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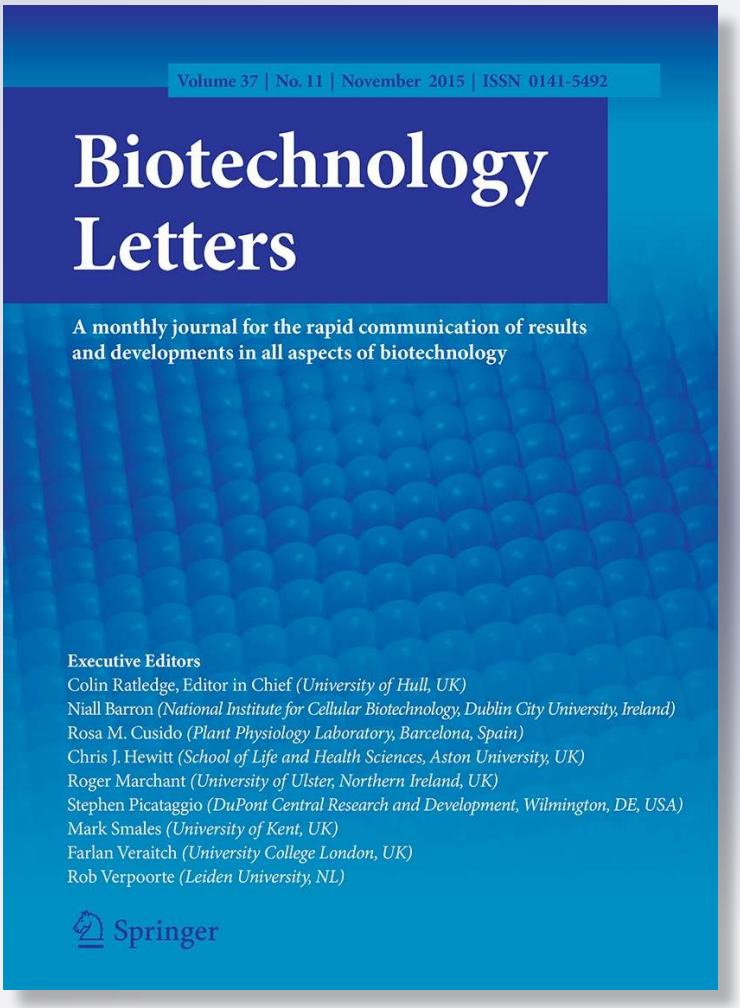

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REVIEW

Fungi as an efficient mycosystem for the synthesis of metal nanoparticles: progress and key aspects of research

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Abstract Nanotechnology is an emerging cutting-edge technology, which involves interdisciplinary subjects, such as physics, chemistry, biology, material science and medicine. Different methods for the synthesis of nanoparticles have been discussed here. Although physical and chemical methods have been successfully used to synthesize nanoparticles, the use of hazardous chemicals and synthesis at high temperature is a matter of concern. Hence, there is a necessity to develop eco-friendly techniques for the synthesis of nanoparticles. Biosynthesis of nanoparticles by fungi, bacteria, actinomycetes, lichen and viruses have been reported eco-friendly. Moreover, the fungal system has emerged as an efficient system for nanoparticle synthesis as fungi possess distinctive characters including high wall binding capacity, easy to culture and simpler biomass handling, etc. In this review, we have discussed fungi as an important tool for the fabrication of nanoparticles. In addition, methods and mechanism for synthesis of nanoparticles and its potential applications have also been discussed.

Keywords Biosynthesis · Fungi · Green chemistry · Nanoparticles · Nanotechnology

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Introduction

Nanotechnology is an important area that has demonstrated multiple applications in opto-electronics, textiles, medicine, agriculture, and environment, etc. (Dasgupta et al. 2015; Nanda and Majeed 2014) due to the unique properties of nanomaterials. Among the medical fields, high prevalence of resistance to antimicrobial agents in different microorganisms (Shelar and Chavan 2014), resistance of arthropods to insecticidal agents (Jayaseelan and Rahuman 2012), cancer cells to antitumor therapy (Daenen et al. 2014) and many others have attracted attention of researchers towards metal nanoparticles as an alternative class

of agents with antibacterial (Beyth et al. 2015; Franci et al. 2015; Naseem and Farrukh 2015), antiviral (Gaikwad et al. 2013; Galdiero et al. 2011), antiprotozoal (Said et al. 2012), antifungal (Kim et al. 2012), larvicidal (Arjunan et al. 2012; Muthukumaran et al. 2015), acaricidal (Jayaseelan and Rahuman 2012; Marimuthu et al. 2013), anthelmintic (Garg and Chandra 2012) and antitumor activities (Cabeza et al. 2015; Ortega et al. 2015).

Many unique properties of nanoparticles, such as physicochemical, optical, mechanical, magnetic, etc., make them suitable for their use in various applications. Considering the importance of nanomaterials in key future technologies, many countries have been investing in nanotechnology. The concept of nanotechnology was first given by Richard Feynman during his famous talk 'There's Plenty of Room at the Bottom' (The title of classic talk at the annual meeting of the American Physical Society, the California Institute of Technology in 1959).

Various methods of synthesis, viz. chemical, physical and biological, are commonly used for the fabrication of metal nanoparticles. Among these, chemical methods are used because of their advantage in producing large quantities of nanoparticles in a relatively short time with a fairly good control on the size and distribution (Alkilany et al. 2015; He et al. 2015). Moreover, in chemical methods the variety of shape of nanoparticles could be synthesized by adjusting the concentration of reacting chemicals and controlling the reaction conditions. Apart from these advantages, chemical methods are energy intensive, employ toxic chemicals and produce hazardous wastes that are major risk to environment. Similarly, in physical methods, various options, such as sputter deposition, laser ablation or cluster beam deposition, microwave-assisted synthesis, etc., are available for the synthesis of metal nanoparticles. But, due to the involvement of high temperature, radiations and pressure associated with these methods (Alzahrani et al. 2015; Dzido et al. 2015), biogenic synthesis is gaining ground. The development of experimental protocols for the synthesis of nanoparticles of specific size and shape is a necessary advancement of nanotechnology (Duran et al. 2010; Vala et al. 2014). Hence, researchers are foreseeing biological systems that can be used as an efficient system for the fabrication of different metal nanoparticles (Kar et al. 2014). Biological systems such as microorganisms (bacteria, fungi, algae, cyanobacteria, actinomycetes, myxobacteria) and plants are being efficiently used either for intracellular or extracellular synthesis of different metal nanoparticles (Adil et al. 2015; Ahmed et al. 2015; Chen et al. 2014; Patel et al. 2015; Singh et al. 2015a) Among the microbial systems, fungi are most commonly used because they are ubiquitously distributed in nature and play a crucial role in synthesis of metal nanoparticles. There are several reports on synthesis of metal nanoparticles by fungi (Kar et al. 2014; Qian et al. 2013; Rai et al. 2015a). In 2009, Rai and his co-workers proposed the term "Myconanotechnology" to point out research on synthesis of nanoparticles using fungi (Rai et al. 2009b). Thus, myconanotechnology is an integrated discipline of mycology and nanotechnology.

Biosynthesis: a novel and eco-friendly approach

As mentioned above, there are different methods for the synthesis of metal nanoparticles including physical, chemical and biological. But, the physical and chemical methods for the synthesis of nanoparticles involve chemical reduction of metal ions in aqueous solutions with or without use of stabilizing agents, chemicals and photoreduction in reverse micelle, chemical radiation and thermal decomposition in organic solvents (Rai et al. 2008; Sharma et al. 2009; Thakkar et al. 2010). Unfortunately, these methods involve a large amount of heat and energy, use of elevated temperature and toxic chemicals. (Sanghi and Verma 2009). Thus, development of clean, non-toxic, environment-friendly and biocompatible methods for the synthesis of nanoparticles is needed. The use of microorganisms for the synthesis of nanoparticles is gaining impetus due to the ease in synthesis of nanoparticles; moreover, the rate of success is much higher (Adil et al. 2015; Chen et al. 2014; Patel et al. 2015; Shelar and Chavan 2014; Singh et al. 2015a, b). Synthesis of nanoparticles by microbes and plants is a green approach, and biogenic nanoparticles can be used as antimicrobial agents, biosensors and bionanocatalysts (Chen et al. 2014; Narayanan and Sakthivel 2010; Rai et al. 2009a; Singh et al. 2011).

