Ram Prasad · Vivek Kumar
Manoj Kumar · Shanquan Wang *Editors*

Fungal Nanobionics: Principles and Applications

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Foreword

In the twentieth century, humans acquired skills to connect fungi to protect human health (through antibiotics, antimicrobial, immunosuppressive agents, etc.), which led industrial scale production of enzymes, alkaloids, detergents, acids, biosurfactants, etc. With the establishment of modern nanotechnology in the 1980s, fungal-based innovations continued to excite by providing greener (non-toxic, eco-friendly, cost effective, non-hazardous and sustainable route) alternative to chemically synthesized nanoparticles. The merger of antibacterial and antifungal assets, coupled with their intrinsic "green" and facile synthesis, makes smart biogenic nanostructures for future applications in nanomedicine ranging from topical ointments and bandages for wound healing to coated stents.

The present book on *Fungal Nanobionics: Principle and Applications* is a very timely publication, which intends to provide state-of-art information in the area of nanotechnology, broadly involving fungal-based innovation and applications. The book comprises twelve chapters. The first chapter by Cotica et al. reviews nanobiocomposites: synthesis and environmental applications in the development of processes and methods for remediation and monitoring of systems contaminated with chemical wastes. Chapter 2 highlights the increased productivity and eco-friendly synthesis of nanoparticles by fungi for applications in both pharmaceutical and cosmetic industries presented by El Enshasy et al. In Chap. 3, Abdel-Aziz et al. described microorganisms have a promising role in biosynthesis of nanoparticles, especially fungi secrete enzymes and proteins as reducing agents which can be used for synthesis of metal nanoparticles from metal salts with great significance. Chapter 4 highlights the potential of fungus-originated nanomaterials in mycoremediation of waste and toxic materials discussed by Gholami-Shabani et al. In Chap. 5, Romero et al. highlighted on the potential of biogenic metal nanoparticles as an antimicrobial agent, other potential applications such as their cytotoxic activity against cancer cell lines and several biomedical, pharmaceutical, and agricultural applications. Chapter 6 highlighted on recent advances in biomedical applications of chitosan and its functional nano-derivatives by Rajkumari and Siddhardha. In Chap. 7, Salvadori et al. approach new perspectives for the biosynthesis of nanomaterials by fungal dead biomass, and at the same time it has the advantage to be a

low-cost effective bioremediation process. In Chap. 8, Boddula et al. emphasized on nanofabrication as the future of metal nanoparticles processing for its wide scale practical and industrial applications. In Chap. 9, Shasmita et al. described a comprehensive explanation of the strategies used for increasing the production of secondary metabolites in different in vitro culture system through fungal elicitors. Siddhardha and Parasuraman discussed that fungal-mediated synthesis of metal and metal oxide nanoparticles is cost effective, ecofriendly and fabricated material enhances the antimicrobial and antibiofilm efficacy in Chap. 10. Pandey and Tiwari presented an overview of fungal nanobiotechnology and its application in targeted drug delivery, bio-sensing and development of drugs with enhanced efficiency in Chap. 11. Roy et al. discussed the mycosynthesized nanoparticles and the mechanistic approach involved in the synthesis, the reduction of metal ions in developing low-cost techniques and recovery of nanoparticles. Finally, the application of nanoparticles in food processing industries, i.e. antimicrobial mechanisms etc., has also been mentioned in Chap. 12.

Overall, it is a great effort by Dr. Ram Prasad, his editorial team and experts from ten countries to make this highly resourceful, up-to-date and worthwhile unique book for the students, researchers, scientists and academician in the field of cutting-edge microbial nanotechnology. I hope that readers will find this book highly useful and interesting in fungal nanotechnology field.

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Ashok Pandey

Professor Ashok Pandey is currently Distinguished Scientist at CSIR–Indian Institute of Toxicology Research, Lucknow, India, and Honorary Executive Director at the Centre for Energy and Environmental Sustainability, India (www.cees.in). Formerly, he was Eminent Scientist at the Center of Innovative and Applied Bioprocessing, Mohali, and Chief Scientist & Head of Biotechnology Division at CSIR's National Institute for Interdisciplinary Science and Technology at Trivandrum. His major research and technological development interests are in industrial and environmental biotechnology, which span over biomass to fuels & chemicals, waste to wealth/energy, industrial enzymes, solid-state fermentation, etc. Professor Pandey has ~1250 publications/communications, which include 16 patents, 60 books, ~615 papers and book chapters, etc. with *h* index of 87 and >32,000

