**Biosynthesis of nanoparticles by fungi**

 **Introduction**

 **Microorganism-Mediated Synthesis of Nanoparticles**

Much research has been published on the production of metallic NPs by microbial cells by wet synthesis. In general, microorganisms have the capacity to detoxify heavy metals, which they do mainly with metal-binding proteins and peptides, as a bioremediation process for survival in areas contaminated with heavy metals. This mechanism provides several advantages for the biosynthesis of NPs compared with the traditional chemical synthesis methods (Pantidos and Horsfall 2014). Thus, microorganisms are considered as potential biofactories for the synthesis of NPs of different metals such as gold, silver, platinum, palladium, and titanium. Microorganisms are known as efficient NP biofactories because of their capacity to produce large amounts of proteins, enzymes, amino acids, polysaccharides, and vitamins, which act as reducing and covering agents for reducing metal ions (Prasad et al. 2016). The biosynthesis of metallic NPs by various microbes, such as bacteria, yeasts, algae, and lower fungi, as well as edible mushrooms, has been studied (Prasad et al. 2016).

**Biosynthesis by Fungi**

The biosynthesis of metal NPs using filamentous fungi has been extensively studied and recognized as a green and efficient method for NP production. Fungal cells are highly efficient in the extracellular synthesis of NPs, based on their high capacity to excrete reducing agents that are used in this synthesis (Sawle et al. 2008; Prasad et al. 2016). Fungi are characterized by their high capacity to excrete a wide range of metabolites; this maintains their internal hemostasis and enables their survival under harsh environmental conditions with limited nutrients and in the presence of toxic materials (Vahabi et al. 2011). In the biosynthesis of NPs the metal ions are reduced to inorganic solid metal NPs through the catalytic effect of extracellular enzymes and the release of large amounts of proteins into solution (Vahabi et al. 2011; Ahmad et al. 2005). This phenomenon has been proven to contribute to the biosynthesis of stable NPs without the need to add external stopping agents (Gupta and Bector 2013)*.* Therefore, fungal cells are widely used in NP synthesis since they are tolerant to high metal concentrations during the process and produce good NP dispersion (Vahabi et al. 2011). In addition, for the large-scale production of NPs, fungal cells are recommended as first-choice biofactories owing to their high productivity and low energy consumption. Compared with bacterial cells, fungal cells can be easily separated from the broth through a simple filtration process, thus saving

considerable cost in the downstream process (Vahabi et al. 2011; Prasad 2016, 2017). In conclusion, the possibility of developing a rational, fungal-based method for the synthesis of Ag-NPs and Au-NPs has been reported using a wide range of fungal strains, including *Botrytis cinerea, Trichoderma reesei, Aspergillus clavatus, A.* *fumigatus, A. oryzae* var. viridis*, A. sydowii, A. terreus, Hormoconis resinae,* *Fusarium semitectum, Alternaria alternata*, and *Penicillium brevicompactum*

(Table 2.1). These fungi were simply exposed to solutions of different types of metal or inorganic ions for the single-step synthesis of various types of metal NPs (Park et al. 2016). Ag-NPs and Au-NPs have drawn much attention because of their extensive application in the medical and cosmetic industries.



**Mechanism of Metal Nanoparticle Biosynthesis by Fungi**

Fungi produce NPs as a cellular defense mechanism against the chemical pollutants found in their habitats. Toxic ions are reduced to their metal NPs by various chemical reactions, e.g., precipitation and co-precipitation, complexation, biosorptionion form modification, immobilization, or bio-coupling (Das et al. 2012a, b; Dorcheh and Vahabi 2016). Fungi use their cellular enzymes, proteinaceous molecules, or cell membrane-bound molecules as electron donors during the reduction

process. Once reduced, the toxic ions are easily precipitated as metal NPs, either intracellularly or extracellularly, depending on the mechanism of biosynthesis (Vigneshwaran et al. 2007).

**1-Extracellular Fungal Biosynthesis of Metal Nanoparticles**

Fungal cell membranes play an important role in the extracellular biosynthesis of metal NPs. They contain large amounts of differently bound molecules, e.g., peptides, proteins, polysaccharides, oxidoreductases, and quinones, which participate in the process of metal ion reduction and precipitation (Sharma and Dietz 2006; Keat et al. 2015; Moghaddam et al. 2015). Extracellular reductases are the key enzymes responsible for the biosynthesis and growth of metal NPs (Vahabi and Dorcheh 2014). *F. oxysporum* cells have been reported to produce nicotinamide adenine dinucleotide phosphate (NADPH)- dependent nitrate and sulfite reductases and use them for the biosynthesis.