Why fungi are an efficient mycosystem?

Fungi are non-phototrophic, eukaryotic microorganisms consisting of a rigid cell wall (Bowman and Free 2011; Duran and Nombela 2004). They have simple nutrition requirements being chemo-organotrophs (Holan and Volesky 1995). The majority of these fungi grow in the land and derive their food from dead organic matter. Some fungi also grow as parasites on other organisms. Fungi generally feed by secreting enzymes that digest their food extracellularly and the remaining food is then absorbed and completely digested internally (Madigan and Martinko 2006).

The fungal cell wall is a dynamic structure that provides cell with mechanical strength to endure changes in osmotic pressure and environmental stress (Bowman and Free 2011; Duran and Nombela 2004). The morphological features and the biological activity of the fungal cell wall are mainly due to its chemical composition. The fungal cell wall comprises glycoproteins and polysaccharides, which mainly include glucan and chitin. Polysaccharides represent about 80 to 90 % of dry matter of fungal cell wall (Fig. 1). The glycoprotein, glucan and chitin are exceedingly cross-linked together in a complex network, which forms the structural basis of the fungal cell wall (Bowman and Free 2011; Farkas 1979).

The fungal cell wall causes the adhesion of fungal cells and also serves as a signaling center to activate signal transduction pathways within the cell. Disruption of fungal cell wall depicts profound effect on the growth and morphology of the cell and often leads to lysis and cell death (Bowman and Free 2011). Fungi have an incredible potential and are employed in various biotechnological applications such as remediation of toxic metals, biomining, bioleaching, biomineralization and biocorrosion (Klaus-Joerger et al. 2001; Narayanan and Sakthivel 2010). Hence, among different biological agents harnessed for synthesis of metal nanoparticles, fungi are used predominantly due to their high metal tolerance and ability to bioaccumulate metals. The fungal system possesses high wall-binding capacity and also intracellular metal uptake capability (Hemath et al. 2010; Ingle et al. 2008; Jain et al. 2011; Sanghi and Verma 2009). The fungus, *Verticillium* sp., for example, showed efficient reduction of silver ions leading to the synthesis of silver nanoparticles below the surface of the fungal cells (Mukherjee et al. 2001).

Fungi are easy to grow and synthesize nanoparticles as the handling of biomass is simple (Chan and Mashitah 2012; Honary et al. 2013). The fungal mycelia can withstand high flow pressure, agitation and other conditions in bioreactor compared to other microbes and plants (Soni and Prakash 2012). Further advantages include economic viability as large scale synthesis is possible using a small amount of biomass (Ingle et al. 2008; Vala et al. 2014). Fungi secrete a large amount of extracellular enzymes required for synthesis and higher yield of nanoparticles (Alani et al. 2012; Birla et al. 2009; Kumar et al. 2007b; Narayanan and Sakthivel 2010). Furthermore, the nanoparticles precipitated outside the cell are devoid of cellular components and hence can be directly used for different applications (Narayanan and Sakthivel 2010).

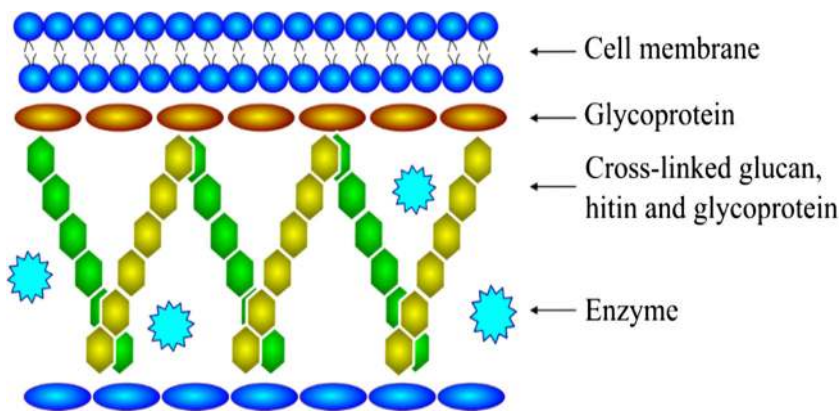

Fig. 1 Representation of the fungal cell wall

Progress in mycofabrication of metal nanoparticles

The nanoparticles can be synthesized both intra- and extra-cellularly in nanoscale dimensions with exquisite morphology (Gholami-Shabani et al. 2013; Li et al. 2012). In intracellular synthesis, nanoparticles are synthesized inside the fungal cell (Chan and Mashitah 2012; Vala et al. 2014). In this method, the fungal biomass is treated with a metal salt solution and incubated for 24 h in the dark. In extracellular synthesis, the fungal filtrate is treated with a metal salt solution and observed for the synthesis of nanoparticles (Duran et al. 2005, 2006; Mukherjee et al. 2008; Nanda and Majeed 2014). The synthesis of metal nanoparticles using the extracellular method is much faster as compared to the intracellular method (Narayanan and Sakthivel 2010). Moreover, nanoparticles synthesized using intracellular methods are smaller in size compared to the extracellularly synthesized nanoparticles (Narayanan and Sakthivel 2010; Thakkar et al. 2010). The difference in size could be possibly due to the nucleation of particles inside the fungus. However, as the synthesis takes place inside the cell, its downstream processing becomes difficult and hence the cost of nanoparticle synthesis increases (Dhillon et al. 2012; Gade et al. 2008; Zhang et al. 2011). On the other hand, extracellular synthesis does not require extensive downstream processing, which offers easier and cost-effective synthesis. Therefore, extracellular methods for nanoparticle synthesis are mostly preferred (Devi and Joshi 2015). There are some reports on intra- and extracellular synthesis of metal nanoparticles briefly discussed below.

There are only a few studies that have been carried out on the intracellular synthesis of nanoparticles; they include the intracellular synthesis of silver nanoparticles in the size range of 2–25 nm within *Verticillium* sp. with the deposits of the metal clearly bound to the surface of the cytoplasmic membrane (Sastry et al. 2003). Similarly, Mukherjee et al. (2001) demonstrated the intracellular synthesis of gold nanoparticles using the same fungus. In another study by Chen et al. (2003) the fungus *Phoma* sp. 32883 was used for the intracellular synthesis of silver nanoparticles. Moreover, some other fungi such as *Trichothecium* spp. (Ahmad et al. 2005), *Verticillium luteoalbum* (Gericke and Pinches 2006), *Penicillium chrysogenum* (Sheikhloo and Salouti 2011) for gold, *Fusarium oxysporum* f. sp. *lycopersici* for platinum (Riddin et al. 2006), *Aspergillus flavus* (Vala et al. 2014) for silver, etc. have been used for the intracellular synthesis of various metal nanoparticles.

