biomedical to the food industry. NPs possess a plethora of applications and act as antimicrobial agents, anticancer agents, drug delivery agents, and sensing agents, etc.

7.5.1 Antimicrobial Agents

Applications in the field of medicine include formulations of many potential antimicrobial agents that are effective against human pathogens, including multidrug-resistant bacteria (Ingle et al. 2008). Multidrug-resistant strains of bacteria have become a serious public health problem (Wright 2005). The emerging resistance of bacteria and the high cost of advanced antimicrobial drugs have encouraged scientists to search for effective, economically viable, and broadly applicable drugs (Jones et al. 2004). Therefore, the development of novel antimicrobial compounds or modification of the available ones to combat resistant pathogens is urgently needed. AgNPs produced by microorganisms are good candidates for a new generation of antimicrobial materials (Rai et al. 2009). This study focused on the biogenic synthesis of metal NPs by acidophilic actinobacteria strain HGG16n and evaluation of their antimicrobial activity. The physico-chemical characteristics of the biosynthesized AgNPs were also determined.

Microbial infections represent serious threats to human health. In addition, extensive usage of antimicrobial agents such as antibiotics render the antimicrobial-resistant microbes ineffective (Raghunath and Perumal 2017). In such scenarios,

applications of microbial NPs show strong antimicrobial cells and are safe to humans (Sirelkhatim et al. 2015; Syed et al. 2010; Ren et al. 2009; Kim et al. 2007) NPs may act against microbial infections by inducing oxidative stress in addition to non-oxidative mechanisms in the targeted microbes that act against them, providing a substitute for antibiotics and subsequently multiple drug resistance (Das et al. 2017; Wang et al. 2017).

Microbial production of AgNPs was achieved by using *Streptacidiphilus durhamensis*. NPs showed highest antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis* (Buszewski et al. 2018). Copper, tungsten carbide, and Ag showed strong antimicrobial effects against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria (Bankier et al. 2018).

7.5.2 Anticancer Agents

Biogenic synthesis of Ag NPs was achieved using microalgal secretory carbohydrates (Ebrahiminezhad et al. 2016). The synthesized NPs showed anticarcinogenic properties as novel anticancer and antimicrobial agents.

Nanoparticle-bound enzymes with peroxidase activity have dual application; first, the detection (selective quantitation and colorimetric analysis) of cancer cells, and second, cancer therapy by activating oxidative stress. Both the detection and therapeutic processes are selective to cancer cells, indicating the high specificity and robustness of the hybrid NP conjugate, proving it to be a promising candidate for clinical cancer diagnostics and treatment and their targeted drug delivery approach (Nasrabadi et al. 2016).

Nanoparticle-immobilized serratiopeptidase conjugate was developed to improve therapeutic benefit (Venkatpurwar and Pokharkar 2010). Conjugate was characterized by using UV-visible spectroscopy, TEM, X-ray diffractometry, and Fourier transform infrared spectroscopy. In vitro enzymatic activity and in vivo anti-inflammatory activity of the synthesized serratiopeptidase capped gold NPs complex confirmed the retention of biological activity. The tri-functional role of serratiopeptidase was reported, such as reduction, stabilization, and therapeutic activity, which demonstrated the viable nanocarrier for oral administration with improved therapeutic benefit (Venkatpurwar and Pokharkar 2010).

Recently, the green synthesis of biogenic NPs from microbial sources has become an emerging field owing to their safer, eco-friendly, simple, fast, energy-efficient, low-cost, and less toxic nature. Interestingly, NPs play a key role in the diagnosis of tumors at the initial stage by allowing cellular visualization (Barabadi et al. 2017).

Nanoparticles are the ideal nanocarrier for the delivery of anticancer agents, showing anticarcinogenic properties. Recently, the anticancer activity of biogenic AuNPs synthesized by using marine bacteria *Enterococcus* sp. was demonstrated against cancerous cell lines derived from lung (A549) and liver (HepG2) cells (Rajeshkumar 2016).

7.5.3 *Drug Delivery Agents*

Microbial NPs possess unique properties and biocompatibility, and are considered to be the most efficient nanocarriers for drug delivery (Xin et al. 2017; Pelaz et al. 2017). Nanomaterials possess unique properties because of the nanosized and quantum effects (Kong et al. 2017).

Nanoparticles have been employed to target cancer cells (Baskar et al. 2018). AuNPs were covalently bound to the fungal enzyme asparaginase. Molecular characterization of the NP-bound biocatalyst was done using Fourier transform infrared spectroscopy and proton nuclear magnetic resonance spectroscopy techniques. The cytotoxicity studies of lung and ovarian cancerous cells demonstrated very promising results for lung cancer cells compared with ovarian cancer cells. A therapeutic enzyme named superoxide dismutase was immobilized onto NPs that demonstrated antioxidant properties against free radicals (Golchin et al. 2018). NP-immobilized serratiopeptidase enzyme was employed for oral drug delivery and demonstrated efficient anti-inflammatory activity (Venkatpurwar and Pokharkar 2010).

