Chapter 11
Intervention of Fungi in Nano-Particle Technology and Applications

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Abstract Biosynthesis of nanomaterial is of particular attention for material scientists due to its environmentally benign perspective and durability in a natural medium. Nanoparticles synthesized by using the whole cell, either inside the biological entity (intracellular) or extract/lysate/peptide-template (extracellular) believed to have a wide range of biological application. The chapter focuses primarily on the mechanistic investigation of metal and metal oxide nanoparticle synthesis and their potential applications in the agricultural and biomedical sector. So far fungus is explored more for silver nanoparticle synthesis among all other nanoparticles and their use as an antimicrobial agent either bare nanoparticles or as a synergetic agent with existing counterparts. In addition, fungus-nanotechnology explored for the synthesis of agriculturally important nutrient for native phosphorus mobilization and enhancement in photosynthetic activity.

1 Introduction

Fungi, belongs to the group of eukaryotic organism, have been extensively used to produce industrial chemical and enzymes for various purposes, notably from food to medicine (Carlile et al. 2001; Prasad et al. 2015). With the advent of modern nanotechnology, researchers have practiced harnessing fungal strains to provide an

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alternative greener approach of nanoparticle production. A nanoparticle or nanostructure is an intentionally created, engineered particle of 1–100 nm at least in one dimension. Due to extremely small size, nanoparticles have high surface area to mass ratio that brings extraordinary physicochemical properties than its bulk counterpart (Suman et al. 2010; Prasad 2014; Aziz et al. 2015). Nanoparticle research is currently an area of intense scientific interest of the community due to a wide variety of potential applications in agriculture, biomedical, energy, environmental, electronic and optical fields (Prasad et al. 2014; NSF 2015).

Producing nanomaterial's sustainably in part means using less harmful chemicals in nanoparticle production (Murphy 2008). One of the ways nanoparticles can be created without using harmful chemicals is by exploiting natural biological processes, and fungus is one of the organisms used in nanoparticle technology to create nanoscale objectives. To the best of authors' literature survey in the scientific domain, historically, Dameron et al. (1989) biosynthesized nanometer scale (2 nm) semiconductor quantum crystallites of cadmium sulfide (CdS) using yeasts *Candida glabrata* and *Schizosaccharomyces pombe*, cultured in the presence of cadmium salts. The biosynthesized quantum CdS crystallites were more monodisperse than CdS nanoparticles synthesized chemically. Later, in 2001, a team of National Chemical Laboratory, India, used fungus *Verticillium* to reduce aqueous silver nitrate solution into silver nanoparticle of 25 nm (Mukherjee et al. 2001a). The reduction of the metal ions occurs on the surface of the mycelia (extracellular) leading to the formation of silver nanoparticles of fairly well-defined dimensions and tolerable monodispersity. In-spite of different proposed synthesis mechanism (intracellular vs. extra-cellular), both of these early landmark studies suggested that protein/peptide play a key role in the reduction of metal salt.

The advantage of biosynthesis reaction is obvious from the sustainability point of view (e.g., recycling or utilization of bio-based objects for chemical production) (Murphy 2008). The fungal mediated synthesis of nanoparticles has many advantages over other biosynthesis approaches (using whole cell(s), secretion, extract or lysate of bacteria, plant or animal source) such as ease of scale-up process, minimum downstream processing, economically viable, easy to maintain the culture and handling of biomass (Prasad et al. 2015). Compared to bacteria, fungi are saprophytic in nature, the large surface area covered by mycelia (help in intracellular synthesis) and also known to secret much higher amounts of protein (help in extracellular synthesis), thereby fungi has significant attention to using as nanoparticle production.

