

Chapter 5

Fungal Nanotechnology: A New Approach Toward Efficient Biotechnology Application



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Abstract Nanotechnology is a wide developing area of the biotechnology since the important applications of nanoparticles (NPs) in different technologies. The NPs produced by green technologies have many advantages such as greater surface area and high catalytic activity, in addition to providing a suitable contact between the metal salt and enzyme. Fungi secrete proteins, enzymes, and reducing agents which can be used for the synthesis of metal NPs from metal salts.

The biosynthesis of metal NPs by fungi has been explored in recent years, evaluating the extracellular and intracellular chemistry of formation. Emphasis has been given to the potential of metal NPs as an antimicrobial agent to inhibit the growth of pathogenic bacteria and fungi and other potential applications such as their cytotoxic activity against cancer cell lines. Further, the metal NPs are being explored as promising candidates for several biomedical, pharmaceutical, and agricultural applications.

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5.1 Introduction

Nanotechnology is a wide developing field of biotechnology due to its great range of applications in different areas. Nowadays a large variety of methods are used for nanoparticle (NP) synthesis which in turn are able to deliver nanomaterial with desirable characteristics. A large number of NPs are continuously produced to be used in different areas/processes as biomedicine, fine chemical synthesis, cosmetics, electronics, and information technology and recently utilized as catalysts, semiconductors, optical devices, biosensors, and encapsulation of drugs (Bhargava et al. 2016; Bhushan 2010). According to their chemical nature, NPs can be classified in two major groups: organic and inorganic. The first group refers to NPs consisting mainly of carbon, while the second group refers to those composed of a noble metal as silver or gold and others as aluminum, zinc, titanium, palladium, iron, fullerenes, and copper (Boroumand Moghaddam et al. 2015). Conventional chemical methods for NP synthesis have shown certain limitations. These limitations are the use of poisonous chemicals which are responsible for different biological hazards and the use of high energy levels which increase manufacturing costs. In this context, the need to develop environmentally friendly procedures usually known as “green technologies” is a current concern.

Nanobiosynthesis is considered a green technology since it includes biological methods which have great possibilities for nanoparticle synthesis through natural biomineralization. Biomineralization is an eco-friendly and sustainable biological process that provides water-soluble particles with well-defined properties produced by a highly reproducible process (Bhargava et al. 2016; Golinska et al. 2016; Ahmed and Ikram 2016). NPs developed by green technologies have more advantages than those produced by chemical synthesis such as greater surface area and higher catalytic activity, in addition to providing a suitable contact between the metal salt and enzyme (Bhattacharya and Mukherjee 2008; Prasad et al. 2016).

Although plant tissues are an important source of NPs, microorganisms are currently explored as new biofactories of metallic NPs following simple processes such as metal reductions (Vigneshwaran et al. 2007; Sharma et al. 2009). The biomass extracts are used as extracellular or intracellular reductants (Ammar and El-Desouky 2016; Kubo et al. 2016), being the extracellular method the most popular because it facilitates the downstream process of NP recovery. Microbial nanobiosynthesis is currently the focus of interest mainly because of the production of tiny particles on a large scale to a relatively high morphology control (Salunke et al. 2016; Prasad et al. 2016).

Among microorganisms, fungi have been reported as one of the best NP producers. The synthesis of NPs using fungi has been reported by several authors (Table 5.1).

Table 5.1 Synthesis of different nanoparticles (NPs) by fungi

Fungi	NPs	Size (nm)	Shape	Pathway of biosynthesis	Application	Reference
<i>Aspergillus terreus</i> HAIN	AgNPs	10–18	Spherical	Extracellular	Antifungal activity	Ammar and El-Desouky (2016)
<i>Aspergillus niger</i>	AgNPs	20	Spherical	Extracellular	–	Ghazwani (2015)
<i>Aspergillus niger</i>	AgNPs	43–63	Spherical	Extracellular and intracellular	Antibacterial activity	Vanaja et al. (2015)
<i>Aspergillus niger</i> PFR6	AgNPs	8.7	Spherical	Extracellular	–	Devi (2015)
<i>Aspergillus</i> sp.	AgNPs	–	–	Extracellular	Antimicrobial activity	Prabavathy and Vaishnavie (2015)
<i>Aspergillus tamarii</i> PFL2	AgNPs	3.5	Spherical	Extracellular	–	Devi (2015)
<i>Penicillium expansum</i> HA2N	AgNPs	14–25	Spherical	Extracellular	Antifungal activity	Ammar and El-Desouky (2016)
<i>Penicillium chrysogenum</i>	AgNPs	30–150	Spherical	Extracellular and on the hyphal surface	–	Mohammadi and Salouti (2015)
<i>Penicillium expansum</i>	AgNPs	50–200	Spherical	Extracellular and on the hyphal surface	–	Mohammadi and Salouti (2015)
<i>Penicillium spinulosum</i> (OC-11)	AgNPs	25	Spherical	Extracellular	Promoted wound healing antibacterial activity	Wen et al. (2016)
<i>Penicillium decumbens</i> (MTCC-2494)	AgNPs	30–60	Spherical	Extracellular	Antimicrobial activity	AbdelRahim et al. (2017)
					Anticancer activity against human lung cancer cell line	
<i>Penicillium ochrochloron</i> PFR8	AgNPs	7.7	Spherical	Extracellular	–	Devi (2015)
<i>Fusarium oxysporum</i>	AgNPs	34–44	Spherical	Extracellular	Antibacterial activity	Hamed et al. (2016)
<i>Fusarium oxysporum</i>	AgNPs	5	Spherical	Extracellular	–	Ghazwani (2015)

(continued)

Table 5.1 (continued)