The extracellular synthesis has been extensively studied using various fungi. For example, green synthesis of gold nanoparticles using *Alternaria* sp. In this study, the authors demonstrated the effect of different concentration of chloroaurate solution on the size of nanoparticles. TEM analysis revealed the formation of spherical, rod, square, pentagonal and hexagonal shape nanoparticles for 1 mM chloroaurate solution. However, quasi-spherical and spherical nanoparticles/heart-like morphologies with size range of about 7–13 and 15–18 nm were observed for lower molar concentrations of 0.3 and 0.5 mM gold chloride solution, respectively (Dhanasekar et al. 2015). In another study, Devi and Joshi (2015) used three endophytic fungi, *Aspergillus tamarii* PFL2, *Aspergillus niger* PFR6 and *Penicillium ochrochloron* PFR8 isolated from an ethno-medicinal plant *Potentilla fulgens* L., for the synthesis of silver nanoparticles. The nanoparticles synthesized using the fungus *A. tamarii* PFL2 had the smallest average particle size (3.5 ± 3 nm) compared to the nanoparticles synthesized by other two fungi *A. niger* PFR6 and *P. ochrochloron* PFR8 with average particle sizes of 8.7 ± 6 and 7.7 ± 4.3 nm, respectively.

Similarly, a number of fungi such as *F. oxysporum* (Duran et al. 2005; Kumar et al. 2007a; Namasivayam et al. 2011), *F. acuminatum* (Ingle et al. 2008), *F. solani* (Ingle et al. 2009), *F. semitectum* (Basavaraja et al. 2007), *Trichoderma asperellum* (Mukherjee et al. 2008), *A. flavus* (Jain et al. 2011), *A. niger* (Gade et al. 2008), *Phoma glomerata* (Birla et al. 2009), *A. clavatus* (Verma et al. 2010), *Aspergillus* sp. (Pavani et al. 2012), *Trichoderma viride* (Fayaz et al. 2009), *Pestalotia* sp. (Raheman et al. 2011), *A. terreus* (Li et al. 2012), *Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum canisetc* (Moazeni et al. 2012), *Helminthosporium tetramera* (Shelar and Chavan 2014), *P. gardeniae* (Rai et al. 2015a), etc. have proved their ability for extracellular synthesis of nanoparticles. Table 1 summarizes the list of some fungi used for intra- and extracellular synthesis of various nanoparticles.

Table 1 List of some fungi that synthesize metal nanoparticles or other metallic nanostructures

Fungi	Mode of Synthesis	Nanoparticles	References
Verticillium sp.	Intracellular	Au	Mukherjee et al. (2001)
Fusarium oxysporum	Extracellular	CdS	Ahmad et al. (2002)
Phoma sp. 3.2883	Intracellular	Ag	Chen et al. (2003)
Colletotrichum sp.	Extracellular	Au	Shankar et al. (2003)
Fusarium oxysporum	Extracellular	Zirconia	Bansal et al. (2004)
Trichothecium sp.	Extra/Intra	Au	Ahmad et al. (2005)
Fusarium oxysporum	Extracellular	Si, Ti	Bansal et al. (2005)
Fusarium oxysporum	Extracellular	Ag	Duran et al. (2005)
Fusarium oxysporum, Verticillium sp.	Extracellular	Magnetite	Bharde et al. (2006)
Aspergillus fumigates	Extracellular	Ag	Bhainsa and D'Souza (2006)
Fusarium oxysporum f. sp. *lycopersici*	Intra- & Extracellular	Pt	Riddin et al. (2006)
Verticillium luteoalbum	Intracellular	Au	Gericke and Pinches (2006)
Fusarium semitectum	Extracellular	Ag	Basavaraja et al. (2007)
Fusarium oxysporum	Extracellular	CdSe quantum dots	Kumar et al. (2007a)
Fusarium oxysporum	Extracellular	Ag	Kumar et al. (2007b)
Fusarium oxysporum	Extracellular	Ag	Mohammadian et al. (2007)
Aspergillus niger	Extracellular	Ag	Gade et al. (2008)
Fusarium acuminatum	Extracellular	Ag	Ingle et al. (2008)
Trichoderma asperellum	Extracellular	Ag	Mukherjee et al. (2008)
Penicillium sp.	Extracellular	Ag	Sadowski et al. (2008)
Phoma glomerata	Extracellular	Ag	Birla et al. (2009)
Fusarium solani	Extracellular	Ag	Ingle et al. (2009)
Coriolus versicolor	Extracellular	Ag	Sanghi and Verma (2009)
Aspergillus clavatus	Extracellular	Ag	Verma et al. (2010)
Penicillium sp.	Extracellular	Ag	Hemath et al. (2010)
Fusarium oxysporum	Extracellular	Ag	Namasivayam et al. (2011)
Aspergillus flavus NJP08	Extracellular	Ag	Jain et al. (2011)
Neurospora crassa	Extracellular	Ag, Au, Bimetallic	Castro-Longoria et al. (2011)
Aspergillus niger	Extracellular	Au	Soni and Prakash (2012)
Aspergillus terreus	Extracellular	Ag	Li et al. (2012)
Pycnoporus sanguineus	Intracellular	Ag	Chan and Mashitah (2012)
Schizophyllum commune			
Lentinus sajor caju			
Aspergillus foetidus MTCC8876	Extracellular	Ag	Roy et al. (2013)
Penicillium citrinum	Extracellular	Ag	Honary et al. (2013)
Fusarium oxysporum	Extracellular	Ag	Gholami-Shabani et al. (2013)
Helminthosporium tetramera	Extracellular	Ag	Shelar and Chavan (2014)
Aspergillus flavus	Intracellular	Ag	Vala et al. (2014)
Nigrospora oryzae	Extracellular	Au	Kar et al. (2014)
Penicillium glabrum	Extracellular	Ag	Nanda and Majeed (2014)
Phanerochaete chrysosporium	Extracellular	CdS	Chen et al. (2014)
Aspergillus tamari, Aspergillus niger,	Extracellular	Ag	Devi and Joshi (2015)
Penicillium ochrochloron			
Fusarium oxysporum	Extracellular	Ag	Krishnakumar et al. (2015)
Phoma gardeniae	Extracellular	Ag	Rai et al. (2015a)

Other biological systems used for nanoparticle synthesis

A vast array of biological resources for nanoparticle synthesis have been harnessed, and include plants and their products, bacteria, algae, fungi, yeast, actinomycetes and viruses showing both intracellular and extracellular synthesis of metal nanoparticles (Adil et al. 2015; Ahmed et al. 2015; Chen et al. 2014; Golinska et al. 2014; Nanda and Majeed 2014; Patel et al. 2015; Singh et al. 2015a). Some of the recent reports are briefly discussed here.

The phytosynthesis of silver nanoparticles include use of leaf extract of *Prosopis farcta* (Miri et al. 2015), *Musa balbisiana* (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (Banerjee et al. 2014), *Phyllanthus niruri* (Kathireswari et al. 2014), seed extract of *Brassica nigra* (Pandit 2015), etc. Gold nanoparticles were produced from the leaves extract of *Salvia officinalis*, *Lippia citriodora*, *Pelargonium graveolens* and *Punica granatum* (Elia et al. 2014), *Nepenthes khasiana* (Bhau et al. 2015), *Cucurbita pepo* (Gonnelli et al. 2015), etc. Copper nanoparticles were synthesized from *Citrus medica* (Shende et al. 2015), *Nerium oleander* (Gopinath et al. 2014), copper oxide nanoparticles—from *Gloriosa superba* extract (Naika et al. 2015), iron nanoparticles—from the extract of *Lawsonia inermis* and *Gardenia jasminoides* (Naseem and Farrukh 2015).

Singh et al. (2015a) reviewed the role of various bacteria for the intra- and extra-cellular synthesis of silver nanoparticles. Similarly, there are many reports available on the synthesis of silver nanoparticles by bacteria that include *Escherichia coli* (Kushwaha et al. 2015), *Bacillus* spp. (Das et al. 2014; Malarkodi et al. 2013) and *Bacillus stearothermophilus* (El-Batal et al. 2013). Gold nanoparticles were synthesized from *E. coli* K12 (Srivastava et al. 2013), *Geobacillus* sp. strain ID17 (Correa-Llanten et al. 2013). Copper nanoparticles from *Pseudomonas fluorescens* (Shantkriti and Rani 2014) and *Salmonella typhimurium* (Ghorbani et al. 2014).

