

## Chapter 2

# Innovation of Strategies and Challenges for Fungal Nanobiotechnology

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**Abstract** Nanotechnology involves the study and use of materials under the 100 nm scale, exploiting the different physiochemical properties exhibited by these materials at the nanoscale level. Microorganisms are the best model and role of action for the nano/biotechnological applications. This technology has become increasingly important for the biotechnology and the related sectors. Promising applications have been already employed in the areas of drug delivery systems using bioactive nanoencapsulation, biosensors to detect and quantify pathogens, chemical and organic compounds, alteration of food compositions, and high-performance sensors and film to preserve fruits and vegetables. Moreover, the taste of food and food safety can be improved by new nano-materials from the microbiological sources. The huge benefits from this technology have led to increases in the market investments in nanoscience and nanoproducts in several areas.

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Fungi are the common source of industrial enzymes by cause of their excellent capacity for extracellular protein production. These industrial enzymes are applied in pulp and paper chemical and biomedical products, food, starch, textile, drinks, baking, leather, detergents and animal feed. For industrial application, immobilization of enzymes has advantages due to their improvement in the stability and storage ability because of reuse, easy separation of enzymes from the reaction mixture, a possible increase in pH and thermal stability and low product cost. The reusability and the cost of immobilized enzymes display a great advantage comparing to those of free enzymes. Using nanoscale structures for immobilization is preferred due to an increase in the functional surface area to maximize enzyme loading and reducing diffusion limitations. In addition, the physical characteristics of nanostructure such as enhanced diffusion, thermal stability, irradiation resistance and support mobility can impact catalytic activity of immobilized enzymes. This chapter deals with the strategies, challenges, applications and benefits of fungal nanobiotechnology in different areas and, also, antifungal activity of nanoparticles from the microbial sources.

Fungal nanobiotechnology based agro-industries and environmental spheres created the enormous range of possible applications of fungi. The successful and promising studies in these areas have provided a better understanding of fungi in nanobiotechnological disciplines. The utilization of fungi in the environmental biotechnology is a more recent development with many advantages related to bioremediation, treatment of industrial wastes and biotransformation of specific compounds. The objective of this chapter is to summarize recent developments in fungal nanobiotechnology and fungal synthesis of nanoparticles.

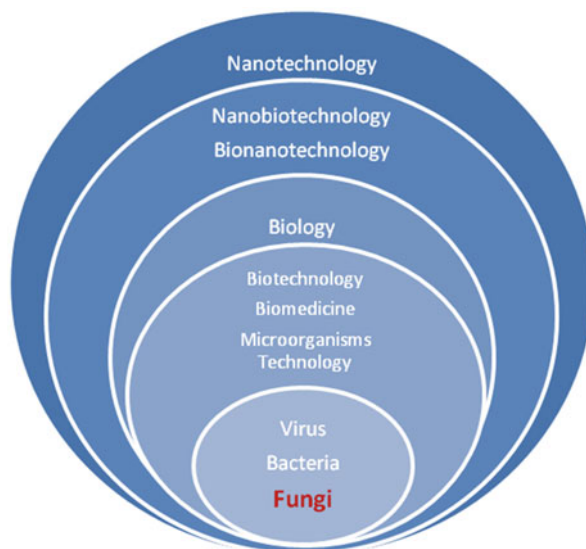
The manufacture and use of dyes are widespread industries. The utilization of these pigments is an integral part of almost all manufacturing processes. Wastewaters are produced during the synthesis and use of dyes. Decolorization of water is a significant and a critical part of wastewater treatment processes. Furthermore, metal contaminated industrial wastewater treatment is also acknowledged as one of the bionanotechnological issues. Microorganisms, especially fungi, are possible and strong candidates for heavy metal removal from wastewaters due to its binding ability to a toxic metal or metal ion. Thus, nanobiotechnological aspects of fungal studies in wastewater treatment applications are explained in a separate section in this chapter.

Nanoparticles can be used in various areas such as medicine, biosensors, environmental treatment and so on. These could be produced by conventional chemical and physical methods although conventional methods have some disadvantages. Therefore, a relatively simple, economical and nonhazardous (i.e., eco-friendly) method must be used in order to synthesize various nanoparticles. Biotechnological methods have several advantages over conventional ones. Nanoparticles can be synthesized by using various organisms such as fungi and bacteria. Here, some of the fungi used in the synthesis of nanoparticles are reviewed. The mechanism of bionanoparticle synthesis and biological activity of these nanoparticles are also discussed.

## 1 Introduction

Nanotechnology is known as the art of the creation and modification of materials in the general size up to 100 nm and this science is greatly affected by various disciplines especially physics, chemistry and biology (Fig. 2.1). This technology provides great opportunities by changing the significant properties of materials when they are in nano size. Because, when the dimension of a material is getting closer to nanometer, quantum physics becomes to play an important role instead of conventional laws of physics. This situation brings different and unique properties to the material. In other words, nanoscience helps us understand these new behaviours that emerge in nanometer size due to quantum theories. Thus, nanotechnology lets us to design and synthesize nanostructures and provides us to use these extraordinary properties in order to generate new material forms and new application areas such as medicine, environment, green technological methods, pharmacology, electronics etc. (Rai et al. 2009; Suman et al. 2010; Gupta et al. 2012; Aziz et al. 2015). Nanobiotechnology is a new branch of nanotechnology, combining biological principles with physical and chemical procedures to generate nanoscale particles with specific functions and structures.