Fungi	NPs	Size (nm)	Shape	Pathway of biosynthesis	Application	Reference
<i>Rhizopus stolonifer</i>	AgNPs	2.86	Spherical	Extracellular	–	Khalid AbdelRahim et al. (2017)
<i>Arthroderma fulvum</i>	AgNPs	15.5	Spherical	Extracellular	Antifungal activity	Xue et al. (2016)
<i>Lentinius edodes, Pleurotus ostreatus, Ganoderma lucidum, Grifola frondosa</i>	AgNPs	5–20	Irregularly spheres	Extracellular	–	Vetchinkina et al. (2017)
<i>Alternaria solani</i>	AgNPs	25	Spherical	Extracellular	–	Ghazwani (2015)
<i>Mucor hiemalis</i>	AgNPs	5–15	Spherical	Extracellular	Antibacterial and antifungal activities	Aziz et al. (2016)
<i>Guignardia mangiferae</i>	AgNPs	5–30	Spherical	Extracellular	Antimicrobial activity	Balakumaran et al. (2015)
<i>Curvularia lunata</i>	AgNPs	10–50	Spherical	Extracellular	Cytotoxicity against human cancer cells	Ramalingam et al. (2015)
<i>Pestalotiopsis microspora VJI/ VSI</i>	AgNPs	2–10	Spherical	Extracellular	Antibacterial activity	Netala et al. (2016)
<i>Sclerotinia sclerotiorum MTCC 8785</i>	AgNPs	10	Spherical	Extracellular	Antioxidant activity	Saxena et al. (2016)
<i>Rhizopus oryzae</i>	AuNPs	16–43	Spherical	Extracellular (purified surface proteins)	Cytotoxicity activity	Kitching et al. (2016)
<i>Aspergillus aculeatus</i>	AuNPs	60–140	Flower	Extracellular (purified surface proteins)	Antibacterial activity	Kubo et al. (2016)
<i>Aspergillus sydowii</i>	AuNPs	8.7–15.6	Microtubules	Depositing AuNPs on the surface of fungi biotemplates	–	Vala (2015)
	AuNPs		Spherical	Intracellular and extracellular	–	

Fungi	NPs	Size (nm)	Shape	Pathway of biosynthesis	Application	Reference
<i>Cladosporium oxysporum</i> AJP03	AuNPs	72-32	Quasi-spherical	Extracellular	Degradation of rhodamine B	Bhargava et al. (2016)
<i>Penicillium brasilianum</i>	AuNPs	80–500	Microtubules	Depositing AuNPs on the surface of fungi biotemplates	–	Kubo et al. (2016)
<i>Lenitius edodes</i> , <i>Pleurotus ostreatus</i> , <i>Ganoderma lucidum</i> , and <i>Grifola frondosa</i>	AuNPs,	5–50	Spherical	Intracellular or on the surface of the hyphae	–	Vetchinkina et al. (2017)
<i>Alternaria</i> sp.	AuNPs	7–18	Spherical, square, pentagonal, hexagonal	Extracellular	–	Dhanasekar et al. (2015)
<i>Pycnoporus sanguineus</i>	AuNPs	10–500	Spherical, triangular, pentagonal	Intracellular	Degradation of 4-nitroaniline	Shi (2015)
<i>Penicillium</i> sp.	Fungus-Fe ₃ O ₄	<50	Spherical	Magnetic core and a mycelia layer	–	Ding et al. (2015)
<i>Lenitius edodes</i> , <i>Pleurotus ostreatus</i> , <i>Ganoderma lucidum</i> , and <i>Grifola frondosa</i>	Silicon NPs	50–250	Spherical	On the hyphal surface	–	Vetchinkina et al. (2017)
<i>L. edodes</i> and <i>G. frondosa</i>	SeNPs	20–200	Spherical	Intracellular or on the hyphal surface	–	Vetchinkina et al. (2017)
<i>Fusarium oxysporum</i>	Tb ₂ O ₃ NPs	10	Spherical	Extracellular	Cytotoxicity activity	Iram et al. (2016)

5.2 Biosynthesis of NPs by Fungi

Fungi are eukaryotic organisms present in nature, known typically as decomposer organisms since they possess the ability to synthesize numerous extracellular enzymes that hydrolyze complex macromolecules. The metabolic capacity of fungi and their use in bioprocesses have stimulated a great interest in the fungi application as metallic NPs producers (Bhargava et al. 2016; Kitching et al. 2016; Dhillon et al. 2012; Jain et al. 2015; Prasad 2016, 2017).

Most of fungi are capable of high wall-binding and intracellular metal uptake (Volesky and Holan 1995). Their metabolic mechanism could participate either directly as in the case of on-cell/intracellular nanoparticle synthesis or indirectly by the extracellular nanoparticle synthesis mediated by secreted metabolites (Jain et al. 2011; Bhargava et al. 2015). Thus, metal NPs can be nano- or mesostructured, according to the path of synthesis, the intra- or extracellular reducing enzymes, and the biomimetic mineralization. These possibilities are related with the cell tolerance and metal bioaccumulation capability (Kitching et al. 2016; Sastry et al. 2003). In this connection, fungal strains isolated from metal-rich environments are the better source for biosynthesis of metal nanoparticle (Jain 2013).

5.2.1 Silver Nanoparticles

Synthesis of silver NPs has become an important scientific field applied since it is used mainly in pharmaceutical industry. Generally, the methods employed for its preparation include chemical treatments where high temperatures and chemical reducing agents are critical procedures. Reducing agents have to be able to donate electrons to the Ag^+ resulting in a reverting Ag^+ to Ag^0 (Mishra et al. 2015).

One of the main characteristics of the silver NPs is their excellent antimicrobial ability against a large range of pathogenic strains. The anti-biofilm activity of silver NPs has been demonstrated, showing good biocompatibility in cell viability studies in human keratinocyte HaCat cells suggesting its potential application in chronic wound healing. Antioxidant properties and their remarkable toxicity to cancer cell lines as Hela and A549 cells even at very low concentration make silver NPs a possible anticancer agent (Du et al. 2016).

Different fungal species have been utilized for the extracellular synthesis of silver NPs, due to great particle stability and excellent biocompatibility (Table 5.1).

Endophytic fungi is an interesting group for the synthesis of silver and gold NPs. *Pestalotiopsis microspora* VJ1/VS1 isolated from leaves of *Gymnema sylvestre* showed an efficient and eco-friendly approach for the synthesis of silver NPs using aqueous culture filtrate of the fungus, due to the higher enzymatic activity present in the cell-free extract. Nanoparticle synthesis was evidenced by the observation of a characteristic absorption peak at 435 nm (UV-visible). Silver NPs showed antioxidant effects by the effective radical scavenging activity against 2,2'-diphenyl-1-

picrylhydrazyl and H_2O_2 radicals as well as exhibited significant cytotoxic effects against different cancer cell lines (Netala et al. 2016). Golinska et al. (2016) observed a significant antimicrobial activity by silver NPs synthesized by the myco-endophyte *Guignardia mangiferae*. Besides, the authors informed that if NPs were used in combination with common antibiotics, antimicrobial activity was enhanced. In addition, silver NPs were found to be highly biocompatible, so it could be used in biomedical/pharmaceutical and agricultural industries (Golinska et al. 2016; Balakumaran et al. 2015; Ramalingam et al. 2015; Rekha et al. 2012).