Other microorganisms including yeast, actinomycetes and algae have also been used for the synthesis of metal nanoparticles. Dameron et al. (1989) reported synthesis of quantum crystallites in yeasts *Candida glabarata* and *Schizosaccharomyces pombe* cultured in the presence of cadmium salt. Extracellular synthesis of silver nanoparticles was reported in silver tolerant yeast strains MKY3 when challenged with 1 mM soluble silver in the logarithmic phase of growth (Kowshik et al. 2003). Other studies on biosynthesis of silver nanoparticles from yeasts have also been published (Mourato et al. 2011; Namasivayam et al. 2011). Actinomycetes are also potential synthesizers of nanoparticles (Golinska et al. 2015) and includin extremophilic actinomycetes *Thermomonospora* sp. (Sastry et al. 2003), *Rhodococcus* sp. (Ahmad et al. 2003) and *Streptomyces viridogens* (Balagurunathan et al. 2011); they have been used for the extra and intracellular synthesis of gold nanoparticles. Abdeen et al. (2014), Narasimha et al. (2013), Saminathan (2015) and others have demonstrated the synthesis of silver nanoparticles from actinomycetes.

Many algae, such as cyanobacteria (Patel et al. 2015), *Caulerpa racemosa* (Kathiraven et al. 2015) and marine brown macroalgae (Sunitha et al. 2015), etc., have been used for the synthesis of silver nanoparticles. In addition, *Spirulina platensis* (Kalabegishvili et al. 2012), green algae (Parial et al. 2012), blue- green algae (Suganya et al. 2015) and others were used for the synthesis of gold nanoparticles.

Mechanism of mycosynthesis of metal nanoparticles

The dissimilatory properties of eukaryotic microorganisms, such as fungi, may be used to biosynthesize nanoparticles. Fungi have the ability of producing extracellular metabolites that serve as agents for their own survival when exposed to different environmental stresses like toxic materials (such as metallic ions), predators and temperature variations (Mehra and Winge 1991). During the synthesis of metal nanoparticles by a fungus, the fungal mycelium is exposed to the metal salt solution, which prompts the fungus to produce enzymes and metabolites for its own survival. In this process, the toxic metal ions are reduced to the non-toxic metallic solid nanoparticles through the catalytic effect of the extracellular enzyme and metabolites of the fungus (Vahabi et al. 2011).

For the extracellular synthesis of nanoparticles, a number of mechanisms have been proposed (Duran et al. 2011; Ingle et al. 2008; Mukherjee et al. 2008). Ahmad et al. (2003) for the first time proposed mechanism involved for the synthesis of silver

nanoparticles by *F. oxysporum*. They performed the protein assay to detect the presence of NADH-dependent reductase and reported that this enzyme was responsible for the reduction of Ag ions and the subsequent formation of silver nanoparticles. Duran et al. (2005) worked out the mechanism of biosynthesis of silver nanoparticles and reported that the synthesis of silver nanoparticles occurred in the presence of anthraquinone and NADPH-nitrate reductase. In this case, the electron required to fulfil the deficiency of aqueous silver ions (Ag^+) and convert it into Ag neutral (Ag^0) was donated by both quinone and NADPH. Kumar et al. (2007b) reported that the process of formation of silver nanoparticles require the reduction of −NADPH to $-NADP^+$ and the hydroxyquinoline probably acts as an electron shuttle transferring the electron generated during the reduction of nitrate to Ag^+ ions converting them to Ag^0. They further reported that the action of hydroxyquinoline is similar to that of quinones in the electron transport taking place in the mitochondria or the chloroplast. The presence of nitrate reductase enzyme was confirmed from the SDS-PAGE. Similarly, based on the previous studies Ingle et al. (2008) proposed a mechanism for the synthesis of silver nanoparticles from *F. acuminatum*. The authors supported the hypothetical mechanism where the cofactor NADH and nitrate reductase enzyme were responsible for the synthesis of silver nanoparticles. They also confirmed the presence of nitrate reductase in the fungal cell filtrate.

Mukherjee et al. (2008) also suggested Michaelis–Menten type of mechanism for the synthesis of nanoparticles where the reaction initially exhibits pseudo-zero order kinetics and then follows higher-order kinetics. Thus, at initial phase when the concentration of silver nitrate is higher, the reaction is rather slow and as the reaction proceeds the concentration of silver nitrate lowers down considerably. The authors proposed that bioreduction of metal nanoparticles was brought about by protein extract containing amino acid with -SH bonds and most likely cysteine undergoes dehydrogenation on reaction with silver nitrate to produce silver nanoparticles. While, the free amino acid groups possibly serve as a capping for silver nanoparticles.

The involvement of polypeptides/proteins in the bioreduction of metal ions was also reported by Das et al. (2009). In this study, FTIR spectra of fungal culture containing $AuCl_4^-$ (auric chloride) revealed the presence of amide I, II and III groups and the disappearance of carbonyl groups present in the mycelia. The shifting of peaks from 1034 to 1075 cm^{-1} illustrated the role of phosphate bonds in the reduction process. Thus, the authors hypothesized that the surface bound protein molecules acted as reducing and stabilizing agent. Silver nanoparticles synthesized by *Coriolus versicolor* also showed the reduction of silver ions by amide I and amide II groups (Sanghi and Verma 2009). The stabilization of nanoparticles was attained by fungal protein.