Nowadays, metal nanoparticles like silver, gold and platinum have great popularity because of their many advantageous properties in several fields of application. Furthermore, metals and metal oxides can be used as antimicrobial agents. During the studies with antimicrobial nanoparticles, the developing resistance of microorganisms against nanoparticles was not evident (Mühling et al. 2009).



**Fig. 2.1** Nanotechnology and its related areas

Silver nanoparticles are one of the most researched and popular ones among the other metal nanomaterials. Its usage in wound healing applications and treatments of some infections makes them very preferable and attainable particularly in biomedical field. Even if they are widely utilized in many areas, these nanoparticles have many side effects especially against environment because of their toxicity (Rai et al. 2009; Gupta et al. 2012). Therefore, new eco-friendly approaches have been improved for green biosynthesis of nanoparticles. Fungal nanotechnology has emerged due to these requirements and studies. In addition, energy shortage and malnutrition problems have occurred with increasing population. Environmental pollution is also getting a threatening issue for human life and, recently, nanotechnology has appeared as a promising tool to solve such environmental- and health-related issues.

Nanobio/Bionanotechnology is playing a significant role for solving current difficulties, many scientists specifying that the studies of microorganisms would provide precious contributions. Fungal nanotechnology can be defined as the fabrication of nanoparticles with green synthesis by fungi, which bring significant advantages to the nanomaterials. Because of the unsatisfactory researches that include both the activity of microorganisms and nanotechnology for solving the problems that mentioned above is the main reason for focusing recently more on fungal approaches in nanobiotechnology.

Fungi have a number of advantages for green synthesis of nanoparticles compared with other organisms, particularly for their easy isolation and ability of extracellular enzyme secretion (Singh et al. 2014; Prasad et al. 2015). Also, many of the proteins that secreted by fungi can transform metal ions rapidly with non-hazardous processes. Therefore, there is an increasing interest in using fungi for these processes, and they may have a significant potential to provide a quick and safe process for production of metallic nanoparticles (Rai et al. 2009). For example, an endophytic fungus, *Penicillium* sp., isolated from the leaves of turmeric (*Curcuma longa*) was studied for producing silver nanoparticles and this successful study was used as a weapon against *Staphylococcus aureus* and *Escherichia coli* in a facial way (Singh et al. 2014).

Enzymes are among the most important products obtained for human needs through microbial sources, especially fungi. The enzymes produced from white rot fungi (manganese peroxidase, lignin peroxidase, superoxide dismutase, ligninase and laccase etc.) generally associated with the lignin degrading processes. There is a popular interest in improving the biodegradative abilities of white rot fungi for treating contaminated water and soil and bioremediation of water contamination with colored substances (Bumpus 2004) including recalcitrant chemical molecules, for example, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls and dioxins, phenols and its derivatives, lignin, pesticides and azo dyes. Especially, laccase enzyme has an industrial importance for decolorization of reactive coloring substances by being immobilized to nanocomposites. One of the significant studies was to use free and immobilized laccase enzymes against an industrial textile dye, Reactive Red 5 (Ilk et al. 2016). As a result, optimum time studies resulted in 120 min for free laccase and 90 min for immobilized laccase. The productivity of free laccase was measured as 33 % while immobilized laccase was 65 %. Also, during the reapplication of laccase complex to the nanostructure for 10 times over a period of 3 days; it was found that 77.3 % of the initial productivity was maintained. In another similar study, Cetin et al.

(unpublished data/submitted paper) focused on *Phanerochaete chrysosporium* loaded monolithic composite cryogel columns for the removal of mercury ( $\text{Hg}^{2+}$ ). The columns showed a great efficiency about biosorption of  $\text{Hg}^{2+}$ .

Based on these studies and aspects, because of these products are really hazardous, their removal from clean sources with conventional methods is not sufficient nowadays, so fungal nanotechnology may contribute to these refining studies. These effective, economical, eco-friendly and quick nanobiotechnological methods can provide a new perspective to these researches.

In brief, nanotechnology brings new aspects to biological applications and provides many advantages unlike the conventional methods. Bionano/Nanobiotechnological studies include the scientific researches with living organisms; bacteria, yeasts, fungi etc. and when nanotechnological studies are observed from fungal aspects, these new approaches will contribute a lot to the conventional nanotechnological methods (Prasad et al. 2015). The significant roles of fungi in green technology and its biological activities in many areas make these unique organisms to provide a variety of bio-engineering applications. Fungal nanotechnology also creates more effective and eco-friendly synthesis methods for metal nanoparticles and the other toxic and hazardous methods are about to become invalid nowadays. Additionally, nano-sized fibres created with electrospinning method are getting popular in encapsulation of microorganisms and in microbial biosensors; some microorganisms being able to detect comprehensive chemical substances. Dar and Soyong (2014) indicated the ability of electrospinning method for the encapsulation of anti-fungal compounds. For example, they encapsulated the active compounds from *Chaetomium* species that are widely known as an effective anti-fungal agent. Also, El-Newehy et al. (2012) tested polyvinyl alcohol (PVA) and polyethylene oxide (PEO) nanofibers against pathogenic fungi such as *Penicillium notatum*, *Aspergillus niger* and *Aspergillus flavus*. The results showed that these nanofibers are able to exhibit zone of inhibitions.

As mentioned above, because of the insufficient studies related to environmental/green nanotechnological methods with using living organisms and the increasing importance of nanotechnology in industrial applications, fungal nanotechnology is emphasized within this chapter by utilising both general nanotechnology description and its fungal applications.