Even though the synthesis of NPs using fungi has been widely reported, the more important issue to reach this achievement is the optimization of the parameters used in the synthesis protocol (Golinska et al. 2016). For instance, the size of silver NPs could be controlled by temperature and $AgNO_3$ concentration (AbdelRahim et al. 2017).

The aqueous silver ions can be reduced to silver NPs by mixing with fungal filtrates. For example, the evidence of Ag NPs formation was detected using the aqueous mycelial extract from *Rhizopus stolonifer* observing a surface plasmon band around 420 nm. The smallest size of Ag NPs (2.86 ± 0.3 nm) was obtained with 10^{-2} M of $AgNO_3$, at 40 °C.

Cultures of *Aspergillus terreus* HA1N and *Penicillium expansum* HA2N incubated for 72 h in the dark at 30 °C showed the change of color in the medium which would be produced by the excitation of surface plasmon vibrations in the metal NP (Ammar and El-Desouky 2016). Similarly, cultures of *Penicillium decumbens* (MTCC-2494) have shown a dark brown color in the culture flask suggesting the extracellular biological synthesis of silver NPs which was confirmed by UV-spectrophotometric analysis. As was expected, silver nanoparticle displays anti-cancer effects and a broad antimicrobial activity including a synergistic effect with carbenicillin, piperacillin, cefixime, amoxicillin, ofloxacin, and sparfloxacin (Majeed et al. 2016). Extremophile fungi also exhibit a high capacity for synthesis of mineral NPs (Beeler and Singh 2016). Thus, *Aspergillus fumigatus*, a thermophilic microorganism, was able to produce stable silver NPs (15–45 nm). These NPs showed to have capping proteins and the NADH reductase was the mechanism to reduce Ag^+ (Alani et al. 2012).

5.2.2 Gold Nanoparticles

Gold NPs represent a key area of nano-research since they show finely tunable surface plasmon resonance that allows applications in a wide array of biomedical sciences (Khan et al. 2014; Karthika et al. 2017).

Extracellular synthesis of gold NPs using metal-tolerant fungi has been widely reported. *Cladosporium oxysporum* AJP03 has been found to produce gold NPs by extracellular synthesis. The highest tested concentration of extracellular metabolites (1:5, biomass/water ratio) and 1 mM precursor salt concentration at physiological

pH (7.0) favored the synthesis of well-defined gold NPs with maximum yield (Bhargava et al. 2016).

Extremophile fungi exposed to higher concentration of gold chloride produced smaller NPs. Thus, *Aspergillus sydowii*, a halophilic marine fungus, has been recognized as a biofactory capable of producing highly specific gold NPs (Vala 2015; Gunde-Cimerman 2014). Regulating gold chloride concentration in a potato dextrose medium at 27 °C, *Aspergillus sydowii* was capable of modulating the size and changing the mechanism from intra- to extracellular production pathway. *Fusarium oxysporum* showed several extracellular enzymes, naphthoquinones (Medentsev and Akimenko 1998; Duran et al. 2002; Bell et al. 2003) and anthraquinones (Baker and Tatum 1998), which possess redox properties to reduce the metal ions (Newman and Kolter 2000). In the biosynthesis process of metallic NPs, the fungal mycelium is exposed to a solution of metal salt, where the metal ions are reduced to NPs by the action of metabolites and extracellular enzymes (Siddiqi and Husen 2016).

The synthesis of NPs not only is made by extracellular metabolites but also by proteins bound to the cell surface which showed a significant biomineralization activity. In fact, several studies have shown that cell surface proteins of *Rhizopus oryzae* play a crucial role in biomineralization of Au (III) to produce gold NPs. Cell surface proteins are able to reduce Au³⁺ to later produce the nucleation and growing of Au crystals (Kitching et al. 2016). Other proteins act as capping agents, thereby controlling the size of the gold NPs (Kitching et al. 2016; Das et al. 2009).

5.2.3 Magnetic Nanoparticles

In the last years has been observed an increasing interest in the development of procedures for the synthesis of magnetic NPs, due to their potential application in areas such as storage devices (Matsunaga 1991), ferrofluids (Raj et al. 1995), enhancement in magnetic resonance imaging (Schüler and Frankel 1999), and drug delivery.

Bharde et al. (2006) focused their studies in the biosynthesis of magnetic NPs. The authors observed that when *Fusarium oxysporum* and *Verticillium* sp. were exposed to an aqueous solution of a 2:1 molar mixture of K₃[Fe(CN)₆] and K₄[Fe(CN)₆], extracellular magnetite was synthesized. The proteins secreted by *Fusarium oxysporum* and *Verticillium* sp. hydrolyzed iron precursors extracellularly to form iron oxide prevailing in the magnetite (Fe₃O₄) at room temperature. Particularly, the fungi were capable of hydrolyzing metal ion precursors under acidic conditions. Protein analysis suggests the induction of two proteins of molecular weights of 55 and 13 kDa, which might be responsible for the hydrolysis of magnetite precursors and/or the capping of magnetite particles. The magnetite biosynthesis mentioned above presents a simple and aerobic route for the magnetic nanomaterial synthesis.

New techniques for the synthesis of magnetic NPs have been evaluated. One of them consisted in the growing up of magnetite NPs (nano-Fe₃O₄) on the mycelium

of fungus *Penicillium* sp. (Ding et al. 2015). Given a large number of functional groups naturally present on the mycelium surface, the assembly of nano-Fe₃O₄ is a relatively simple process. More important to this is that nano-Fe₃O₄ could self-assemble on the fungus template accompanied with forming mycelia pellet in a mild case, whereas the chemical synthesis to obtain similar product requires extreme pH and temperature environment. Moreover, *Penicillium* sp. formed mycelia pellets under submerged shaking cultivation conditions which enrich functional groups on the surface (Mishra 2013).

Fungus-Fe₃O₄ presented a composite structure by nano-Fe₃O₄ particles uniformly adhered on the surface of *Penicillium* sp. which improve the dispersion and stability of nano-Fe₃O₄ particles, avoiding the pollution resulting from the nano-Fe₃O₄ particles (Ding et al. 2015).

Similar studies have been made with the white rot fungus. The external membrane of fungus has abundant negatively charged functional groups. So, it was able to grab various positively charged inorganic particles through both physical adherence and chemical bonding, and nano-functionalized fungus could be assembled (Ding et al. 2015).

5.2.4 Other Metal Nanoparticles

Although the silver, gold, and magnetic NPs are the most studied NPs synthesized by fungi, there are other NPs with interesting properties. In fact, the first NP synthesis by fungus was reported as CdS NPs in addition to the formation of PbS, ZnS, and MoS₂ NPs (Iram et al. 2016).