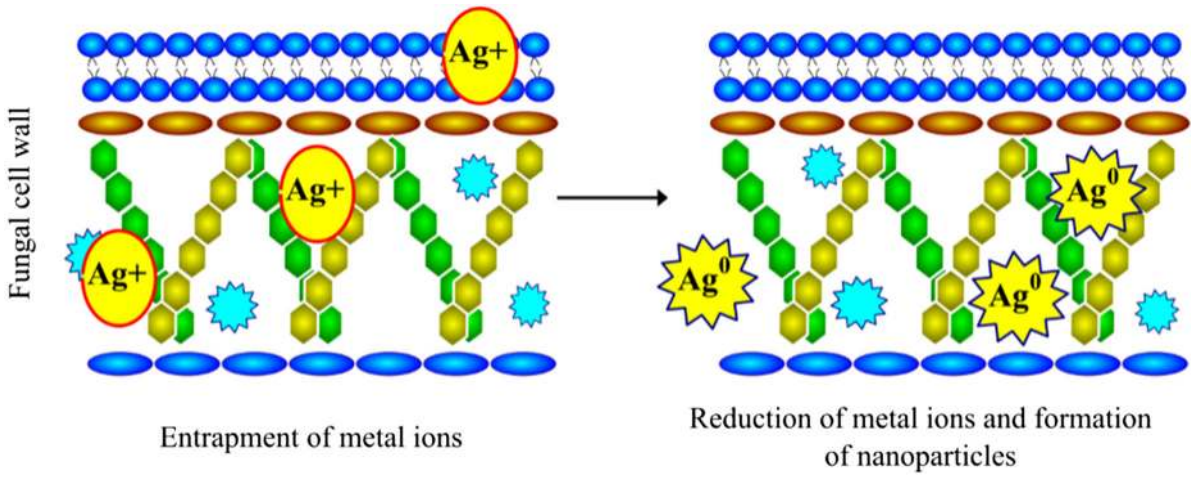

Fig. 2 Hypothetical mechanism for intracellular synthesis of nanoparticles

Jain et al. (2011) reported a two-step hypothetical mechanism for synthesis of silver nanoparticles. In the first step, reduction of bulk silver ions to silver nanoparticles takes place by a 32 kDa protein, which might be a reductase secreted by *Asp. flavus*. In the second step, silver nanoparticles were capped by a 35 kDa protein that binds with the nanoparticles and confers stability. Similar results were reported with *F. oxysporum* showing the presence of two extracellular proteins with molecular weight of 24 and 28 kDa responsible for the synthesis of zirconian oxide nanoparticles (Bansal et al. 2004; Duran and Seabra 2012). Chan and Mashitah (2012) exploited three different macro fungi (*Pycnoporus sanguineus, Schizophyllum commune* and *Lentinus sajor-caju*) for synthesis of silver nanoparticles. The authors supposed that the reduction of silver ions was possibly due to the presence of diketone compound, which was also confirmed by GC–MS analysis. Li et al. (2012) also inferred that a NADH-dependent reductase released by *A. terreus* might have accounted for the synthesis of silver nanoparticles. In the process, NADH acted as an electron carrier, and the silver ions obtained electrons from NADH via the NADH-dependent reductase, and then were reduced to silver nanoparticles. However, it is hypothesized that the intracellular synthesis of metallic nanoparticles is through electrostatic attraction of the ions by the enzymes or proteins in the fungal cell wall, then reduced by the enzymes. Other possibility is the migration of ions to cytoplasmic membrane and then reducing in that site. These data were summarized by Kashyap et al. (2013) who indicated that the synthesis of silver nanoparticles require the reduction of NADPH to $NADP^+$ and the hydroxyquinone acts as an electron shuttle transferring the electrons generated during the reduction of nitrate to Ag^+ ions converting them to Ag^o. It can be concluded that the electrostatic interaction and specific enzyme of fungi (e.g. NADPH dependent reductase enzyme, hydroxyquinone, phytochelatin etc.) are major factors in the mycosynthesis of nanoparticles.

The actual mechanism of mycosynthesis of nanoparticles, however, is still not fully understood. According to Mukherjee et al. (2001), in intracellular synthesis, metal nanoparticles are synthesized below the cell surface, which is possibly due to the reduction of metal ions by enzymes present in the cell membrane. Synthesis proceeds firstly by the entrapment of metal ions on the surface of fungal cell, which occurs due to the electrostatic interaction between lysine residues and metal ions (Riddin et al. 2006). The second step in the synthesis is the enzymatic reduction of metal ions, which leads to aggregation and formation of nanoparticles. The cell-wall sugars also play a major role in the reduction of metal ions (Mukherjee et al. 2001). Although the mechanisms for the intracellular synthesis of other metals are not available, the reduction of other metals may occur in a similar pattern as described for silver and gold nanoparticles (see Fig. 2).

Application of nanoparticles

Over the past few decades, inorganic nanoparticles have demonstrated unique electromagnetic, optical and catalytic properties (Dhillon et al. 2012; Rai et al. 2008; Singh et al. 2011). This has elicited much interest of researchers to allow better usage of nanoparticles in a number of applications (Gholami-Shabani et al. 2013). Applications of nanoparticles in medicine, agriculture and environment have been discussed here.

Biomedical applications of nanoparticles

The metal nanoparticles in general and noble metal nanoparticles in particular such as silver, gold and platinum, have huge biomedical applications. Out of these, silver and gold nanoparticles are preferentially used. Silver nanoparticles showed strong antimicrobial potential, whereas gold nanoparticles showed their applications in drug delivery for many important diseases including cancer (Rai et al. 2015b).

Silver nanoparticles

Studies that emphasize the antimicrobial properties of silver nanoparticles against bacteria, viruses, and fungi have been explored extensively. Here, we have briefly focused on these activities. Naqvi et al. (2013) demonstrated the effect of silver nanoparticles and commercial antibiotics on *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus* sp. and *E. coli*. The results confirmed the potential activity of silver nanoparticles compared to antibiotics used in the study. Many other researchers have reported the

antibacterial activity of silver nanoparticles on Gram positive as well as Gram negative bacteria (Rai et al. 2009a). Morones et al. (2005) studied the effect of silver nanoparticles on *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholerae*. Further, they reported the size-dependent activity of silver nanoparticles. According to them, binding strength of nanoparticles depend on the surface area to the volume ratio resulting into the changes in the local electronic surface, which ultimately enhance the reactivity of surface. Taglietti et al. (2012) studied the minimal inhibitory concentration (MIC) for silver nanoparticles against Gram positive (*Staphylococcus aureus*) and Gram negative (*E. coli*) bacteria, which were 180 and 15 μg/ml, respectively. Kanmani and Lim (2013) and Lee et al. (2014) also studied the effect of silver nanoparticles against various bacteria.

There are some studies that have shown promising results for the control and prevention of viral diseases by the use of silver nanoparticles. The latter have demonstrated inhibitory activity against influenza A H1N1 virus, which was proved through hemagglutination-inhibition tests and embryo inoculation assays (Xiang et al. 2011). Silver nanoparticles of 1–10 nm can bind to HIV-1 in a size-dependent manner (Elechiguerra et al. 2005). The authors also reported a spatial relationship, which can be explained using the structural information of HIV-1 envelope. The HIV-1 is composed of two subunits out of which a surface glycoprotein subunit gp120 is exposed to the exterior and the transmembrane glycoprotein subunit gp41 connects the gp120 subunit to the interior p17 matrix protein. The principle function of gp120 is to bind with CD4 receptor sites on the host cells (Lara et al. 2010a). The silver nanoparticles in concentration of 24 μg/ml significantly inhibited HIV-1 infection in CD_4^+MT2 cells and cMAG HIV-1 receptor cells (Elechiguerra et al. 2005; Lara et al. 2010a).

In another study by Lara et al. (2010b) polyvinylpyrrolidone (PVP)-stabilized silver nanoparticles were employed for the development of a gel used to avoid contamination by viruses, especially HIV, during sexual intercourse. The results were favorable to prevent HIV infection in sexually active women, providing protection up to 48 h after application. Tefry et al. (2012) reported the development of a method to evaluate the activity of silver nanoparticles against pseudo-typed HIV-1-based viruses. Their aim was to develop a system that could also be used to test the activity of other nanoparticles on other pseudo viruses.

Gaikwad et al. (2013) demonstrated the antiviral activity of biologically synthesized silver nanoparticles against herpes simplex virus type 1 and 2 (HSV 1 and 2) and human parainfluenza virus type 3 (HPIV-3) in dose-dependent manner. In another method, the infectivity of virions was inhibited when viral aliquots were incubated with silver nanoparticles for 2 h at 37 °C. In a similar study against adenovirus type 3, Chen et al. (2013) reported the cytotoxicity of chemically-synthesized silver nanoparticles at 50 μg/ml. Hu et al. (2014) explained the inhibition mechanism of silver nanoparticles on HSV2. Silver nanoparticles formed bonds with the glycoprotein membrane of HSV2, which contains sulfhydryl groups. This interaction prevents the internalization of the virus by inhibiting the interaction of glycoprotein and receptor. Silver nanoparticles at concentration 50 and 25 μg/ml significantly inhibited the HSV 2 progeny.

Antifungal activity of silver nanoparticles has been less explored compared to their antibacterial activity. Kim et al. (2008) reported significant antifungal activity of silver nanoparticles against various strains of *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *Trichophyton mentagrophytes*. Gajbhiye et al. (2009) reported effectiveness of biosynthesized silver nanoparticles against *Phoma glomerata*, *P. herbarum*, *F. semitectum*, *Trichoderma* sp. and *C. albicans*. Some other reports include antifungal activity of silver nanoparticles against *A. flavus* and *C. albicans* (Kandile et al. 2010), *C. albicans* and *Saccharomyces cerevisiae* (Nasrollahi et al. 2011), *A. flavus, A. niger, Curvularia* sp., *Fusarium* sp. and *Rhizopus* sp. (Savithramma et al. 2011), *Rhizoctonia solani*, *A. flavus* and *Alternaria alternata* (Kaur et al. 2012), *C. albicans, Trichophyton rubrum* and *A. fumigatus* (Tile and Bholay 2012), against keratitic fungi such as *Fusarium*, *Aspergillus* and *Alternaria* (Xu et al. 2013), *C. albicans* (Dar et al. 2013).