## 2 Biosynthesis of Metallic Nanoparticles by Fungi

### 2.1 Filamentous Fungi

Several products obtained from the filamentous fungi are produced at commercial scale, including organic acids, antibiotics and enzymes. Moreover, fungi and their secondary metabolites are important biological agents for industrial applications such as biodegradation, bioremediation, bioaugmentation and biotransformation (Çabuk et al. 2013; Grimm et al. 2005). Currently, fungi are used for the fabrication of nanoparticles.

Progresses in the field of nanobiotechnology have resulted in the production of different metal nanoparticles, which have found a decent ground for several applications. Production of nanoparticles by fungi has some practical advantages. Fungi that are typically not exposed to large concentrations of metal(s) can be transformed to a form that can tolerate high concentrations of metal ions owing to their inherent ability to yield higher concentrations of proteins which aids in the reduction of metal ions to less toxic forms (Sastry et al. 2003; Prasad et al. 2015).

Additionally, fungal mycelial mesh can withstand flow pressure and agitation and other conditions in bioreactors or other chambers compared to bacteria. These are fastidious to grow and easy to handle and produce. The extracellular secretions of reductive proteins are more common and can be easily handled in downstream processing. Also, since the nanoparticles precipitated outside the cell is devoid of unnecessary cellular components, it can be directly used in various applications (Narayanan and Sakthivel 2010). Therefore, several fungi have been utilized for many biotechnological processes on the industrial scale; the utilization of waste mycelium would be promising for feasible, eco-friendly and cost effective biosynthesis of nanoparticles. Nowadays, fungi are excellent candidates for the synthesis of various metals and metal sulfides nanoparticles (Sastry et al. 2003; Sadowski et al. 2008).

Metallic nanoparticles have potential applications in various fields, such as electronics, cosmetics, coatings, packaging and medical applications (Prasad et al. 2014). Nanoparticles can be induced to amalgamate into a solid at relatively lower temperatures, often without melting, leading to the improvement of easy-to-create coatings for electronics applications. Nanoparticles possess a wavelength below the critical wavelength of light, which renders them transparent, a property that makes them very useful for applications in cosmetics and packaging. Nanoparticles can be employed as an efficient tool to explore the finest processes in various biotechnologies including biomedical sciences (Hutten et al. 2004; Safarik and Safarikova 2002). Apart from this, nanoparticles play an essential role in drug delivery, diagnostics, imaging, sensing, gene delivery and tissue engineering (Morones et al. 2005; Arruebo et al. 2007; Prasad 2014; Prasad et al. 2014).

*Verticillium* sp. and *Fusarium oxysporum* are known to produce nanoparticles when challenged with aqueous solutions of metal ions either intracellularly or extracellularly (Mukherjee et al. 2001a; Gericke and Pinches 2006). *Fusarium*, *Aspergillus*, and *Penicillium* have capable potential for the extracellular production of different metal nanoparticles. The biosynthesis of nanoparticles by various strains of filamentous fungi is presented in Table 2.1 and some examples of nanoparticles are described below.

## 2.2 Gold Nanoparticles

In the study of Castro-Longoria et al. (2011), the filamentous fungus *Neurospora crassa* was screened and found to be successful for the production of mono and bimetallic Au/Ag nanoparticles. Scanning electron microscopy (SEM), energy

**Table 2.1** The list of nanoparticles synthesized from different fungi

Nanoparticle type	Fungus name	Size of the nanoparticle (nm)	Production location	References
Gold	<i>Aspergillus oryzae</i>	10–60	Extracellular	Binupriya et al. (2010)
	<i>Verticillium luteoalbum</i>	10	Extracellular	Gericke and Pinches (2006)
	<i>Trichothecium</i> sp.	5–200	Extracellular	Ahmad et al. (2005)
	<i>Collitotrichium</i> sp.	20–40	Extracellular	Shankar et al. (2003)
	<i>Fusarium oxysporium</i>	20–40	Extracellular	Mukherjee et al. (2002)
	<i>Collitotrichum</i> sp.	20–40	Extracellular	Shankar et al. (2003)
	<i>Verticillium Luteoalbum</i>	<10–100	Intracellular	Gericke and Pinches (2006)
	<i>Verticillium</i> sp.	2–20	Intracellular	Mukherjee et al. (2001a), Mukherjee et al. (2001b)
Silver	<i>Rhizopus nigricans</i>	35–48	Extracellular	Ravindra and Rajasab (2014)
	<i>Trichoderma</i> sp.	5–40	Extracellular	Fayaz et al. (2010)
	<i>Alternaria alternata</i>	20–60	Extracellular	Gajbhiye et al. (2009)
	<i>Phoma glomerata</i>	60–80	Extracellular	Birla et al. (2009)
	<i>Penicillium fellutanum</i>	5–25	Extracellular	Kathiresan et al. (2009)
	<i>Penicillium brevicompactum</i>	58.35 ± 17.88	Extracellular	Shaligram et al. (2009)
	<i>Fusarium solani</i>	16.23	Extracellular	Ingle et al. (2009)
	<i>Humicola</i> sp.	5–25	Extracellular	Syed et al. (2013)
	<i>Cladosporium cladosporioides</i>	10–100	Extracellular	Balaji et al. (2009)
	<i>Aspergillus fumigates</i>	5–25	Extracellular	Gade et al. (2008); Prabhu et al. (2009)
	<i>Volvariella volvacea</i>	Ag and Au-Ag 15 and 20–150 nm	Extracellular	Daizy (2009)
	<i>Fusarium semitectum</i>	10–60	Extracellular	Basavaraja et al. (2008)
	<i>Aspergillus niger</i>	20 nm/3–30 nm	Extracellular	Gade et al. (2008); Jaidev and Narasimha (2010)
	<i>Aspergillus flavus</i>	8.92	Extracellular	Vigneshwaran et al. (2007)
	<i>Penicillium purpurogenum</i>	5–200	Extracellular	Nayak et al. (2011)
	<i>Penicillium atramentosum</i>	5–25	Extracellular	Sarsar et al. (2015)