2 Fungal Mediated Nanoparticle Synthesis and Mechanistic Aspects

Fungi are drawing the attention of nanotechnology researchers for the bottom up and top down synthesis of nanoparticles due to metal tolerance, bioaccumulation, and saprophytic ability (Sastry et al. 2003; Thakkar et al. 2010; Raliya et al. 2013). Over the several nanoparticle biosynthesis sources such as bacteria, plant, and

Table 11.1 Synthesis of metal and metal oxide nanoparticles using fungi

Fungi species/name	Type of nanoparticles	Size (nm)	References
Verticillium sp.	Ag, Au	25 ± 12	Mukherjee et al. (2001a, b) and Sastry et al. (2003)
Fusarium oxysporum	Ag, Au, CdS, Zirconia, $BaTiO_3$, Ag-Au bi-metallic	4–40	Ahmad et al. (2002, 2003), Mukherjee et al. (2002), Senapati et al. (2005) and Bansal et al. (2006)
Aspergillus oryzae	Fe	10–24.6	Raliya and Tarafdar (2013a)
Aspergillus fumigatus	Ag	5–25	Bhainsa and D'Souza (2006)
Aspergillus terreus	Ag	2.5	Raliya and Tarafdar (2012)
Aspergillus flavus	Ag	8.92 ± 1.61	Vigneshwaran et al. (2007)
Aspergillus tubingensis	TiO_2, Phosphorous	1.5–30	Tarafdar et al. (2012, 2013)
Rhizoctonia bataticola	Au	6.2	Raliya and Tarafdar (2013a, b, c, 2014)
Pochonia chlamydosporium, Aspergillus fumigatus, Curvularia lunata, Chaetomium globosum, Aspergillus wentii, Aspergillus tubingensis, Aspergillus flavus, Aspergillus terreus	Zn, Ti, Mg and Fe	12–95	Kaul et al. (2012), Raliya and Tarafdar (2014) and Raliya et al. (2014a, b)

higher kingdom animal tissues, fungi are a potential candidate to scale-up nanoparticle synthesis process due to economic viability (Prasad et al. 2015). Table 11.1 summarizes the metal and metal oxide nanoparticles synthesize using fungi. Scientific literature survey suggests that fungi have been used primarily for metal and metal oxide nanoparticles, in particular, silver (Ag), gold (Au), zinc (Zn), zinc oxide (ZnO) and titanium di oxide (TiO_2) nanoparticles synthesis (Table 11.1).

The fungus can synthesize nanoparticles both inside the cell (intracellular) and outside the cell (extracellular). One of the early report on the use of fungus for intracellular nanoparticle synthesis by using *Verticillium sp.* was reported by Mukherjee et al. (2001a). Author exposed fungal biomass with aqueous silver nitrate suspension. As a result of the intracellular reduction of the metal ions, 20–30 nm silver nanoparticles were observed below the cell wall surface (Fig. 11.1). It was also observed in the same study that silver nanoparticle didn't exert any toxic effect rather increased biomass after biosynthesis of silver nanoparticles. Similarly, the same *Verticillium sp.* used for the bioreduction of gold chloride and found similar to the size of the silver nanoparticle as reported above (Mukherjee et al. 2001b). The authors explained that internalization of a precursor salt of gold ions on the surface of fungal