Silver nanoparticles are used as biocides to prevent infections in burns, traumatic wounds and ulcers (Franci et al. 2015; Jain et al. 2011; Rai et al. 2009a; Rai et al. 2009b). Furthermore, they are also employed as water disinfectant (Bhattacharya and Mukherjee 2008). Other applications of silver nanoparticles include nanocrystalline silver dressings, silver nanoparticles impregnated surgical masks, gloves,

Table 2 Antibacterial activity of combination of mycofabricated nanoparticles and antibiotics

Fungus used for biosynthesis	Nanoparticles type and size	Test object	Antibiotics in combination	Observed effect in the presence of nanoparticles	Ref
Phoma glomerata	Silver, 60–80 nm	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*	Ampicillin, gentamicin, kanamycin, streptomycin and vancomycin	Activity of vancomycin was the most increased. An increase of activity was more prominent against Gram-negative bacteria.	Birla et al. (2009)
Trichoderma viride	Silver, 5–15 nm	*S. aureus, E. coli, Micrococcus luteus, Salmonella typhi*	Ampicillin, erythromycin, kanamycin and chloramphenicol	All tested combinations showed beneficial effect, especially in amoxicillin. An increase in activity was more significant against Gram-negative bacteria.	Fayaz et al. (2009)
Ganoderma lucidum	Silver, 10–70 nm	*S. aureus, E. coli*	Tetracycline	Activity increased	Karwa et al. (2011)
T. viride	Gold, 4–15 nm	*S. aureus, E.coli,* VRSA	Vancomycin	Against VRSA activity increased; appeared activity against *E. coli*	Fayaz et al. (2011)
Pestalotia sp.	Silver, 10–40 nm	*S. aureus, S. typhi*	Gentamycin and Sulphamethizole	Activity of both antibiotics increased, especially of gentamycin	Raheman et al. (2011)
Trichoderma harzianum	Silver, 30–50 nm	*S. aureus, E. coli*	Cefazolin	Activity of both antibiotics increased	Singh et al. (2011)
Aspergillus terreus, Paecilomyces lilacinus, Fusarium sp.	Silver, 5–50 nm	*S. aureus, Streptococcus pyogenes, Salmonella enterica, Enterococcus faecalis*	Erythromycin, methicillin, chloramphenicol, ciprofloxacin	Increase in activity of erythromycin, methicillin, chloramphenicol and ciprofloxacin, more prominent against *S. aureus* and *S. pyogenes*	Devi and Joshi (2012)

catheters, etc. (Kumar and Yadav 2009; Rai et al. 2009a).

Combined use of mycofabricated silver nanoparticles and antibiotics to combat antibiotic resistance

Although many studies on antibacterial activity of silver nanoparticles have been made, the exact mechanism is still to be elucidated. Silver nanoparticles may damage the structure of bacterial cell membrane and suppress the activity of some membranous enzymes, which cause the bacteria to die (Li et al. 2010). Xiu et al. (2012) presented data that silver nanoparticles themselves do not significantly exert direct particle-specific toxicity on bacteria, which is still an open question, since contrary results to this were previously reported stating that the silver ions as the final active effector against bacteria are quite clear. The grade of penetration is the question that plays an important role in this action. Silver nanoparticles are extremely effective at penetrating microorganisms compared to silver ions. But it is very important to note that studies by Fayaz et al. (2011) and Devi and Joshi (2012) must be taken with certain caution when extrapolating this mechanistic inference to other biological systems. In addition, their significant antimicrobial effects have been clearly demonstrated (Birla et al. 2009; Karwa et al. 2011; Lima et al. 2013). The use of this nanomaterial in combination with antibiotics is therefore a new and important area.

One of the possible applications of metallic nanoparticles is the enhancement of activity of other antimicrobial agents. Toxic effects of nanoparticles on

mammalian cells limit their broad use with therapeutic purposes (Lewinski et al. 2008) but these effects depend on several other factors (Lima et al. 2013). However, synergistic combinations between nanoparticles and other antimicrobials make it possible to lower dosages thus reducing toxic effect. Another important benefit of such combinations is the prolonged activity of highly effective antibiotics owing to fewer chances of development of resistance to a combination of drugs compared with the use of single components, and combinations are important also in restoring activity of previously effective antibiotics but which lose their clinical application owing to development of bacterial resistance (Allahverdiyev et al. 2011).

Research on combinations between antibiotics and metallic nanoparticles of any origin are scarce and, especially, for mycofabricated nanoparticles, as this is a new and developing area (Table 2). Most studies are conducted with silver nanoparticles produced by fungi; some or all tested combinations demonstrate beneficial enhancing effects; therefore, combinations of mycofabricated nanoparticles with antibiotics should be further evaluated in detail against different bacterial models and especially, multi-drug resistant bacteria. Mechanisms of synergistic interactions between metal nanoparticles and antibiotics can be explained by increasing local concentration of antibiotic. With the accumulation of nanoparticle-antibiotic complexes at the site of bacterium-antibiotic interaction, it facilitates the binding reaction between antibiotic and bacterial surface, and increases permeability of the bacterial cell wall for nanoparticles. For example, beta-lactam antibiotics acted synergistically with silver nanoparticles enhancing subsequent influence of nanoparticles on inner bacterial structures (Li et al. 2005); in another study, synergistic effect was obtained in the combination of silver nanoparticles and polymyxin B against Gram-negative bacteria (Ruden et al. 2009).

The formation of biofilm is associated with resistance to antimicrobial agents and chronic bacterial infections. In this direction, silver nanoparticles have demonstrated antibiofilm activities. To prove this, an ATPase inhibitor assay, permeability assay and hydroxylradical assay were studied. Antibacterial activity of silver nanoparticles was influenced by these factors and not by permeability of the outer membrane (Hwang et al. 2012).

The antibacterial activities of amoxicillin, erythromycin and vancomycin were increased in the presence of silver nanoparticles against *S. aureus*; also activity of gentamicin, tetracycline and carbenicillin was increased against *P. aeruginosa*. The highest enhancing effects were observed for vancomycin and amoxicillin against *S. aureus* and for carbenicillin and gentamicin against *P. aeruginosa* (Sattari et al. 2012). The biogenic silver nanoparticles showed enhanced quorum sensing activity against *S. aureus* biofilm and prevention of biofilm formation. The synergistic effect of silver nanoparticles along with antibiotics in biofilm sensing was found to be effective (Chaudhari et al. 2012).

Silver nanoparticles functionalized with ampicillin were effective, broad-spectrum bactericides against Gram-negative and Gram-positive bacteria (Brown et al. 2012). The activity of different antibiotics evaluated against selected human bacterial pathogens such as *S. aureus, S. epidermidis, E. coli, P. aeruginosa,* and *Bacillus cereus* by disc diffusion method showed the maximum increase with vancomycin against *P. aeruginosa* and *E. coli* and kanamycin against *S. epidermis* (Thangapandiyan and Prema 2012). Biosynthesized silver nanoparticles alone and in combination with antibiotics demonstrated excellent antimicrobial activity against MRSA. Antimicrobial activity was studied with biogenic silver nanoparticles and cephalexin, which showed a synergistic effect against *S. aureus* and *E. coli* (Vivekanandan et al. 2012). Piperacillin and erythromycin showed a significant increase in their activities in the presence of biogenic silver nanoparticles. A synergism was observed for chloramphenicol or vancomycin against *P. aeruginosa* and streptomycin against *E. coli* (Ghosh et al. 2012).