(continued)

**Table 2.1** (continued)

Nanoparticle type	Fungus name	Size of the nanoparticle (nm)	Production location	References
Silver	<i>Fusarium oxysporium</i>	5–50 nm/8–14 nm Au-Ag	Intracellular/extracellular	Senapati et al. (2004); Senapati et al. (2005)
	<i>Aspergillus clavatus</i>	550–650	Extracellular	Saravanan and Nanda (2010)
	<i>Neurospora crassa</i>	11 nm for silver and 32 nm for gold	Extracellular	Castro-Longoria et al. (2011)
	<i>Trichoderma asperellum</i>	13–18	Extracellular	Mukherjee et al. (2008)
	<i>Tricoderma viride</i>	2–4	Extracellular	Mohammed-Fayaz et al. (2009)
	<i>Trichoderma reesei</i>	5–50	Extracellular	Vahabi et al. (2011), Mansoori (2010)
	<i>Alternaria alternata</i>	20–60	Extracellular	Gajbhiye et al. (2009)
	<i>Fusarium oxysporum</i>	30	Extracellular	Duran et al. (2007)
	<i>Fusarium oxysporium</i>	3–11	Extracellular	Bansal et al. (2005)
	<i>Aspergillus terreus</i>	54.8–82.6	Extracellular	Baskar et al. (2013)
	<i>Fusarium oxysporium</i>	9–15/5–20	Extracellular	Ahmad et al. (2002), Kumar et al. (2007)
	<i>Fusarium oxysporium</i>	5–15	Extracellular	Bansal et al. (2005)
	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	10–100	Intracellular/Extracellular	Riddin et al. (2006)
	<i>Fusarium oxysporium</i>	6–13	Extracellular	Bansal et al. (2005)
	<i>Fusarium oxysporium</i>	20–50	Extracellular	Bharde et al. (2006)

dispersive X-ray spectroscopy (EDS), and transmission electron microscopy (TEM) confirmed the biosynthesis of nanoparticles by *N. crassa*. The size of nanoparticles was found to be average diameter of 32 nm for gold when the fungus was exposed to the aqueous solutions of HAuCl<sub>4</sub>. The results obtained indicate that *N. crassa* can be a potential “nanofactory” for the synthesis of metallic nanoparticles.

Vala (2015) produced gold nanoparticles using a marine-derived fungal isolate, *Aspergillus sydowii*. The mode of biosynthesis (extracellular/intracellular) depended on supplied gold ion concentration. Higher concentrations supported synthesis of smaller particles. The particles biosynthesized at 3 mM gold chloride were found to be spherical and nearly monodisperse in nature. The particles were found to be in the size range of 8.7–15.6 nm with a mean diameter of 10 nm (Vala 2015). Roy and



co-workers studied the extracellular biosynthesis of gold nanoparticles (GNPs) using the fungal species *Aspergillus foetidus*. X-ray diffraction (XRD) results revealed distinctive formation of face centered cubic crystalline GNPs. The spherical and polydispersed GNPs in the range of 10–40 nm were observed by TEM analysis (Roy et al. 2016).

Gopinath and Arumugam (2014) investigated the extracellular synthesis of gold nanoparticles from *Fusarium solani* culture filtrate. Synthesized gold nanoparticles were characterized by UV–VIS, FTIR, XRD, AFM, and TEM analysis. TEM results revealed that the gold nanoparticles were highly stable in the diameter range between 20 and 50 nm. Mukherjee et al. (2002) also studied *Fusarium oxysporum* strain for producing gold nanoparticles using green chemistry approach. The same researchers utilized *Trichoderma asperellum* for biosynthetic route to nanocrystalline silver particles (Mukherjee et al. 2008).

### 2.3 Silver Nanoparticles

Microorganisms may play a significant role in toxic metal remediation through reduction of metal ions. Studies demonstrated that silver ions might be reduced extracellularly using *Fusarium oxysporum* to generate stable gold or silver nanoparticles in water. These nanoparticles synthesized by some microbes can be incorporated with several kinds of materials such as cloths. These cloths with silver nanoparticles are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria such as *S. aureus*. Duran et al. (2007) investigated the extracellular production of silver nanoparticles by *F. oxysporum* and its antimicrobial effect when incorporated with cotton fabrics against *S. aureus*. In conclusion, it was demonstrated the application of biological synthesis to silver nanoparticles production and its incorporation in cloths, providing them sterile properties. Similar results were also obtained by Ahmad et al. (2002). Basavaraja et al. (2008) produced highly stable and crystalline silver nanoparticles (10–60 nm) in solution by treating the filtrate of the fungus *F. semitectum* with the aqueous silver nitrate solution.