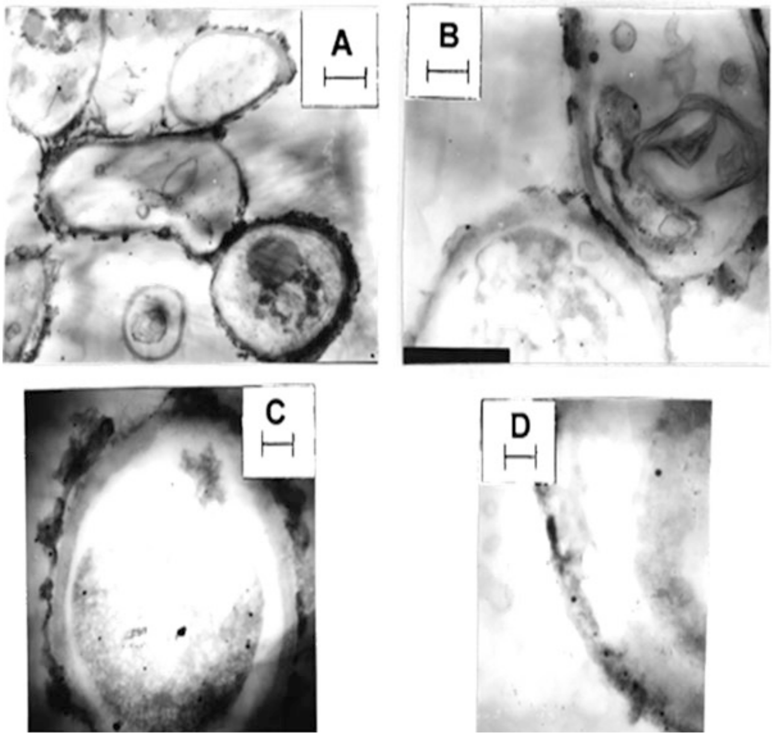

Fig. 11.1 TEM micrograph *Verticillium* cells after reaction with silver nitrate at different magnifications (**a–d**). The scale bars in (**a–d**) correspond to 1 μm, 500, 200, and 100 nm respectively (Mukherjee et al. 2001a)

cells perhaps occurs by electrostatic interactions with positively charged groups such as lysine residues in enzymes that existed in the mycelial cell wall. In addition to *Verticillium, Fusarium oxysporum* has also been used for the synthesis of CdS (Ahmad et al. 2002), $BaTiO_3$ (Bansal et al. 2006), Ag (Ahmad et al. 2003), Au (Mukherjee et al. 2002) and Ag-Au bi-metallic (Senapati et al. 2005). Diversion to the extracellular synthesis of nanoparticles using fungi is believed to simplify the purification steps for synthesized nanoparticles.

Mechanistically, it is proved that molecular machinery and various cellular proteins are involved in the bioreduction of the metal salt. Barwal et al. (2011) explained that ATPase, sedoheptulose-1, 7-bisphosphatase, carbonic anhydrase, ferredoxin NADP+ reductase, superoxide dismutase, oxygen evolving enhancer protein ribulose bisphosphate carboxylase and nuclear histone are involved in the bioreduction of silver nitrate by the unicellular algae *Chlamydomonas reinhardtii.* Raliya and Tarafdar (2012) explained the mechanism of extracellular synthesis of silver nanoparticles using the fungus *Aspergillus terreus* (Fig. 11.2). Authors also explained that protein capping enabled nanoparticle stability, monodispersity and

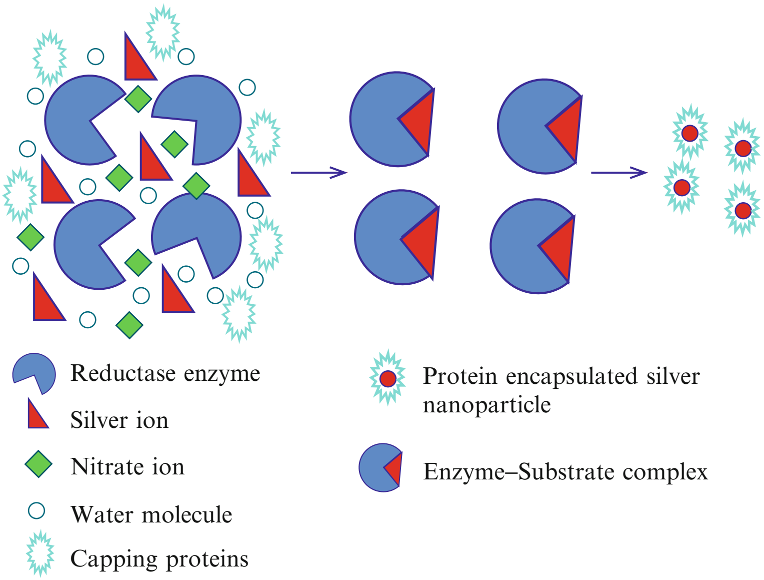

Fig. 11.2 Mechanism for biosynthesis of silver nanoparticles using *A. terreus* (Raliya and Tarafdar 2012)

environmentally benign (Raliya and Biswas 2015). In the recent development of nanoparticle biosynthesis, peptides (isolated from bio-source or in vitro synthesized) are being used for the precursor metal ion reduction. A patent granted to Belcher et al. (2015), showed a method for producing magnetic nanocrystals by using a biological molecule that has been modified to possess an amino acid oligomer that is capable of specific binding to a magnetic material. Unal Gulsuner et al. (2015) described multidomain (modular) peptides, which direct a cascade reaction is coupling the synthesis and surface functionalization of gold nanoparticles in a single step (Fig. 11.3). Synthesized gold particles have improved colloidal stability on the counter approach of nanoparticle synthesis. Design and construct of biological macromolecules control the assembly of inorganic material (metal and metal oxide). Peptide-mediated synthesis approach opened the door for the scale up of engineered nanoparticles.