Tao et al. (2015) investigated silica-coated NPs, which exhibited both oxidase- and peroxidase-mimicking activities and imparted reactive oxygen species (ROS) reactions. Antibacterial properties were demonstrated against both Gram-negative and Gram-positive bacteria. Biocompatibility of NPs, with ease of their biological and chemical nature, mimic the function of some enzymes, including super oxide dismutase (SOD), esterase, peroxidase, glucose oxidase, for various therapeutic applications such as tissue regeneration (Golchin et al. 2018).

Nanoparticle-based targeted drug deliveries have considerable ability to overcome the limitations of traditional therapeutics (Xin et al. 2017; Daraee et al. 2016). For example, many drugs are manifestly stuck owing to their inability to cross the blood–brain barrier. The ability of NPs to deliver across this barrier is enormously promising because NPs can cross several biological barriers for sustained delivery of therapeutic agents for difficult-to-treat diseases such as brain tumors (Bosio et al. 2016; Nazir et al. 2014; Hainfeld et al. 2013).

Serratiopeptidase (STP), a proteolytic endopeptidase bioenzyme is recognized as one of the most important therapeutic enzymes, having anti-inflammatory activity (Salamone and Wodzinski 1997). Traditionally, therapeutic enzyme delivery has been limited because of their poor uptake and vulnerability to degradation inside the gastrointestinal tract. For efficient drug delivery, today, NPs such as the AuNP complex have immense potential from the therapeutic perspective of biomedicine formulation. In this, the prerequisite is the nanocarrier, which plays an important role in the bioavailability of the pharmaceutical active compound and efficiently improves absorption across the gastrointestinal mucosa (Dykman and Khlebtsov 2017). Maji et al. (2015) developed a new nanostructured hybrid as a mimetic enzyme for in vitro detection and therapeutic treatment of cancer cells. In vitro studies demonstrated enhanced cytotoxicity to HeLa cells. However, it was safe for normal cells; the treatment caused no damage, proving the selective killing effect of the NPs on cancer cells.

It is inferred from the recent studies discussed above of the application of NPs that a nanocarrier-based approach such as NP-immobilized enzymes represents an important modality within therapeutic and diagnostic biomedical applications, including cancer, cardiovascular diseases, and neurological diseases.

7.5.4 Sensing Agents

Owing to the indiscriminate use of pesticides, the agricultural sector is highly contaminated by the excessive misuse of pesticides (Ghormade et al. 2011). In this area, nanosensors can detect on-site soil monitoring of real-time concentrations of pesticide residues and confirm the health of the soil for agricultural usage (Periasamy et al. 2009; Prasad et al. 2014, 2017). Nanosensors play a vital role in the real-time monitoring of pesticide concentrations in the agricultural sector. Zhang et al. (2015) developed nanosensors for the detection of organophosphorus and non-organophosphorus pesticides with a higher sensitivity limit within the range 0.5 μM to 1 μM. Da Silva et al. (2014) developed nanosensors using an atomic force microscopy tip for detection of the acetolactate synthase-inhibitor herbicides metsulfuron-methyl and imazaquin. Nanosensors were developed by coating an atomic force microscopy tip with acetyl co-enzyme A carboxylase (Amarante et al. 2014; Franca et al. 2011). Molecular modeling techniques were employed to measure the interaction at the molecular level. Gan et al. (2010) developed nanosensors for the detection of the organophosphorus pesticide at the limit of 50 pgl^{-1}. Ramanathan et al. (2009) developed nanosensors with the detection limit of 34 μM for paraoxon.

The food industry is facing challenges with the increasing incidence of food contamination by pathogenic microorganisms (Verma 2017b). Nanosensors have become the prerequisite of quality assurance in food industry. They play a crucial role in the early detection of contaminants in food samples that can avoid future loss to the food industry. NP-immobilized enzymes are being used to check the quality of food samples (Perez-Lopez and Merkoci 2011). Nanosensors were developed for biomolecule detection in food samples by immobilizing enzymes to check sugar molecules such as glucose, lactose, and fructose in food (Ozdemir et al. 2010). Nanosensors are being used in many applications in the food industry. For example, Pal et al. (2014) developed nanosensors for choline detection in milk samples within the range 0.5 μM to 2 mM; Devi et al. (2012) for the detection of the contamination of fish meat with xanthine residues at a limit of 0.1 μM; Miranda et al. (2011) for the early detection of microbial contamination; and Li et al. (2011) for aflatoxin B_1 detection at a limit of 1.6 nM.

7.6 Conclusion and Future Prospects

Biosynthesis of NPs using microorganisms offers multifarious advantages. However, to economize NP production on a commercial scale, factors such as the time-consuming production process and the cost-intensive downstreaming process need to be addressed so that cost-effective methods of production can be developed.

This write-up concludes that the production of microbial NPs is the most sought-after bioprocessing technology, which has the possibility for scaling up NP production. Microbial production of NPs demonstrates a sustainable approach for the large-scale production of NPs.

The chapter concludes with discussions on the current limitations and prospects of the biogenic production of NPs.

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