In another study, the biosorption of silver in the form of nanoparticles by the fungus *Aspergillus flavus* was demonstrated. These nanoparticles were found to be stable in aqueous for more than three months, which could be attributed to surface binding of stabilizing materials secreted by the fungus. The average size of the nanoparticles was determined to be  $8.92 \pm 1.61$  nm. Vigneshwaran et al. (2007) concluded that process of nanoparticle production is eco-friendly as it is free from any solvent or toxic chemicals. Gade et al. (2008) also performed biosynthesis of silver nanoparticles by *Aspergillus niger* isolated from soil. The nanoparticles characterized by TEM exhibited spherical silver nanoparticles with a diameter of around 20 nm. The silver nanoparticles showed remarkable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The reduction of the silver ions might have occurred by a nitrate-dependent reductase enzyme and a shuttle quinone extra-

cellular process. Potential of fungal-mediated biosynthesis of silver nanoparticles may be important for the development of effective antibacterial agents showing resistance to drugs available in the market. Another *Aspergillus* strain, *A. fumigatus*, was also used for extracellular biosynthesis of silver nanoparticles, synthesis process of which was quite fast and silver nanoparticles formed within minutes of silver ion coming in contact with the cell filtrate. TEM micrograph showed formation of well-dispersed silver nanoparticles in the range of 5–25 nm (Bhainsa and D'souza 2006).

Vahabi et al. (2011) reported the extracellular biosynthesis of silver nanoparticles (AgNPs) by using a fungus named *Trichoderma reesei*. In the biosynthesis of AgNPs by this fungus, the fungus mycelium is exposed to the silver nitrate solution. That prompts the fungus to produce enzymes and metabolites for its own survival. In this process the toxic  $\text{Ag}^+$  ions are reduced to the non-toxic metallic AgNPs through the catalytic effect of the extracellular enzyme and metabolites of the fungus. They suggested that this process is an excellent candidate for industrial scale production of silver nanoparticles

## 2.4 Other Nanoparticles

Bansal et al. (2005) showed that *Fusarium oxysporum* secreted proteins capable of hydrolyzing aqueous zirconia ions extracellularly at room temperature. Particularly gratifying is the fact that the fungus is capable of hydrolyzing tough metal halide precursors under acidic conditions. To use as biosorbent agent, the combination of  $\text{SiO}_2$ -nanoparticles (N-Si) with *Penicillium funiculosum* for the formation of (N-Si-Pen) was investigated as a solid sorbent phase. This biosynthesized nanoparticle was utilized to adsorb Pb(II). The maximum capacity value was  $1266.7 \mu\text{mol g}^{-1}$  for N-Si-Pen combined particle, at pH 5. Sorption equilibrium was established in about 20 min.

## 2.5 White Rot Fungi

White rot fungi are able to degrade a wide variety of environmentally pollutants such as organopollutants and xenobiotics by means of laccase enzyme (Birhanli and Yesilada 2010; Birhanli et al. 2013; Yesilada et al. 2014). Due to their many well-known advantages, various researchers are interested in the development of white rot fungi technology for biodegradation of pollutants (Cihangir and Saglam 1999), decolorization of textile dyes (Yesilada et al. 2003) and biodesulphurization of coal (Aytar et al. 2008).

There is a great interest in metallic nanoparticles because of their various applications in several areas such as medicine, biosensors preparation, water purification, food packaging and cosmetic industry (Li et al. 2011; Das and Thiagarajan 2012).

Various metallic nanoparticles including silver, iron, gold, cadmium, selenium and copper could be synthesized by physical and chemical methods. However, these methods are not eco-friendly as well as having some disadvantages. These nanoparticles must be produced via environment-friendly and safe methods due to their possible uses, especially in biomedical applications. Therefore, there is need to produce this metallic nanoparticles via safe approaches. A biological method, which is the green way of synthesizing, may be an alternative and eco-friendly method for producing metallic nanoparticles. There have been serious attempts to develop biological processes for nanoparticle production. The main advantage of biological methods is that they are safe and eco-friendly. In this green way, biological systems such as fungi, bacteria, plants and also enzymes can be used to produce these nanoparticles. Due to the pathogenic properties of various fungi, the use of safe fungi is important. The solution could be white rot fungi. These fungi are non-pathogenic and many of them have also medicinal properties. There is limited study on the production of nanoparticles by white rot fungi. The extract, mycelia or culture filtrate of white rot fungi can be used to synthesize metallic nanoparticles with various sizes via bioreduction process (Table 2.2).

**Table 2.2** Some nanoparticles (AgNPs) produced by white rot fungi and their applications

Fungus	NP	Shape	Size (nm)	Application	References
<i>P. sajor caju</i>	AgNP	Spherical and well distributed without aggregation	40	Decolorization	Nithya and Rangunathan (2011)
<i>P. sajor-caju</i>	AgNP	Spherical	5–50	Antibacterial	Nithya and Rangunathan (2009)
<i>P. florida</i>	AgNP	Spherical	20 ± 5	Antibacterial	Bhat et al. (2011)
<i>P. ostreatus</i>	AgNP	–	100	Antibacterial	Mirunalini et al. (2012)
<i>P. ostreatus</i>	AgNP	Polydispersed with spherical	4–15	Anticandidal, Anticancer	Yehia and Al-Sheikh (2014)
<i>G.neo-japonicum</i> Imazeki	AgNP	Spherical and well dispersed	5–8	Anticancer	Gurunathan et al. (2013)
<i>Pleurotus djamor</i> var. <i>roseus</i>	AgNP	Spherical	90–370	Anticancer	Raman et al. (2015)
<i>Pleurotus cornucopiae</i> var. <i>citrinopileatus</i>	AgNP	Spherical	20–30	Anticandidal	Owaid et al. (2015)