citations (Goggle Scholar). He is the recipient of many national and international awards and fellowships. Professor Pandey is Founder President of the Biotech Research Society, India (www.brsi.in) and is currently International Coordinator of International Bioprocessing Association (www.ifibiop.org), and Chairman of the International Society for Energy, Environment & Sustainability (www.isees.in). Professor Pandey is Editor-in-Chief of *Bioresource Technology*, Honorary Executive Advisors of *Journal of Water Sustainability* and *Journal of Energy and Environmental Sustainability*, Subject editor of *Proceedings of National Academy of Sciences* (India) and editorial board member of several international and Indian journals.

Preface

The use of fungi to synthesize functional nanoparticles has been of great interest. Fungi can change the oxidation state of metals, and these fungal processes have opened up new opportunities for us to explore novel applications, for example, the biosynthesis of metal nanomaterials. In contrast to chemical and physical methods, microbial processes for synthesizing nanomaterials can be achieved in aqueous phase under gentle and eco-friendly benign conditions. This approach has become an attractive focus in current green bionanotechnology research toward resource-efficient and sustainable development. The book covers the synthesis of nanoparticles by fungi, the mechanism involved in such biosynthesis, and a unique template for synthesis of tailored nanoparticles targeted at therapeutics, diagnostics, bioremediation, agriculture, and industries.

This book should be immensely useful to biological sciences specially microbiologists, nanotechnologists, researchers, technocrats, and scientists of fungal nanobiotechnology. We have honored that the leading scientists who have extensive, in-depth experience and expertise in fungal system and nanotechnology took the time and effort to develop these outstanding chapters. Each chapter is written by internationally recognized researchers/scientists so the reader is given an up-to-date and detailed account of our knowledge of the nanobiotechnology and innumerable applications of fungi.

We are indebted to the many people who helped to bring this book to light. Editors wish to thank Dr. Mamta Kapila, Senior Editor, Springer; Mr. Ramkumar John, Project Coordinator, Springer Nature; and Ms. Ishrath Ara, Project Manager, SPi Global for generous assistance, constant support, and patience in initializing the volume. Dr. Ram Prasad gives special thanks to his exquisite wife Dr. Avita for her constant support and motivations in putting everything together. Dr. Prasad in particular is very thankful to Professor Ajit Varma, Amity University, for constant

encouragement. He also gives special thanks to his esteemed friends and well-wishers and all faculty colleagues of School of Environmental Science and Engineering, Sun Yat-Sen University, China, and AIMT, Amity University, India.

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About the Editors

Dr. Ram Prasad is associated with Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, India since 2005. His research interest includes plant-microbe-interactions, sustainable agriculture and microbial nanobiotechnology. Dr. Prasad has more than hundred publications to his credit, including research papers, review articles and book chapters and five patents issued or pending, and edited or authored several books. Dr. Prasad has 12 years of teaching experience, and he has been awarded the Young Scientist Award (2007) and Prof. J.S. Datta Munshi Gold Medal (2009) by the International Society for Ecological Communications; FSAB fellowship (2010) by the Society for Applied Biotechnology; the American Cancer Society UICC International Fellowship for Beginning Investigators, USA (2014); Outstanding Scientist Award (2015) in the field of Microbiology by Venus International Foundation; BRICPL Science Investigator Award (ICAABT-2017) and Research Excellence Award (2018). Previously, Dr. Prasad served as Visiting Assistant Professor, Whiting School of Engineering, Department of Mechanical Engineering at Johns Hopkins University, USA, and presently, working as Research Associate Professor at School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou, China.