Gold nanoparticles

Like silver nanoparticles, gold nanoparticles also have tremendous biomedical applications. They are mainly used for the diagnosis and treatment of cancer, AIDS, tuberculosis and other diseases through drug delivery. Huo et al. (2012) demonstrated that size of nanoparticles exerts great influence on the penetration and retention behavior of nanoparticles entering in tumors. They studied the effect of 50 and 100 nm gold-coated Au@tiopronin nanoparticles using MCF-7 breast cells as a model system. The results showed that

nanoparticles of 50 nm penetrated more deeply into tumor spheroids. In contrast, larger gold-coated nanoparticles (100 nm) were primarily localized in the periphery of the tumor spheroid and around blood vessels, hindering deep penetration into tumors. Johnson et al. (2013) reviewed the potential role of nanotechnology in therapeutic approaches for breast cancer. Madhusudhan et al. (2014) reported that conjugates of anticancerous drug doxorubicin and gold nanoparticles are more effective as compared to the use of doxorubicin alone. Similarly, Ashiq et al. (2013) reported the use of gold nanoparticles in breast cancer therapy by the technique known as laser-induced coulomb explosion of gold nanoparticles.

Radiotherapy is mostly used to treat various cancers. It involves the use of bombardment of ionizing radiations containing high energy particles on target tissue. These rays, however, are non-specific as they cannot discriminate between the normal and cancerous cells. Thus, there are many chances of damaging the normal cells during the process. The tumour specific nanoparticles can be used with radiation therapy to reduce the exposure of normal cells (Hainfeld et al. 2010). Functionalized particles like gold-coated, lanthanide phosphate nanoparticles were used for radiotherapy (McLaughlin et al. 2013). Hwang et al. (2014) demonstrated the potential use of small sized gold nanoparticles (6 nm) for the photothermal therapy in cancer. Ali et al. (2014) investigated the anticancer activity of rifampicin conjugated gold nanoparticles in multidrug resistant (MDR) cancer cells. Rifampicin enhances the accumulation of anticancer drugs in cancer cells and the authors reported that rifampicin-conjugated gold nanoparticles significantly enhanced the rate and efficiency of endocytosis and also increased their concentration inside the cancer cells. Cell viability test showed a remarkable enhancement in the photothermal therapeutic effect of gold nanoparticles in presence of rifampicin. Hence, it will help to decrease the demand on the overall amount of gold nanoparticles needed for treating cancer and thus decreasing its toxicity.

Gold nanoparticles are also efficiently used for the treatment of AIDS. Berry et al. (2007) demonstrated the role of biocompatible gold nanoparticles of different sizes functionalized with the HIV-1 tat integral protein transduction domains (PTD) to develop nuclear targeting agents. The functionalized gold nanoparticles were tested in vitro with a human fibroblast cell line. Nanoparticles at 5 nm were easily transferred across the plasma membrane. Larger nanoparticles (30 nm), however, were retained in cytoplasm, suggesting entry was blocked via nuclear pores dimension. This study concluded that gold nanoparticles 5 nm or smaller can be used as a vehicle for the drug delivery for AIDS. In another study, multivalent mercaptobenzoic acid-coated gold nanoparticles of 2 nm were synthesized and conjugated with SDC-1721, a derivative of TAK-779 and a known CCR5 antagonist, which is principal entry co-receptor for most commonly transmitted strains of HIV-1 (Bowman et al. 2008). The authors reported that free SDC-1721 alone had no inhibitory effect on HIV infection; however, the SDC-1721-gold nanoparticles conjugate showed higher inhibition. Consequently, the conjugation of small molecules (SDC-1721) on gold nanoparticles surface can convert inactive drugs into potent therapeutics. Other reports have indicated the efficacy of gold nanoparticles against cancer (Arnaiz et al. 2012; Chiodo et al. 2014; He et al. 2014; Kesarkar et al. 2012; Zheng et al. 2012).

Until recently, only PCR-based molecular methods have been available for the direct identification and susceptibility testing of mycobacteria (Costa et al. 2010; Hussain et al. 2013). But, today, various types of gold nanoparticle-based sensors have been developed for the detection of *Mycobacterium* infections. Duman et al. (2009) demonstrated activity of biosensor developed in combination with gold nanoparticles, which is also used for the detection of *M. tuberculosis*. Similarly, Thiruppathiraja et al. (2011) developed DNA electrochemical biosensor using gold nanoparticles for detection of genomic DNA of *Mycobacterium* sp. Gajendiran et al. (2014) developed a gold conjugated poly(lactic-co-glycolic acid)-polyethylene glycol (PEG)-succinic anhydride (SA)-polyethylene glycol (PEG)-poly(lactic-co-glycolic acid) (i.e. PLGA–PEG–SA–PEG–PLGA) multiblock copolymer nanoparticles, loaded with the tuberculosis drug, rifampicin, and administered in the experimental model. Liu et al. (2014) developed a new DNA-based biosensor for the highly sensitive detection of the specific IS6110 DNA sequence of *M. tuberculosis*. They also found that DNA biosensor show stability,

possessed high specificity and also provided a new strategy for early detection of *M. tuberculosis*.

Gold nanoparticles are now used in vaccine delivery. The current field is still developing but there are reports on the use of gold nanoparticles in vaccine delivery in some diseases due to their small size and ability to enter the cell easily. Fujita and Taguchi (2011) proposed that gold nanoparticle-based vaccines can be developed by two ways; the first one includes addition of functional components (T cell epitopes, cell-penetrating peptides and lipophilic moieties) and the second one is known as the synthetic approach, which is achieved by using size-defined nanomaterials (self-assembling peptides, non-peptidic dendrimers and gold nanoparticles) as antigen-displaying platforms.

Gold nanoparticle- based DNA vaccines are more effective than the conventional vaccines. Zhou et al. (2008) demonstrated that gold nanoparticles conjugated with low molecular weight chitosan induce an enhanced serum antibody response ten-times more potent than naked DNA vaccine. Park et al. (2013) reviewed the role of nanotechnology in the field of immunotherapy. According to them this field allows the application of vaccine adjuvants and immunomodulatory drugs that improve clinical outcomes for immunological diseases (vaccines in cancer immunotherapy). Similarly, Lee et al. (2012) and Ahn et al. (2014) developed imageable antigen-presenting gold nanoparticle vaccines and tumor-associated self-antigens as a potential vaccine for effective cancer immunotherapy. Cao-Milan and Liz-Marzan (2014) have reviewed the recent advances of conjugated gold nanoparticles in various clinical applications including delivery of vaccines in infectious diseases.

Application of nanoparticles in agriculture

Nanotechnology has the potential to revolutionize different sectors of the agriculture (Goel 2015; Rai and Ingle 2012; Rai et al. 2015c). Apart from its major application as antimicrobial agents for the management of plant pathogens, nanoparticles can serve as nano-pesticides, nano-insecticides and nano-fertilizers. Nanomaterials are also useful for the development of nanobiosensors used for the preparation of devices, which can be applied in precision farming.

Precision farming

Precision farming generally involves the use of devices made of biosensors which helps in agriculture. Nanobiosensor is a modified version of a biosensor, which may be defined as a compact analytical device or unit incorporating a biological or biologically-derived sensitized element linked to a physico-chemical transducer (Turner 2000). Rai et al. (2012a, b) reported that nanobiosensors can be effectively used for sensing a wide variety of fertilizers, herbicide, pesticide, insecticide, pathogens, moisture and soil pH. According to Mousavi and Rezaei (2011), the concept of precision farming includes a system controller for each growth factor such as nutrition, light, temperature, etc. Also, these systems should have information for planting and harvest time, which can be controlled by satellite systems. These systems allow the farmer to know the best time for planting and harvesting to avoid of encountering bad weather conditions.

Nano-fungicides

Fungi are most common plant pathogens compared to bacteria and viruses. There is a large number of fungal genera, that are common plant pathogens: species of *Fusarium, Phoma, Aspergillus, Phytopthora, Phyllosticta,* etc. (Ingle and Rai 2011). All can be managed by nanomaterials (Ingle et al. 2014; Singh et al. 2015a).