3 Applications of Nanoparticles Synthesized by Fungus

The nanoparticle is being used for various applications in biomedical engineering, medicine, environment, manufacturing and material, energy and electronics, pesticides and fertilizers. Owing to high inputs of energy and use of harmful chemicals; physical and chemical methods are less preferred. More emphasis has been given

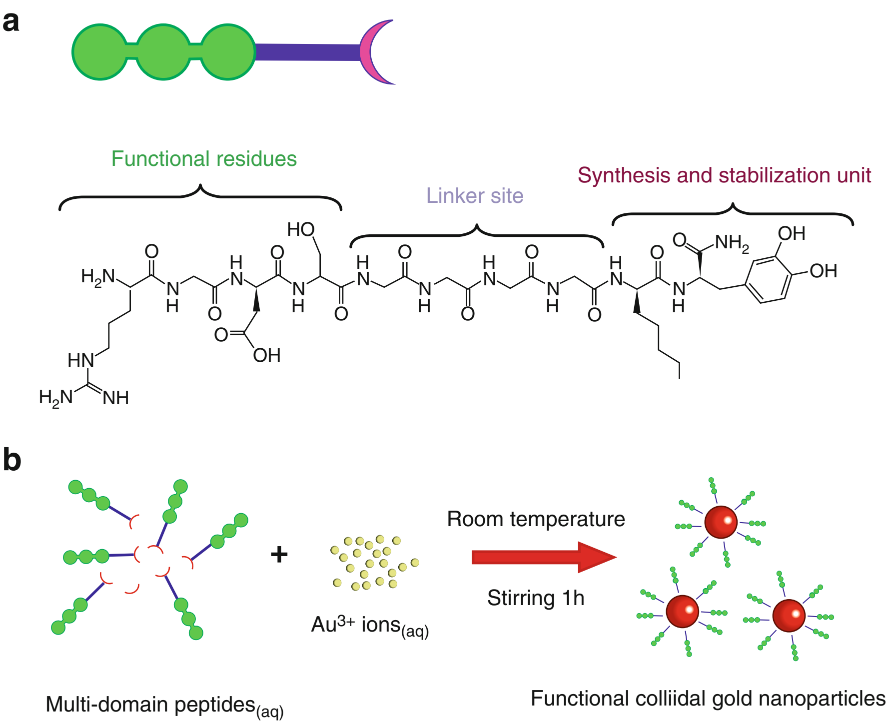

Fig. 11.3 Schematic illustration of the MDP design for one-step synthesis and surface modification of AuNPs. (**a**) Chemical sketch of a proof-of-concept MDP, RGDSGGGGKDopa-Am, where Dopa and Lys serve as synthesis and stabilization units, Gly4 functions as a steric linker to Arg-Gly-Asp-Ser, an integrin-binding peptide sequence. (**b**) Proposed synthesis and surface modification (capping) of AuNPs with the peptides (Unal Gulsuner et al. 2015)

currently for the synthesis of nanoparticle in a sustainable approach like using of fungi bacteria and plants. With advantages of fewer inputs of energy and devoid of harmful chemicals, use of fungi for nanoparticles synthesis becomes a prime choice. Another possible reason is perhaps due to recent advancement in the use of fungus for the scale of nanoparticle synthesis. There are very few reports on the application of fungus mediated nanoparticle synthesis. So far, metal, in particular, Ag and Au, and metal oxide (ZnO, MgO, and TiO_2) are dominant nanoparticles synthesized and explored by researchers (Table 11.2).