(continued)

**Table 2.2** (continued)

Fungus	NP	Shape	Size (nm)	Application	References
<i>T.trogii</i>	AgNP	–	<10	–	Unpublished data
<i>Pleurotus</i> sp.	FeNP		–	–	Mazumdar and Haloi (2011)
<i>Stereum hirsutum</i>	CuNP and copper oxide	Monodispersed and spherical	5–20	–	Cuevas et al. (2015)
<i>Lentinula edodes</i>	AuNP	Spherical	5–50	–	Vetchinkina et al. (2013)
<i>Ganoderma</i> sp.	AuNP	Monodispersed and spherical	20	Biocompatibility	Gurunathan et al. (2014)
<i>Pycnoporus sanguineus</i>	AuNP	Various shapes	Several to several hundred nm	Biodegradation	Shi et al. (2015)
<i>P. ostreatus</i>	CdS	Spherical	4–5	–	Borovaya et al. (2015)

### 3 Biosynthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) have various applications including therapeutic ones. Therefore, their production by biotechnological approach is very important. There are some studies on AgNP production potential of white rot fungi. Vigneshwaran et al. (2006) used the mycelial mat of *Phanerochaete chrysosporium* to synthesize the stable AgNPs. The workers stated that the extracellular proteins of this fungus keep the NPs stable by minimizing the aggregation with capping. They reported the peak maximum at 470 nm instead of 413 nm which might be due to a decrease in particle size.

The same group also produced uniform AgNPs, which remained stable for more than 6 months by spent substrate (stalks) of edible *Pleurotus sajor caju* (Gade et al. 2008). The proteins secreted by this fungus on solid substrate functioned as capping agent and thus stabilized the NPs. AgNPs showed the surface plasmon absorption band at 436 nm. They also had an excellent antibacterial activity (Vigneshwaran et al. 2007). Nithya and Ragunathan (2011) produced spherical shaped AgNPs with an average size of 40 nm by the biomass of *P. sajor caju* and used these *P. sajor caju* NPs to decolorize congo red dye. They emphasized that these *P. sajor caju* NPs could be used for treatment of dyes. The biosynthesis of spherical shaped AgNPs, with an antibacterial activity against Gram positive and Gram negative bacteria, using *P. sajor caju* culture filtrate has also been reported by Nithya and Ragunathan (2009).

It was shown that stable AgNPs could be synthesized by culture filtrate or mycelium of *Coriolus versicolor* MUCL (Sanghi and Verma 2009a). The reduction capacity of filtrate (third day) was faster than the mycelium (fourth day) at pH 5.5–6.0. Under alkaline conditions (pH 10) the reaction was much faster and only 60 min was enough for fast reduction of silver ions. They attributed this effect to the increase in the reducing power of the responsible proteins at alkaline conditions. The fungal proteins acted as reducing and capping agents in NPs production process and spherical shaped NPs were obtained. The pH of the solution was reported to be an important factor affecting the morphology of the nanoparticle obtained.

Mycosynthesis of AgNPs was also achieved using the *Pleurotus florida* mushroom extract as reducing agent (Bhat et al. 2011). The spherical shaped NPs formed by the photo-irradiation technique using the extract as bioreductant were in a size range of  $20 \pm 5$  nm. They showed antimicrobial activity against various pathogenic microorganisms. The workers claimed that their approach was biologically safe and eco-friendly due to the biosynthetic nature of this process.

Various extracts of *Pleurotus ostreatus* are also able to synthesize AgNPs. Mirunalini et al. (2012) described the biosynthesis of AgNPs by *P. ostreatus* mushroom extract. The authors stated that the extract containing proteins could act as the reducing and capping agents, and the NPs with antimicrobial activity against *Staphylococcus aureus* could be obtained. Both Devika et al. (2012) and Yehia and Al-Sheikh (2014) demonstrated that *P. ostreatus* extract reduced silver nitrate solutions and produced AgNPs with the diameter of about 50 and 450 nm, respectively. These NPs were stable due to capping by proteins. The AgNPs exhibited antimicrobial activity against various pathogenic microorganisms. Devika et al. (2012) also verified that the antimicrobial activity of AgNPs increased when the mixture of AgNP and antibiotic were used. Yehia and Al-Sheikh (2014) reported the dose dependent antiproliferative effect of biologically synthesized AgNPs against human breast carcinoma cells (MCF-7)

Mycosynthesis of AgNPs by various extracts of *Ganoderma lucidum* was also described by Mirunalini et al. (2012), Karwa et al. (2011) and Paul et al. (2005). Mirunalini et al. (2012) and Paul et al. (2005) used the extract of mushroom form directly, while Karwa et al. (2011) preferred the extract of mycelia. The bioreduction of silver ions was achieved by *Ganoderma lucidum* mushroom and silver ions were completely reduced to AgNPs with an average size of 50 nm after 48 h (Mirunalini et al. 2012). These NPs were found to have antibacterial activity against *S. aureus*. Paul et al. (2005) also reported the fast bioreduction activity of the extract of *G. lucidum* mushroom, with the presence of large amounts of polyphenols in the sample. The authors incorporated the AgNPs in cotton fabrics and their bacteriostatic activity against various bacteria was also tested. The fabrics incorporated with AgNPs showed a high antibacterial activity against various pathogens. Karwa et al. (2011) also described the biosynthesis of stable AgNPs with the average size of 45 nm by mycelial extract of *G. lucidum*. FT-IR analysis confirmed that large particles may be due to the protein capping of the particles and the authors concluded that the extracellular enzymes were responsible for the reduction. The obtained NPs showed antibacterial activity against *S. aureus* and *E. coli*. The combined effect of

NPs and tetracycline antibiotic was also studied. The results demonstrated a higher antibacterial activity of the mixture than the tetracycline alone. This indicates the synergistic effect of NPs and antibiotic.