Zirconia nanoparticles have been produced by *F. oxysporum* with aqueous ZrF₆⁻² anions. Extracellular protein-mediated hydrolysis of the anionic complexes at room temperature results in the synthesis of nanocrystalline zirconia (Bansal et al. 2004). A strain of *F. oxysporum* f. sp. *lycopersici* was screened and successfully produced intra- and extracellular platinum NPs. Riddin et al. (2006) reported the synthesis of zinc, magnesium, and titanium NPs by using six *Aspergillus* by employing several salt precursors of nitrates, sulfates, chlorides, and oxides.

The synthesis of luminescent lanthanide NPs has shown an increasing attention, since the presence of efficient luminescent groups such as samarium, europium, and terbium (Blasse 1979). This makes the lanthanide compounds to present extraordinary temporal and spectral properties such as sharp emission bands, long lifetime, and large Stokes shifts which make them especially useful in time-resolved luminescence bioassays, wherein they can effectively be distinguished from the background noise (Yuan and Wang 2006; Eliseeva and Bunzli 2010). In the case of terbium, most of the work has been focused on its chemical synthesis (Iram et al. 2016).

Iram et al. (2016) report for the first time the synthesis of terbium oxide NPs using *Fusarium oxysporum*. The biocompatible terbium oxide NPs (Tb₂O₃ NPs) were synthesized by incubating Tb₄O₇ with the biomass of the fungus. Physical characteriza-

tion (UV-V and photoluminescence spectroscopy, TEM, SAED, and zeta-potential) was made to confirm the synthesis, crystallinity, distribution, purity, optical and surface characteristics, shape, size, and stability of the nanoemulsion of Tb_2O_3 NPs. These NPs showed a high degree of biocompatibility and ability to inhibit the growth of bone cancer cells at biologically safe concentrations. They were nontoxic for normal primary osteoblast cells up to a considerably high concentration.

5.3 Mechanisms of Nanoparticle Biosynthesis

Mechanisms of NPs biosynthesis are deeply related to the microorganism since each one may reduce and oxidize the materials of NPs by different ways. In general, methods of synthesis show relationship with the specific survival strategies of the microorganism (Bansal et al. 2012). It has been shown that microorganisms utilize defense mechanisms to reduce the environmental toxicity through different routes of NPs production.

5.3.1 Intracellular Synthesis

During intracellular synthesis metallic ions are attracted to the negatively charged functional groups along the cell wall and nucleated there, initiating the reduction and synthesis of NPs. For instance, Kalabegishvili et al. (2015) demonstrated the biosorption of metallic ions to the cell wall of a fungus, observing that the ions were heterogeneously distributed according to specific binding sites. Moreover, shape, size, and stability of the NPs are also determined by the binding sites of the cell wall (Asmathunisha and Kathiresan 2013; Erasmus et al. 2014).

For some microorganisms, the metallic ions are transferred into the cell via active cellular pumps (ATP-mediated), followed by enzymes that reduce these ions and in occasion cap them. Finally, capping proteins bind to NPs (Fig. 5.1) via open amine groups and cysteine residues, neutralizing its surface charge. The capping proteins act in the prevention the agglomeration and the alteration of NP properties playing an important role also as a site for bioconjugation with other molecules. The protein caps provide stability to biologically synthesized NPs that are not otherwise found in traditional methods unless the surfactants included are very toxic (El-Deeb et al. 2013). Furthermore, the stability decreases the toxicity of the NPs, making them more environmentally friendly (Beeler and Singh 2016; Faramarzi and Sadighi 2013; Stark et al. 2015).

Interesting studies were performed using fungi cells as biotemplate for NPs construction. Self-assembly of NPs on living biotemplate surfaces is a promising route to fabricate nano- or microstructured materials (Kubo et al. 2016). Filamentous fungi, *Aspergillus aculeatus*, *Penicillium brasilianum*, and a *Xylaria* sp., have been used for producing microtubules of gold NPs by the isolation of the growing hypha from the culture medium. Using this methodology, a better morphological control

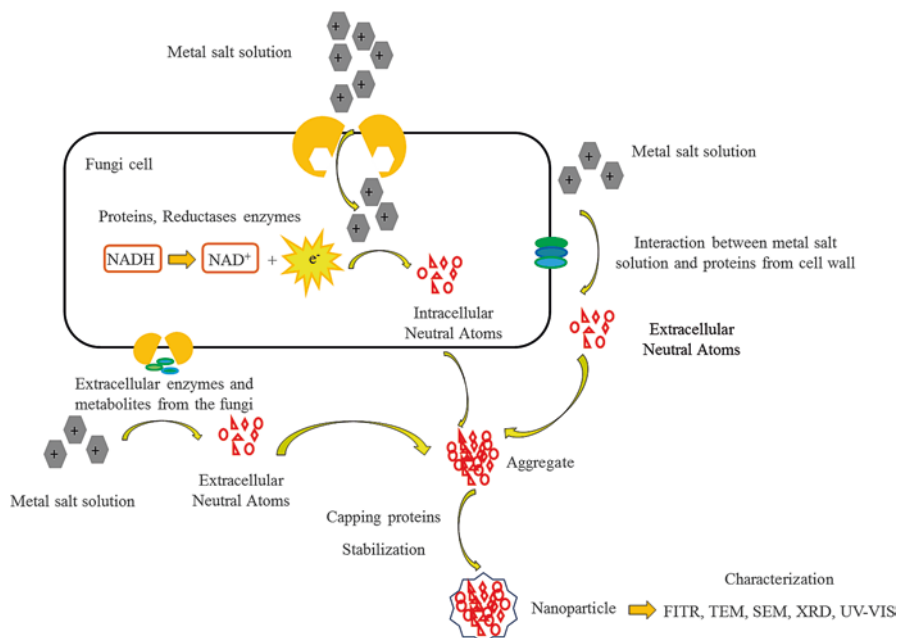


Fig. 5.1 Schematic synthesis of nanoparticles (NPs) by fungi

and faster adsorption kinetics were obtained, allowing the control of microtubule thickness through successive additions of NPs.

It was observed that the secondary metabolites and growth media influence the fungi metabolism producing differences in the adsorption rates due to modifications in the chemical identity of colloidal gold NPs and therefore in NPs biosynthesis (Kubo et al. 2016).