Nanofertilizers

Many problems are associated with agriculture, such as excessive and continuous use of chemical fertilizers and water resources, decrease the fertility of soil and eventually in the crop production. Therefore, nanofertilizers can be the only alternative to regain and protect the fertility of soil with minimum damage to soil. Nanostructured formulation through targeted delivery or slow/controlled release or conditional release of fertilizer according to environmental triggers and biological demands is the important The use of nano-fertilizers leads to an increase in nutrient efficiencies, reduces soil toxicity, minimizes the potential of negative effects associated with over dose of chemical fertilizers. Hence, nanotechnology has a high potential for achieving sustainable agriculture,

especially in developing countries (Naderi and Danesh-Shahraki 2013). Corradini et al. (2010) proposed a concept of incorporation of chemical fertilizers into chitosan nanoparticles for the slow and constant release in adequate amount. This concept is helpful to control the release of chemical fertilizers into soil and also to avoid excess disposal of chemical fertilizers into soil and aquatic environments.

Naturally occurring minerals, such as nano clays and zeolites can be applied as nanofertilizers (Chinnamuthu and Boopathi 2009). Millan et al. (2008) reported that urea- fertilized zeolite chips can be used as slow-release nitrogen fertilizers. Ammonium-charged zeolites have a capacity to raise the solubilization of phosphate minerals and have improved phosphorus uptake and the yield of crop plants. Li (2003) demonstrated the possibility of using surfactant-modified zeolite by application of hexa-decyl trimethyl ammonium as fertilizer carrier to control nitrate release and proposed that surfactant-modified zeolite was suitable sorbent for nitrate, since slow release of nitrate is achievable. In another study, Subbarao et al. (2013) developed the strategies for slow release of potash fertilizer with coating of plaster of Paris, wax etc. It helps in slow release of fertilizers and minimizes the fertilizer loss.

Nano-pesticides

Nano-pesticides include a great variety of products that consist of organic ingredients (polymers) and/or inorganic ingredients (metal oxides) in various forms (particles, micelles). The aims of nanoformulation are generally similar to other pesticide formulations as follows: (i) increasing the apparent solubility of poorly soluble active ingredient, and (ii) releasing the active ingredient in a slow/targeted manner and/or protecting the active ingredient against premature degradation. If one defines nano-pesticides as any formulation that intentionally includes elements in the nanometer size range and/or claims novel properties associated with these small size range, it would appear that some nano-pesticides have already been on the market for several years.

Effectiveness of metal anoparticles against different plant pathogens, insects and pest makes them compatible for their use in the preparation of new formulations including pesticides, insecticides and insect repellants (Barik et al. 2008; Goswami et al. 2010). Liu et al. (2006) used porous hollow silica nanoparticles (PHSNs) loaded with validamycin (pesticide) as efficient delivery system of water-soluble pesticide and for its controlled release. Such controlled release behaviour of PHSNs makes it as promising carrier in agriculture, especially for pesticide controlled delivery whose immediate as well as prolonged release is needed for plants. Wang et al. (2007), reported that nano-emulsions was useful for the formulations of pesticides, which could be effective against various insect pests in agriculture.

Barik et al. (2008) claimed that nano-silica can be used as nano-pesticide. Further, they focused on the mechanism of control of insect pest using nano-silica, and according to them insect pests used a variety of cuticular lipids for protecting their water barrier and thereby prevent death from desiccation, but nano-silica gets absorbed into the cuticular lipids by physiosorption and thereby causes death. Surface-charged modified hydrophobic nano-silica (3 to 5 nm) can control a range of agricultural insect pests (Ulrichs et al. 2005). Goswami et al. (2010) studied the application of different kind of nanoparticles, viz. silver nanoparticles, aluminium oxide, zinc oxide and titanium dioxide, for the control of rice weevil and grasserie disease in silkworm (*Bombyx mori*) caused by *Sitophilus oryzae* and baculovirus *B. mori* nuclear polyhedrosis virus, respectively. Later, it was reported that all the nanoparticles showed significant control of these insect pests.

Application of nanoparticles in environment

The environment is an important factor for existence of life on the earth. Due to human interference air, soil and water are being polluted by many contaminants. For example, carbon monoxide, chlorofluorocarbons and metals, such as arsenic and mercury, and various hydrocarbons are responsible for air pollution. However, NO and SO_2 released from industries cause acid rains leading to soil acidity. Industrial effluents, sewage and oil spills are the major sources of water pollution. There are many technologies being implemented for effective removal of contaminants from environment. But either they lack the efficacy and/or are time-consuming processes. Therefore, there is a need of technology which can cope with this problem and nanotechnology has the great prospects in this

area. Many researchers have focused their research on nanotechnology to save the environment by using various kinds of nanomaterials.

Among all nanomaterials, metal nanoparticles play a vital role in adsorbing water contaminants. For instance, iron nanoparticles transform and detoxify environmental pollutants, such as chlorinated organic solvents, pesticides and polychlorinated biophenyls. They also enhance efficacy of remediation of toxic compounds from environments (Zhang 2003). Zero valent iron nanoparticles can be used for remediation of groundwater so as to make it potable (Rajan 2011).

Metals such as mercury, arsenic and chromium, are among the most harmful contaminants of water and exist in ionic form, which could interact with various biomolecules and alter their structure and functions. Polymer brush functionalised magnetic nanoparticles are highly effective in removing mercury ions (Farrukh et al. 2013) and chromium ions (Telling et al. 2009) from contaminated water. Magnetic iron oxide nanoparticles adsorb arsenic. Moreover, they are highly biocompatible and can be easily degraded in the environment and therefore, they are the safer option for such usage. TiO_2 nanoparticles are promising candidate for photocatalytic degradation of several pollutants. They are effective in degrading pollutants, such as phenols, volatile compounds, dyes, etc. Therefore, they have been used in photocatalytic membranes for decontamination of water at large scale (Chong et al. 2010). Singh et al. (2011) demonstrated that the use of zero-valent iron nanoparticles (nZVI) can be useful for the remediation of Cr(VI) from soil. They reported that 1.5 g nZVI entrapped in alginate beads remove 98 % Cr(VI) from spiked soil within 60 min.

Key areas of research

The mycosynthesis of metal nanoparticles is a green, economically viable and easy approach. Therefore, fungi should be screened for selection of potential strains for the synthesis of nanoparticles. There is a greater need for optimization of conditions for synthesis of nanoparticles. Various mechanisms have been proposed to explain the synthesis of nanoparticles by fungi but a better understanding of the fungal system is still needed. Extensive studies are therefore, required to understand the exact biochemical and molecular mechanism involved in nanoparticle synthesis.

Efforts are needed for large-scale production of nanoparticles. Mycosynthesized nanoparticles can be used as novel nanoantimicrobials with potential to tackle the problem of multi-drug resistance. There is a wide scope of nanoparticles in the fields such as agriculture and environment.

Conclusions

The increasing interest for greener and biological methods of synthesis has led to the development of non-toxic and comparatively more bioactive nanoparticles. Unlike physical and chemical methods of nanoparticle synthesis, microbial synthesis in general and mycosynthesis in particular is cost-effective and environment-friendly. Hence, mycosynthesis of nanoparticles is now an important branch of bionanotechnology and is referred to as myconanotechnology. The fungi involved are efficient due to their innate potential for intracellular and extracellular synthesis of nanoparticles, and therefore, they can be regarded as novel biomills for the synthesis of nanoparticles. However, different aspects, such as the rate of synthesis, monodispersity and downstream processing, need to be improved. Extensive research on the synthesis of nanoparticles using different fungi and the possible mechanisms involved synthesis still needs to be elucidated. There is also an acute need to study the large-scale synthesis of nanoparticles for commercial applications.

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