Gurunathan et al. (2013) described the green synthesis AgNPs by the extract of *Ganoderma neo-japonicum* Imazeki KUM61076. This NPs solution had long-term stability which might be due to the proteins in the extract as capping agent. TEM and DSL analysis showed that most of the AgNPs are spherical in shape and relatively uniform and monodispersed with an average size of 5 nm. The study demonstrated the cytotoxic effect of biologically synthesized AgNPs against MDA-MB-231 human breast cancer cells. It was observed that cell death and membrane leakage were dose-dependent.

Chan and Don (2012) showed that *Schizophyllum commune* and *Pycnoporus sanguineus* could be used for biological synthesis of AgNPs. They used directly mycelia or culture supernatant of these white rot fungi for testing their reduction effect. The mycelia or culture supernatant produced AgNPs with different sizes. The obtained AgNPs were detected as an effective antimicrobial agent against various bacteria and fungi. The authors speculated that AgNPs produced extracellularly as well as by culture supernatant have better antimicrobial activity when compared to AgNPs synthesized intracellularly.

More recently, Raman et al. (2015) and Owaid et al. (2015) described the mycosynthesis of AgNPs by the extract of *Pleurotus djamor* var. *roseus* basidocarps and *P. cornucopiae* var. *citrinopileatus*. When the extract of *P. djamor* var. *roseus* basidocarps exposed to silver ions, the spherical AgNPs with an average size ranging from 5-50 nm could be obtained. Spectral and electron microscopy results revealed that the extract serves as a reducing agent in the biosynthesis of AgNPs. The cytotoxic effects of these nanoparticles against human prostate carcinoma cells (PC3) were also described in this study. These AgNPs significantly inhibited the cell viability and induced cell death in dose-dependent manner. The extract of *P. cornucopiae* var. *citrinopileatus* was also able to synthesis the spherical shaped AgNPs with an average size ranging from 20 to 30 nm. The authors speculated that ions were reduced by the active molecules like polysaccharides and proteins present in this extract. The nanoparticles exhibited antifungal activity against *Candida albicans*, *C. glabrata*, *C. krusei* and *C. pseudotropicalis* especially at a concentration of 60 µg/well.

Yesilada (unpublished data) observed that it was possible to synthesis AgNPs by the culture filtrate of *P. ostreatus*, *T. trogii* 200800 and *T. versicolor* 200801. When *T. trogii* filtrate incubated with AgNO<sub>3</sub> solution, a change in the color of the solution was observed and AgNPs with the diameter below 10 nm were obtained (Table 2.2).

## 4 Biosynthesis of Other Metallic Nanoparticles

White rot fungi can also be used to synthesize different nanoparticles such as iron, copper, gold and cadmium sulphide nanoparticles. Iron nanoparticles have been successfully synthesized by the mycelia of *Pleurotus* sp. (Mazumdar and Haloi

2011). No color change was observed in medium or biomass. The authors suggested that some biochemical changes may occur in the medium due to the oxidation of ferrous ions.

Cuevas et al. (2015) described the use of the extract of *Stereum hirsutum* for preparing copper and copper oxide nanoparticles. In their study, three copper salts ( $\text{CuCl}_2$ ,  $\text{CuSO}_4$  and  $\text{Cu}(\text{NO}_3)_2$ ) were used and the effect of various pH on reduction activity of this extract was investigated.  $\text{CuCl}_2$  (5 mM) gave the highest nanoparticle formation at alkaline conditions. The extracellular protein in this extract may be responsible for nanoparticle formation and stabilization. The obtained nanoparticles (5–20 nm) are spherical in shape, monodispersed and they were embedded in a biopolymer. The XRD analysis also revealed the polycarbohydrate nature associated with stability of the nanoparticles.

Sanghi et al. (2011) compared the utilization of the mycelium and culture filtrate of *P. chrysosporium* for biosynthesis of gold nanoparticles (AuNPs). The rate and shape of particle formation was temperature dependent. Both the mycelium and culture filtrate were ineffective in biosynthesis of AuNPs at room temperature. However, when the temperature increased to 37 °C nanoparticle formation started within 3 min. During the mycelia studies the nanoparticles could only form on the surface of the mycelia and not in the solution (intracellular synthesis); the characteristic absorbance band of 525 nm being detected in the culture filtrate studies (extracellular synthesis). It was possible to obtain stable nanoparticles in the form of spheres and with the diameter of 10–100 nm. Mycelium age influenced the rate and extent of the nanoparticle formation due to the amount of protein secreted. The results of this study demonstrated that the rate of particle formation and size of particles might be controlled by temperature, gold ion concentration and exposure time to  $\text{HAuCl}_4$ . It was also speculated that laccase and lignin peroxidase enzymes are responsible for the extracellularly and the intracellularly bioreduction of gold ions, respectively.

The AuNP biosynthesis activity of *Lentinula edodes* was investigated during the growth under agitated culture conditions. The growing mycelial cells of this fungus produced and accumulated the spherical nanoparticles with 5–50 nm in diameters. The authors emphasized that elemental gold was reduced and accumulated either on the surface or inside the mycelia (Vetchinkina et al. 2013).