Ding et al. (2015) synthesized NPs of fungus Fe_3O_4 using the mycelium pellet of *Penicillium* sp. as biotemplate. SEM images showed uniform decoration of nano- Fe_3O_4 particles on fungus surface. The FTIR analysis showed that nano- Fe_3O_4 were linked to the cellular wall by chemical bonds. The authors highlight the novel synthesis method of fungus- Fe_3O_4 magnetic NPs.

5.3.2 Extracellular Synthesis

There are two possible pathways for extracellular NP synthesis. The first is similar to the intracellular synthesis. Ions pass through the cell membrane via active transporters, and then through reductive enzymes, the synthesis of the NPs is initiated. The proteins bind to the NPs during the reductive process, capping and reducing them through active sites. These NPs, after setting their size and form, are transported outside the

cell (Bansal et al. 2012). Thus, in some microorganisms, the intracellular and extracellular synthesis of NPs can occur in the same cell (Ramanathan 2011) (Fig. 5.1).

The second pathway involves the emission of reducing proteins to the cell solution. This is a result of the whole change in pH of the solution in the presence of the metallic ions. Upon receiving this signal, the cell emits oxidoreductase enzymes that reduce the ions and synthesize mineral NPs. These proteins can cap the NPs, adding stability and additional properties as mentioned above. Thus, the cell-free supernatant from the microorganism culture contains the biomolecules responsible not only for biosynthesis of NPs but also of its dispersion throughout the supernatant (Huang et al. 2015) (Fig. 5.1).

One of the more important minerals in NP study is the silver. One of the more used synthesis mechanism for silver NPs has been the use of fungal extract due to the higher enzymatic activity present in the cell-free extract. NPs resulting from the reduction exposure of fungal filtrates prove to be an important biological component for extracellular biosynthesis of stable NPs. The reduction of ions occurs extracellularly through the enzymes secreted by the fungi in the solution and the interactions between silver and bioactive molecules (cap proteins) (Ammar and El-Desouky 2016). Several authors have shown that filamentous fungi such as *Aspergillus terreus* HA1N and *Penicillium expansum* HA2N (Ammar and El-Desouky 2016), *Fusarium oxysporum* (Ishida et al. 2014), *Fusarium acuminatum* (Ingle et al. 2008), *Aspergillus niger* (Gade et al. 2008), *Amylomyces rouxii* (Musarrat et al. 2010), *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus terreus* (Khalil 2013), *Aspergillus foetidus* (Roy and Das 2015), *Aspergillus oryzae* (Bhimba et al. 2015), and *Penicillium expansum* (Mohammadi and Salouti 2015) are most efficient for silver nanoparticle biosynthesis via extracellular biosynthesis.

Another strategy using cell-free extract is to use bound cell surface proteins, previous extraction from the wall. Kitching et al. (2016) purified cell surface proteins from *Rhizopus oryzae* to conduct the in vitro gold NP synthesis. The author probed the extraction of cell surface proteins using common detergents as sodium dodecyl sulfate (SDS) and Triton X-100 and the reducing agent 1,4-dithiothreitol (DTT) observing gold NPs of different size and shape. These different properties would have occurred due to the protein extraction method which may be so aggressive affecting the morphology and particle size distribution. In fact, the structure and function of the proteins are influenced by pH, temperature, ionic strength, and the presence of surfactants and solvent (Kitching et al. 2016).

5.3.3 Biomolecules Responsible for Nanoparticle Synthesis

In recent years, great attention has been paid to determine which fungal metabolites are involved for the biosynthesis of a nanoparticle. Several reports have been published about the ability for biosynthesis of NPs by a wide range of fungal enzymes (Durán et al. 2007). Although enzymes differ among fungal species, there is a tendency to fall within a common group of enzymes used for the synthesis of NPs by

microorganisms: the oxidoreductase enzymes. Oxidoreductases are a wide class of enzymes involved in redox reactions, shifting electrons from a reductant – the electron donor – to an oxidant, which in the production of NPs would be the inorganic substance, being reduced (NADH-dependent reductase).

The enzyme nitrate reductase showed relevant activity during the biosynthesis of NPs in several fungi. Such is the case of cell-free filtrates of *Fusarium oxysporum*, in which the largest amount of silver NPs synthesized were obtained during the early stationary phase of growth, simultaneously to the higher secretion of extracellular enzymes, particularly nitrate reductase (Hamed et al. 2016). Moreover, it was observed to enhance the nitroreductase synthesis increasing the number of silver atoms in nucleation centers. The presence of nucleation centers enhances the consumption of reducing agents and reduces the possibility of NP uncontrolled aggregation favoring the formation of silver NP clusters (Hamed et al. 2014). Thus, the characteristics of the silver nanoparticle can be controlled and improved by the induction of nitrate reductase enzyme (Hamed et al. 2016).

On the other hand, it was discovered that different quantities of NADH make possible the synthesis of different NPs from diverse compounds. In this sense, Golinska et al. (2016) proposed a mechanism for the synthesis of silver NPs from *Fusarium oxysporum* based on the presence of an NADH-dependent reductase responsible for the reduction of Ag ions and the subsequent formation of silver NPs (Kitching et al. 2015).

Reduction of silver ions could be by electron transfer from the NADH by NADH-dependent reductase as an electron carrier; thus the electron-deficient silver ions (Ag^+) accept the electrons and are reduced to silver neutral (Ag^0). As a consequence, silver NPs are finally synthesized NPs (Golinska et al. 2016).

The biosynthesis of NPs could involve other biomolecules produced by the same fungus. It was reported that the biosynthesis of silver NPs may occur not only in the presence of NADPH-nitrate reductase but also in presence of anthraquinone or hydroxyquinoline molecules (Ahmad et al. 2003; Li et al. 2012). The reduction of NADPH to NADP^+ is required in this process, and electrons generated during the reduction of silver ions are donated from both quinones or hydroxyquinoline and NADPH (Golinska et al. 2016; Balakumaran et al. 2015).

Other oxidoreductase enzymes involved in NPs biosynthesis are hydrogenase enzymes. In this regard, Govender et al. (2009) suggested a mechanism that reduces biologically H_2PtCl_6 and PtCl_2 to platinum NPs by means of filtered hydrogenase enzymes from *Fusarium oxysporum*. The authors suggest that H_2PtCl_6 may act as an electron acceptor during the redox mechanism of the hydrogenase through a direct electron transfer between metal ions and the enzyme. Hydrophobic channels between the active site and the molecular surface serve as a passage for metal ions.