Fungus mediated Ag nanoparticles of various size and shape have been extensively harnessed for its antimicrobial properties either nascent particles or in combination with existing antimicrobial agents (Rai et al. 2009). Silver nanoparticles of 3–30 nm, synthesized by *Aspergillus niger* have antibacterial activity against *Bacillus sp.* and *E. coli* (Jaidev and Narasimha 2010). Mechanistically, silver nanoparticles cause dissipation of proton motive force, and the pitting of the bacterial cell membrane leads to cellular death. Fayaz et al. (2010) studied biogenic synthesis of silver nanoparticles and their synergetic effect with antibiotics against

Table 11.2 Application of biosynthesized nanoparticles

Nanoparticles	Fungus used to synthesize	Size (nm)	Application	Reference
Ag	*Alternaria alternata*	20–60	Activity against pathogenic fungi	Gajbhiye et al. (2009)
Ag	*Trichoderma viride*	5–40	Synergistic effect with antibiotics	Fayaz et al. (2010)
Zn	*Rhizoctonia bataticola*	15–25	Nanofertilizer for pearl millet	Tarafdar et al. (2014)
MgO	*Aspergillus flavus*	5.8	Nanonutrient for *Cyamopsis tetragonoloba*	Raliya et al. (2014a, b)
ZnO	*Aspergillus fumigatus*	1.2–6.8	Enhance native phosphorous mobilization in rhizosphere	Raliya and Tarafdar (2013c) Raliya et al. 2016
TiO_2	*Aspergillus flavus*	18	Physiological improvement in mung bean	Raliya et al. (2015a)

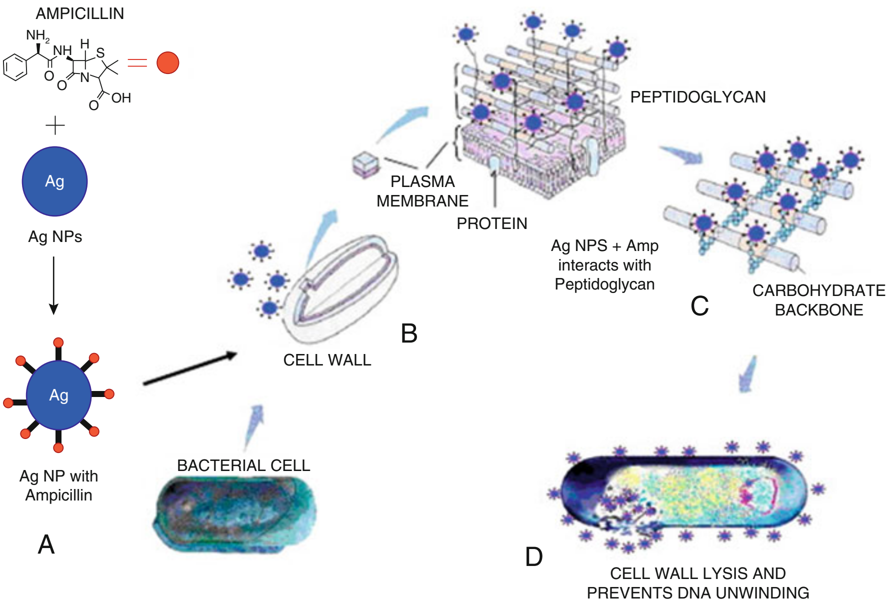

Fig. 11.4 Synergistic activity of AgNPs with ampicillin (Amp) against bacteria. (**a**) Formation of core silver nanoparticles with ampicillin. (**b**) Interaction of silver nanoparticles-Amp complex over the cell wall of bacteria. (**c**) Silver nanoparticles-Amp complex inhibits the formation of cross-links in the peptidoglycan layer (which provides rigidity to the cell wall), leading to cell wall lysis. (**d**) Silver nanoparticles-Amp complex prevents the DNA unwinding (Fayaz et al. 2010)

gram-positive and gram-negative bacteria. Author synthesized silver nanoparticles of 5–40 nm using the fungus *Trichoderma viride*. It was observed that silver nanoparticles exert synergistic antibacterial effect with antibiotics such as ampicillin, kanamycin, erythromycin, and chloramphenicol. Figure 11.4 shows a schematic of synergetic activity of silver nanoparticle with ampicillin.