Gurunathan et al. (2014) reported the extract of *Ganoderma* sp. as a good reducing and stabilizing agent. The proteins in the extract help the biosynthesis of AuNPs. The obtained monodispersed AuNPs with an average size of 20 nm were detected as nontoxic and biocompatible against MDA-MB-231 human breast cancer cells. The authors speculated that the biocompatible AuNPs could be used in catalysis, sensors, electronics and biomedical applications, especially for cancer therapy.

Another study on AuNPs biosynthesis is the bioreduction of gold ions by intracellular protein extract of *Pycnoporus sanguineus* (Shi et al. 2015). The AuNPs with high catalytic activity could be obtained using this extract. The amount of extract and also gold ion concentration influenced the nanoparticle production and particle size. The solution pH and bioreduction rate were also important for particle size distribution and characteristic of nanoparticles. The authors also tested the cata-



lytic activity of the obtained nanoparticles in 4-nitroaniline (4-NP) degradation and reported the complete degradation of 4-NP in 6 min.

Sanghi and Verma (2009b) described a continuous and extracellular formation of cadmium sulphide nanoparticle (CdS) by immobilized *Coriolus versicolor* in a column reactor. The protein was reported as a capping agent. TEM images showed that the embedded nanoparticles in the fungal matrix were well dispersed spherical nanoparticles with uniform size (about 5–9 nm). *P. chrysosporium* was able to synthesize various nanoparticles as stated above. Chen et al. (2014) demonstrated that *P. chrysosporium* synthesized uniform and sphere shaped fluorogenic CdS nanoparticles with the average size of 2.56 nm. The rate of production was dependent on pH level. They also stated the role of proteins and amino acids in the formation of the CdS particles. The mycelium of *P. ostreatus* can also be used for the production of luminescent CdS nanoparticles. It was reported that spherical shaped CdS nanoparticles with a particle size of 4–5 nm could be obtained by the mycelium of this fungus (Borovaya et al. 2015).

The fungal-mediated eco-friendly synthesis approach of nanoparticles has many advantages, for example, the ease with which the process can be scaled up, economic viability, downstream processing, and simpler handling of the biomass and possibility of easily covering large surface areas by appropriate growth of the mycelia (Prasad et al. 2015). A number of filamentous fungi have been successfully used for extracellular biosynthesis of silver and gold nanoparticles and others. Generally, analytical techniques, such as ultraviolet-visible spectroscopy, X-ray powder diffraction, and transmission electron microscopy and zeta potential measurements were applied to characterize the morphology of nanoparticles.

## 5 Biosynthesis of Metallic Nanoparticles by Laccase

Fungi secrete high amount of enzymes in their growth medium. Due to the protein nature of the enzymes, this is an advantage for the biogenic formation of NPs. There are some studies on possible bioreduction role of laccase from fungi (Table 2.3). Faramarzia and Forootanfara (2011) reported that purified laccase enzyme from the ascomycete *Paraconiothyrium variable* is responsible from production of AuNPs. The gold nanoparticles obtained at 70 °C after 20 min of incubation were in the size

**Table 2.3** Nanoparticles (NPs) biosynthesized by laccase enzyme

Source of laccase	NP	Shape	Size (nm)	References
<i>Paraconiothyrium variable</i>	AuNP	Well dispersed	71–266	Faramarzia and Forootanfara (2011)
<i>P. ostreatus</i>	AuNP	Mono dispersed	22–39	El-Batal et al. (2015)
<i>Trametes versicolor</i>	Ag@AgClNP	Spherical	<100	Jose et al. (2013)



range of 71–266 nm and this was the best temperature for this nanoparticle production activity. It was concluded that the reductive groups of the enzyme might be responsible from AuNPs. El-Batal et al. (2015) also described the AuNP production ability of the partially purified laccase enzyme from solid state culture of *P. ostreatus*. The AuNPs were highly mono dispersed nanoparticles with the size range of 22–39 nm. This production activity was found to be dependent on temperature, radiation and substrate concentration. The authors emphasized that laccase performed the reduction as a protein and not as an active enzyme. Durán et al. (2014) described silver and silver chloride nanoparticles production by semi-purified laccase from *Trametes versicolor*. It was also stated that the main pathway for reduction was the interaction of silver ions with T1 site of laccase enzyme, which the sulfhydryl group is the reducing agent.

## 6 Future Prospects

Starting with the last century, microorganisms (especially fungi) have been widely used for medical treatments and disease prevention efforts. For that purpose, several primary and secondary metabolites (antibiotics, immune suppressor substances, enzymes, biosurfactants and organic acids) have been produced at large scales. Considering that we know about a more or less 5 % only of the fungi available in nature, it is highly plausible that there will be an increase in the scientific and technological interest in fungal nanotechnology and the related fields. Since nanoparticles produced by chemical synthesis are highly toxic, a greener alternative (production of nanoparticles via biological pathways) seems to have already gained a strong interest in the scientific community (Jose et al. 2013; Devika et al. 2012; Popescu et al. 2010). On the other hand, functional nanofibrous scaffolds produced by electrospinning have important potential in nanobiotechnology, such as nanomembranes for environmental applications, heavy metals removal from waste water treatment, tissue engineering, enzyme immobilization and drug delivery for biomedical/nanomedicine applications. Thus, all these nanomaterials have a sustainable, biocompatible, biodegradable, antimicrobial and non-toxic of great relevance in nanotechnology (Spasova et al. 2011; Prasad et al. 2015).

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