Vetchinkina et al. (2017) also evaluated the role of phenol oxidase enzymes as laccases, tyrosinases, and Mn-peroxidases. These enzymes were isolated and purified from submerged culture of *Lentinus edodes*. The pure fungal intracellular phenol-oxidizing enzymes were able to form Au^0 NPs. The NPs synthesized by Mn-peroxidase were regular spheres of 5–20 nm. The NPs produced by laccases and tyrosinases were mostly irregular spheroids, with some triangles and tetrahe-

drons from 5 to 120 nm. The structure of Mn-peroxidase enzyme contains one molecule of protoporphyrin IX with an iron (III) atom. The authors believe that extracellular AuCl_4 reduction was performed by the prosthetic group in the enzyme's catalytic center. On the other hand, laccase and tyrosinase reduce gold ions indirectly through forming exogenous hydrogen peroxide. When the enzymes react with molecular oxygen in the presence of a reduced substrate, hydrogen peroxide forms in one of the four active centers of the enzymes (Vetchinkina et al. 2017).

In the bioreduction of metal NPs, the proteins with amino acids with $-\text{SH}$ bonds have a relevant role; most likely cysteine undergoes dehydrogenation in the reaction with the metal ion to produce metal nanoparticle. Besides, the free amino acids possibly serve as a capping for metal NPs (Golinska et al. 2016).

5.4 Nanoparticle Applications

5.4.1 Antimicrobial Activity

Nanoparticles are a hope particularly in the pharmacological industry, because of their antimicrobial properties. It is believed that one of the mechanisms by which the NPs present antimicrobial capacity is due to the use of the NPs negatively charged ions which bind to the microorganism cell wall and break it. Another mechanism that explains this NPs property is the passage of smaller NPs through the cell to cause a direct damage to DNA, inhibiting its replication (El-Deeb et al. 2013). Some authors suggest that NPs release reactive oxygen species (ROS) or free radicals, inducing the cell death (Prasad and Swamy 2013; Beeler and Singh 2016; Mamonova et al. 2015) (Fig. 5.2) since has been observed that NPs are able to attach to the bacterial cell membrane and produce unrest in its normal functioning. Nanoparticles could be accumulated in the cytoplasm or in the periplasmic region producing the cell membrane disruption and consequently the release of the cell contents (Golinska et al. 2016). The alteration of cell membranes involves the binding of NPs to sulfur-containing proteins present in the membrane (Rai and Yadav 2013; Singh et al. 2014; Shahverdi et al. 2007; Ping Li et al. 2005; AshaRani 2009a, b; Brayner et al. 2006). Similarly, sulfur content of intracellular enzymes and DNA makes these molecules the target of the NPs (Raghupathi et al. 2011). In particular, it has been reported that silver NPs specially target pathways of synthesis of bacterial cell wall and nucleic acid and protein synthesis (Marambio-Jones and Hoek 2010) (Fig. 5.2).

The modification of the structure of the bacteria membrane and the possible damage to DNA caused by the NPs may affect the respiratory chain, cell division, and DNA replication, and, finally, the cell death occurs (Golinska et al. 2016; Lara et al. 2010; Andrade et al. 2015; Morones et al. 2005; Aziz et al. 2015). Moreover, silver ions generated from the dissolution of silver NPs could also be involved in the antimicrobial activity since it may complex with electron donor groups (sulfur, oxy-

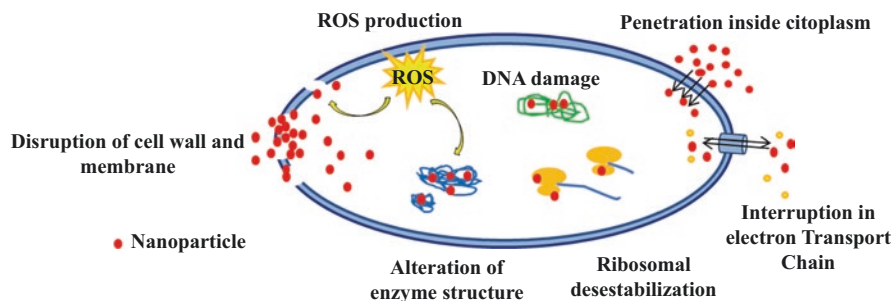


Fig. 5.2 Schematic mechanism of antibacterial activity of nanoparticles (NPs) produced by fungi

gen, or nitrogen atoms) present in phosphates, thiols, amino acids, and nucleic acids (Golinska et al. 2016; Marambio-Jones and Hoek 2010; Louise Meyer et al. 2010).

Another cellular damage produced by NPs is the denaturation of the 30S subunit of ribosomes. These suppress the action of enzymes and other proteins necessary for ATP production (Chauhan et al. 2013).

The antibacterial properties of various metal NPs from fungal cultures have been reported by several publications. However, so far, the best antibacterial activity was observed by silver NPs alone or together with antibiotics (Singh et al. 2014; Louise Meyer et al. 2010; Jung et al. 2008; Aziz et al. 2016). Thus, the antimicrobial efficiency of synthesized silver NPs via the utilization of fungal species against bacteria (Singh et al. 2014; Louise Meyer et al. 2010) and fungal pathogens has been widely demonstrated (Louise Meyer et al. 2010).

Other metallic NPs from fungi used as reducing agents are the nanogold bioconjugate (Kitching et al. 2016). The gold NPs which presented high antimicrobial activity against pathogenic bacteria such as Gram-negative *Klebsiella pneumoniae*; *Escherichia coli*, including MDR *E. coli*; *Pseudomonas aeruginosa*; *Salmonella typhimurium*; *Salmonella typhi*; *Proteus mirabilis*; *Shigella dysenteriae*; *Enterobacter aerogenes*; *Citrobacter* sp.; and Gram-positive bacteria such as *Streptococcus pyogenes*; *Enterococcus faecalis*; *Staphylococcus epidermidis*; *Staphylococcus aureus*, including MRSA; and *Bacillus subtilis* have been reported (Rekha et al. 2012; Raheman et al. 2011; Hullikere et al. 2014; Mukherjee et al. 2001; Mohanpuria et al. 2008; Shankar et al. 2003; Rai et al. 2009; Rahi et al. 2014).