2-**Intracellular Fungal Biosynthesis of Metal Nanoparticles**

The intracellular fungal biosynthesis of metal NPs is mainly attributed to cellular ATPases and hydrogenases. *F. oxysporum* was found to produce Au-NPs intracellularly in cytoplasmic vacuoles, and the reaction was modulated by plasma membrane-ATPase, 3-glucan binding protein, and glyceraldehyde-3-phosphate dehydrogenase (Vahabi and Dorcheh 2014). Hydrogenases function to produce cytoplasmic hydrogen, which is required to precipitate metal NPs (Riddin et al. 2009). Yeast cells have been found to use their intracellular glutathione, and the two metal-binding proteins (phytochelatin and metallothionein) in their detoxification mechanism (Breierovل et al. 2002). This finding was attributed to the fact that these compounds have important redox and nucleophilic characteristics, and thus participate in the bioreduction of metal ions. Additionally, fungal cells have been reported to use their antioxidative systems to detoxify metal ions, to protect the cells from being injured by the oxidative power of these metal ions (Jha and Prasad 2016).

**Characterization of Metal Nanoparticles**

Researchers usually use different techniques to characterize biosynthesized NPs. These techniques are generally employed to give useful information about the size, composition, crystalline type, and chemical state, as well as the optical and magnetic properties, of the biosynthesized NPs (Kulkarni 2015). The techniques employed are classified into different categories, such as microscopy-, diffraction-, spectroscopy-, magnetic properties-, and mechanical properties dependent techniques.

Table 2.2 summarizes the different techniques used to characterize biosynthesized NPs



**1 Electron Microscopy (TEM and SEM*)***

TEM microscopy is normally employed to investigate the morphological characteristics of biosynthesized NPs (Gupta and Bector 2013). These characteristics include nanoparticle shape and size, the formation of aggregates, and symmetrical properties. As the fungal biosynthesis of NPs proceeds via protein capping to provide stability and protection for the formed NPs, TEM can be combined with elemental spectroscopy imaging (ESI) to characterize the capping procedure (Maliszewska et al. 2014). Mukherjee et al. (2001a) used TEM scanning to determine the location of Ag-NPs produced within fungal cells. The elemental characterization of produced NPs was investigated with the help of SEM, accompanied by energy dispersive X-ray spectroscopy (EDS) (Durلn et al. 2005). The presence of nanomaterials within fungal mycelia has been confirmed using SEM combined with energy diffraction analysis (Vigneshwaran et al. 2007).

**2 Spectroscopic Techniques**

 **2.1 UV-Visible Spectroscopy**

The application of the UV-visible spectroscopy technique depends on the development of surface plasmon resonance, which produces strong absorption and scattering of light when the biosynthesized NPs have sizes smaller than or similar to the penetration depth of the electromagnetic field in the metal (Durلn et al. 2010). Plasmon resonance is used to validate the biosynthesis of AgNPs (Netala et al. 2016), and its development can be affected by various parameters, i.e., particle shape and size and the medium dielectric constant. UV-visible spectroscopy is also used to differentiate nanoparticles having aggregate structures from those not forming aggregates, depending on the separation between UV bands (Basavaraja et al. 2008). Moreover, the fact that protein is absorbed at 270 nm, owing to the presence of tryptophan and tyrosine residues, makes it possible to detect protein capping for the synthesized NPs.

 **2.2 Fluorescence and FTIR**

Fluorescence and FTIR are generally employed to evaluate the binding properties of the cellular proteins with the biosynthesized NPs (Durn et al. 2005). Ag-NPs have been excited at 260 nm and found to emit another band at 340 nm. This was attributed to the fact that fungal cellular proteins are attached to the peripheral areas of the NPs, while unbound proteins in the solution remain in their original form. FTIR spectroscopic analysis is also widely employed to investigate protein-NP interactions in terms of the formation of secondary structures. This analysis has also been used to detect protein conformational changes that occur upon binding to the newly synthesized nanomaterials, as well as to confirm the presence of functional groups and thiol derivatives in the excreted proteins (Srivastava and Mukhopadhyay 2015). Such investigations are useful for establishing the mechanism of the reduction process and, hence, the stabilization of the formed nanomaterial.

 **2.3 Photoluminescence**

The capacity of biosynthesized nanoparticles to enhance fluorescence emission has made it possible to use photoluminescence as a powerful tool to investigate the optical characteristics of produced NPs. For example, AgNPs synthesized by *Phanerochaete chrysosporium* were found to emit at 423 nm, while the original silver nitrate solution did not emit at this wavelength (Vigneshwaran et al. 2006).

**.2.4 X-Ray Diffraction (XRD)**

X-ray diffraction has been widely used to determine the particle size, and the particle size distribution, of biosynthesized NPs (Magdi and Bhushan 2015). The crystalline nature of the formed nanomaterials can also be examined by XRD (Khatami et al. 2016). The technique uses the Debye Scherrer equation to calculate particle size. Particle size determined by XRD is closely correlated with measurements obtained from TEM calculations (Basavaraja et al. 2008).