In contrast to antibiotics, fungus originated silver nanoparticle also enhances the activity of antifungal agents. Gajbhiye et al. (2009) synthesized silver nanoparticles of 20–60 nm using the fungus *Alternaria alternata* and evaluated antifungal activity along with commercial counterpart fluconazole. Disk diffusion method was used to evaluate in vitro antifungal activity of fluconazole against pathogenic fungi *Phoma glomerata, Phoma herbarum, Fusarium semitectum, Trichoderma sp.,* and *Candida albicans.* To determine the synergitic antifungal effect, each standard paper disk was saturated with 20 μL of the freshly prepared silver nanoparticles. The antifungal activity of fluconazole increased significantly in the presence of silver nanoparticles. Antimicrobial effect of nanoparticles depends on particle size, concentration, and surface zeta potential that causes reactive oxygen species formation, cellular leakage as a result of membrane pore and electrostatic interaction involved in the binding of nanoparticles on the surface of a microbial agent (Rai et al. 2009).

Recently, fungus mediated nanoparticles in particular metal oxides are being used as nano nutrient fertilizer, delivered either by soil or root application. It is believed that due to smaller size nanoparticles based nutrient uptake rate is quite higher than conventional fertilizer applied through the soil (Wang et al. 2013; Raliya et al. 2015a, b). Enhanced uptake of nutrients by plants may help to avoid eutrophication in the aquatic body, maintain soil health and economically viable too. A group of Indian Council of Agricultural Research isolated a fungus *Aspergillus fumigatus* to synthesize zinc oxide nanoparticles using a precursor salt zinc nitrate (Raliya and Tarafdar 2013c). Zinc act as a cofactor of various phosphorous mobilizing enzymes such as phytase, alkaline, and acid phosphatase, have the potential to mobilize native phosphorous in the rhizospheric soil. It is important to mention that maximum proportion of conventional phosphorous fertilizer applied in soil getting fixed as a stable inorganic complex with calcium, iron or aluminum. Such complex is unavailable to plants for uptake and runoff with water that ultimately causes eutrophication by increasing phosphorous availability in water-body. The zinc oxide nanoparticle (1.2–6.8 nm) synthesized by *A. fumigatus,* significantly improve plant biomass (27.1 %), chlorophyll content (276.2 %), total soluble leaf protein (27.1 %), rhizospheric microbial population (11–14 %), acid phosphatase (73.5 %), alkaline phosphatase (48.7 %), and phytase (72.4 %) activity in clusterbean rhizosphere (Raliya and Tarafdar 2013c). Similar effect were also found in pearl millet (*Pennisetum americanum*) as a result of zinc nanofertilizer applied through foliar spray (Tarafdar et al. 2014).

To enhance solar light absorption by plant leaves to boost plant photosynthesis, fungus originated titanium dioxide nanoparticles and magnesium oxide nanoparticles were used because of their photocatalytic activity and essential part of pigment (chlorophyll) structure, respectively. *Aspergillus flavus* mediated titanium di oxide nanoparticles of 12–15 nm enhances chlorophyll content in the mung bean plant leaves by 46.4 % (Raliya et al. 2015b). Similarly, magnesium oxide nanoparticles (5.8 nm) synthesized by *A. flavus* increase in chlorophyll content by 76.1 % by the application of biologically synthesized MgO nanoparticle at 15 Mg L^{-1} concentration on 2 week old *Cyamopsis tetragonoloba* plants (Raliya et al. 2014b).

4 Conclusions

The fungus is a preferential source for nanoparticle synthesis over other biological sources such as bacterial, animal tissue lysate or plant cell due to easy and scalable mass culture, saprophytic nature, low downstream processing, environmentally benign and economically viable. Fungi used more for metal and metal oxide nanoparticle synthesis. Among the entire synthesized particle, fungus explored more for silver nanoparticle synthesis. Nanoparticle synthesis reaction is mediated by oxidation-reduction reaction mechanism carried out by fungus enzymatic protein and application used as an antimicrobial agent and also exert synergetic effect when to combine with antibiotics or antifungal agents. Rhizospheric fungus harnessed for the synthesis of agriculturally important nanoparticles help to mobilize native nutrient mobilization by boosting plant physiological and metabolic activities.

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