The effect of fungal nanoparticles has been also extensively evaluated against fungal pathogens. Different mechanisms were proposed to discuss its effects on the growth of fungi (Fig. 5.2). Thus, several studies have shown that silver NPs exhibit antimicrobial activity against *Candida* and *Cryptococcus* (Ishida et al. 2014), *Trichophyton mentagrophytes*, *Candida* sp. (Musarrat et al. 2010), *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *Sporothrix schenckii*, *Aspergillus fumigatus*, *Fusarium solani*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Mucor hiemalis* (Ramalingmam et al. 2015; Rai and Yadav 2013; Thakkar et al. 2010; Vardhana

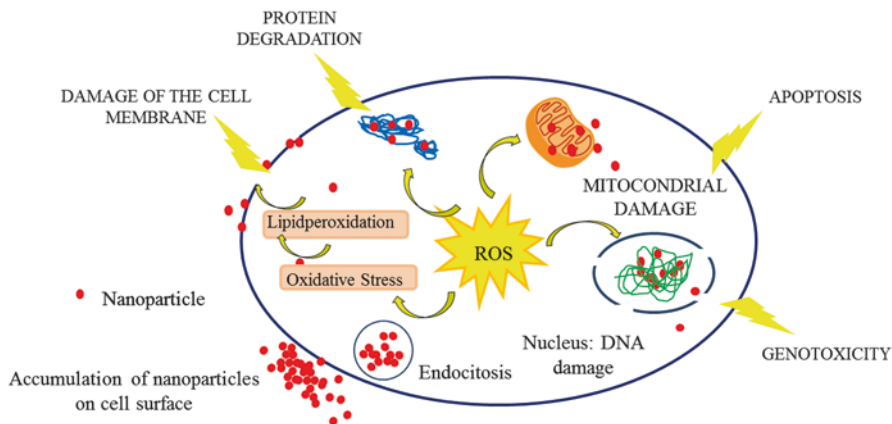


Fig. 5.3 Schematic mechanism of cytotoxic activity of nanoparticles (NPs) produced by fungi

2015; Rahi and Parmar 2014, Aziz et al. 2016) and against the plant pathogens *Colletotrichum* sp., *Aspergillus niger*, *Culvularia lunata*, *Fusarium* sp., *Rhizoctonia solani* (Golinska et al. 2016; Hullikere et al. 2014), *Cladosporium cladosporoides*, *Aspergillus niger* (Pulit et al. 2013), *Aspergillus ochraceus*, and *Aspergillus parasiticus* (Ammar and El-Desouky 2016).

5.4.2 Cytotoxicity

In the last years, the area of diagnosis and treatment of cancer has shown a significant progress. A large variety of nanomaterials has been evaluated to achieve an improved efficacy in cancer therapy as well as to reduce side effects compared to conventional therapies. The toxicity effect of fungal NPs is evaluated mainly by changes in cell morphology and viability, as well as metabolic activity (Ping Li et al. 2005; Prabhu and Poulouse 2012; Oberdorster et al. 2000; Krishnaraj et al. 2014). Nanoparticles have been localized in mitochondria, inducing structural and functional damage as well as oxidative stress (Arora et al. 2008) (Fig. 5.3). It was observed that the functional damage to the mitochondria affects the cellular metabolic inhibition, followed by a decrease in ATP yield, which could affect the mitochondrial respiratory chain. The mitochondrial damage also affects the lactate dehydrogenase activity, which can be used as an indicator of NP success (Golinska et al. 2016; Hullikere et al. 2014; Oberdorster et al. 2000).

Physicochemical characteristics of NPs play a significant role in cytotoxicity effect. The nature and size of NPs, its surface area, and its surface functionalization (capping agents) are important factors that influence their toxicity (Ping Li et al. 2005; Prabhu and Poulouse 2012; Oberdorster et al. 2000). The small-sized NPs are more toxic compared with the larger ones (Golinska et al. 2016; Hullikere et al.

2014). It has been proven that smaller particles diffuse more readily than bigger. Efficient internalization has been observed with particles in the range of 20–50 nm (Iram et al. 2016). Small-sized NPs could also be easily diffused into the nucleus through the pores and bind to DNA (Asharani 2009a, b). In general, inner transition metals are the choice for the synthesis of biogenic NPs, because these metals emit strong fluorescence and are relatively nontoxic to biological systems up to a fairly high concentration (Iram et al. 2016). In this regard, several studies have verified the effect of silver NPs on membrane integrity suggesting that these NPs are targeting cancer cells rather than normal cells. In effect, small silver NPs produced by an oxidative process results in mutagenic 8-hydroxyadenine and 8-hydroxyguanine, inductors of the stability of repetitive sequences. The product of these mutations is the highly reactive and short-lived hydroxyl radicals OH^- (Golinska et al. 2016; Xia et al. 2006).

Netala et al. (2016) biosynthesized silver NPs from fungus *Pestalotiopsis microspora* VJ1/V51 and probe its effects on the following cancer cells: B16F10 (mouse melanoma), SKOV3 (human ovarian carcinoma), A549 (human lung adenocarcinoma), PC3 (human prostate carcinoma), and NPs biocompatible toward normal cells (Chinese hamster ovary cell line). Cytological observations of SKOV3 cells (which were the most susceptible) showed apoptotic changes including pyknotic nuclei, cell membrane blebbing, cell shrinkage, and karyorrhexis followed by destructive fragmentation of nuclei. The mentioned results were very hopeful and provide the bases for the development of versatile biomedical applications of biosynthesized silver NPs for cancer therapy.

Several magnetic NPs have also been developed to improve efficacy in cancer therapy. The interest in this kind of NPs is due to their unique magnetic properties that serve as an extraordinary diagnostic tool, drug carrier, and heat generator for therapy in magnetic resonance imaging. Besides, magnetic NPs have a small size which allows reaching deeper biological tissues.

Currently, iron oxide NPs are the most explored magnetic NPs for magnetic hyperthermia. The use of magnetic NPs as a heat generator could be used in noninvasive cancer treatment to destroy tumor tissues, given that heat promotes cell apoptosis through irreversible physiological changes (Kafrouni and Savadogo 2016; Prasad et al. 2007).

The cytotoxicity effect of magnetic NPs has been associated with ROS production. The decrease in mitochondrial membrane potential in cancerous cells occurs when cells are treated with magnetic NPs, although it is not clearly known as to how it interferes with the normal function of the mitochondria. Since the mitochondria are redox sensitive, they are targeted by NPs (Fig. 5.3). Iron is slowly oxidized, so maybe the mitochondrial membrane potential decreases. The oxidation of iron NPs and generation of ROS are simultaneous processes (Kafrouni and Savadogo 2016; Wei et al. 2015).

Recently, researchers have started to focus on the anticancer activity of lanthanide NPs. The Tb_2O_3 NPs were found to inhibit the propagation of MG-63 and Saos-2 cell lines (IC50 value of 0.102 $\mu\text{g}/\text{ml}$) and remained nontoxic up to a concentration of 0.373 $\mu\text{g}/\text{ml}$ toward primary osteoblasts. Cell toxicity was evaluated by

observing changes in cell morphology, cell viability, and oxidative stress parameters. Morphological examinations of cells revealed cell shrinkage, nuclear condensation, and formation of apoptotic bodies. The levels of ROS within the cells also significantly increase (Iram et al. 2016).

5.4.3 *Fine Chemical and Pharmacology*

The heterogeneous and homogeneous catalysts can be achieved through the use of NPs (Johnson 2003). Nanoparticles provide the benefit of increased surface area which allows for an increased reaction rate (Chng et al. 2013). The NPs catalyst forms a stable suspension in the reaction medium allowing an elevated rate of reaction. One particularly useful and important group of NPs is magnetic NPs.

Magnetic NPs are a highly useful catalyst support enabling immobilization and magnetic recovery of the catalyst (Baig and Varma 2013; Romero et al. 2016). The magnetic NPs may be dispersed in the same form as any nanoparticle in the absence of a magnetic field, provided there is sufficient surface stabilization. But, in the presence of a magnetic field, magnetic NPs can be precipitated selectively. This enables them to be readily removed from the reaction vessel by a simple magnetic separation and may enable them to be re-dispersed and reused.

Magnetic particles have been increasingly used as carriers for enzymes, binding proteins, antibodies, and drugs. Thus, the new biological material can be used directly as affinity ligands to capture or modify target molecules or cells or for a bioassay (Bickerstaff 1997). Immobilization of proteins and enzymes on magnetic NPs is an important area of interest. Several magnetic NPs – magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) – have been evaluated with promising applications.

Over the last few years, significant progress was made in the development of new catalytic systems which are immobilized onto magnetic nano-carriers (Vaghari et al. 2016). Colloidal magnetic NPs are used in drug delivery until the target without interacting with other living cells. In the case of breast cancer (BT 20 cells), polyethylene glycol (Schievano)-coated NPs ranging between 10 and 100 nm were found to penetrate into the cells (Siddiqi and Husen 2016; Mahmoudi et al. 2009).

In the biomedical area was reported a large variety of bimetallic NPs of the type of MFe_2O_4 (where M= divalent Co, Fe, Zn, Cu, Mg, and Ni) containing two metal ions. Their magnetic properties depend on the number of unpaired electrons in the d orbital of transition metal ions. Xu and Sun (2013) have attempted to deliver cisplatin to solid tumor through Fe_3O_4 HMNPs. However, only a few of these compounds have been synthesized by microorganism pathway.

Otherwise, gold NPs may improve the efficiency of PCR, providing an increase in yield and a decrease in reaction time due to their ability to bind negative molecules. The gold NPs bind to single-stranded DNA, adding stability to the DNA strands and preventing mispairing among strands.

In the chemical industry, traditional pigments have been replaced by NPs due to the use of quantum dots that maintain color information despite their small size

(Roduner 2006). This allows the creation of much richer color images. Similarly, it is possible to create customized crystals for LCD screens allowing a sharper more colorful image from the nanoparticle synthesis. They may also be used as UV filters on sunglasses and in sunblock (Beeler and Singh 2016; Stark et al. 2015).

5.4.4 Bioremediation

The biological remediation of organic dyes such as methylene blue, methyl orange, and rhodamine B has received much attention due to their recalcitrant and xenobiotic nature. When dyes are disposed in water bodies high water pollution and eutrophication, and aquatic life alteration is produced (Sharma et al. 2015).

The catalytic property of gold NPs has also been evaluated in the area of degradation of environmentally hazardous chemicals, known in general as bioremediation (Zhao et al. 1998). Among all the methods used for the degradation of organic dyes, reduction by strong agents such as NaBH_4 in the presence of a nanocatalyst may be a viable alternative due to high efficiency and reaction rate (Sharma et al. 2015). The catalysis by gold NPs increases the reaction rate with the mean time in the minute interval (Panáček et al. 2014). Bastus et al. (2014) postulated that the reduction mechanism was a two-step process involving first the accumulation of borohydride ion electrons on the surface of the NPs and the diffusion of the organic dye molecules to the surface of the NPs and their later reduction induced by excess surface electrons. The reaction takes place on the surface of the nanocatalyst due to the nature of the affected capping molecules having reaction kinetics. Bhargava et al. (2016) hypothesized that surface proteins of gold NPs may facilitate the adsorption of organic dyes as amino acids containing aromatic rings to create hydrophobic spaces that can enhance the efficient binding of dye molecules.

5.4.5 Food Safety

With regard to food safety, NPs have been evaluated for use in packaging materials. Zinc NPs have shown antibacterial properties because of having been proposed for produce food packaging and containers (Rajamanickam et al. 2012; Prasad et al. 2014). Thus, the use of NPs in packaging containers would keep food fresh longer and could reduce the chances of foodborne illnesses. In this sense, silver NPs have been shown to better penetrate the biofilms that allow bacteria to survive cleaning and decontamination processes (Huang et al. 2015; Shanthi et al. 2016). The use of these products in industrial food would allow for better control of organisms that maintain survival via biofilms (Beeler and Singh 2016).

5.4.6 Plant Disease Management

In the agriculture area, solutions for protecting food and products from bacterial, fungal, and viral agents are in constant search. Nanotechnology techniques can improve the existing crop control protocols. In this sense, nanomaterials are being developed that offer the opportunity to administer pesticides, herbicides, and fertilizers more efficiently and safely by controlling precisely when and where they are released (Rai and Ingle 2012; Prasad et al. 2017).

Researches have confirmed that metal NPs are effective against plant pathogens, insects, and other pests (Choudhury et al. 2010). In fact, an eco-friendly fungicide is being developed capable of using nanomaterials to liberate its pathogen-killing properties only when it is inside the targeted pathogen (Liu 2006; Alghuthaymi et al. 2015; Bhattacharyya et al. 2016).

On the other hand, pesticides used in agriculture are sometimes harmful to other animals and plants. Their reduction to innocuous chemicals by iron nanoparticle is a simple strategy to make them useful. It was observed that metal NPs can reduce polyhalogenated and nitroaromatic compounds. Also, they can be used for the reduction of nonhalogenated pesticides and azo dyes (Siddiqi and Husen 2016).

Iron oxide nanoparticle (Fe_3O_4) being chemically and biologically neutral has been coated with catalysts, enzymes, or even antibodies to be used as biosensors. Chauhan et al. (2016) have modified Fe_3O_4 nanoparticle using poly(indole-5-carboxylic acid) by preparing nanobiocomposite for its use as a sensor for the determination of pesticides such as malathion and chlorpyrifos in a wide range of concentrations.

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