Dr. Vivek Kumar is Associate Professor, involved in teaching, research and guidance with a pledge to enduring knowledge. Dr. Kumar works at Himalayan School of Biosciences, Swami Rama Himalayan University, Jolly Grant, Dehradun, India. He obtained his master's and doctoral degrees from CCS Haryana Agricultural University, Hisar, Haryana, India. He currently serves on the editorial boards of many prominent international journals, viz., *Environment Asia*, *International Journal of Biological & Chemical Sciences*, *Environmental Sustainability*, *Journal of Advanced Botany and Zoology* and *Journal of Ecobiotechnology*. He is also a reviewer for many prestigious journals such as *Journal of Hazardous Materials*, *Environmental Sustainability*, *Science International*, *Acta Physiologiae Plantarum*, *Environment Science & Pollution Research* and *Rhizosphere*. He has more than 100 publications to his credit, including research papers, review articles, book chapters, and also edited several Springer books. Dr. Kumar also served as a Microbiologist for 8 years at the Department of Soil and Water Research, Public Authority of Agricultural Affairs & Fish Resources, Kuwait. He has been credited with first time reporting and identification of Pink Rot inflorescence disease of date palm in Kuwait caused by *Serratia marcescens*. He has been awarded 'Young Scientist Award' for the year 2002 in 'Agricultural Microbiology' by the Association of Microbiologists of India (AMI). Dr. Kumar's research areas are plant-microbe-interactions, environmental microbiology and bioremediation. He has also organized various outreach activities.

Dr. Manoj Kumar is a scientist with sanguine behavior who is adoring about research and development, with a commitment to lifelong learning. He is determined on high quality science that contributes broadly to both increasing intellectual knowledge of plant development and to increasing the ecological niche. He has a high level of professional desire and intellectual hunt, and the potential to fulfil the dream of his high impact publications and the future recognition of these by academic peers. Dr. Kumar has pursued his Ph.D. in Plant Biotechnology from prestigious

Jawaharlal Nehru University and then awarded two postdoctoral fellowships consecutively: DBT-PDF from IISc Bangalore in 2005 and then NRF-PDF from University of Pretoria and Melbourne. Presently, Dr. Kumar is working as Associate Professor and Head of School of Life Sciences, Central University of Jharkhand, Ranchi, India. He is leading a diverse research group in Life Sciences supported by DBT-BUILDER program, Ministry of Sciences and Technology, Govt. of India. He referees for many more, including *Phytoremediation*, *Journal of Soil Sediments* and many more. Dr. Kumar's research is the integration of microbial genetics with a breadth of plant physiological approaches to enable novel gene discovery and conferring metabolites.

Dr. Shanquan Wang (ALAN) is Associate Professor, School of Civil and Environmental Engineering, Sun Yat-Sen University, Guangzhou, China, from 01/2016 to till date. His area of research focus is on environmental microbiology, especially on organohalide-respiring bacteria and their conversion of halogenated Persistent Organic Pollutants (POPs). He integrates microbial cultivation, metagenomics, molecular techniques and bioreactor operation to gain fundamental insights of our complex biosystems (e.g. bioremediation sites and anaerobic digesters), specifically from molecular-, cellular-, community- to system-levels. The generated knowledge on these reductive processes will be further employed to devise novel methods, techniques and products for our environmental engineering purposes.

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Chapter 1
Nanobiocomposites: Synthesis and Environmental Applications

Luiz Fernando Cotica, Adriana Garcia, Andressa Domingos Polli, Raquel Dosciatti Bini, Thaís de Chaves, Verci Alves de Oliveira Junior, and João Alencar Pamphile

Abstract The nanotechnology has been extensively studied and employed in the environmental area. With the increase of environmental problems, one of the main concerns of biotechnology is the decontamination of environments, a process that can be carried out through bioremediation, aiming at the decontamination of environments by the use of microorganisms such as fungi or their enzymes to degrade toxic substances into nontoxic substances. The union of nanotechnology with the different types of particles and organisms, such as fungi, can provide sustainable ecological alternatives for bioremediation. Among the several types of nanomaterials, the nanoparticles have been widely used in many different applications. In the search for nanoparticles suitable for applications in biological systems, iron oxide nanoparticles are found as good candidates due to its chemical stability and low toxicity. Thus, materials such as nanocatalysts, nanobiocomposites, and bioactive nanoparticles have been increasingly used in the development of processes and methods for remediation and monitoring of systems contaminated with chemical wastes.

Keywords Nanobiocomposites · Nanocatalyst · Microemulsion · Nanoencapsulation · Nanosensor

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1.1 Introduction

Nanotechnology is an emerging and interdisciplinary area of science that involves the engineering of nanometric particles of distinct materials (Khare et al. 2014). This area has grown into a multidisciplinary field, transforming the basic sciences (applied physics, chemistry, mechanics, biological and electrical engineering, robotics, and medicine). Furthermore, it has the potential to revolutionize agriculture, providing solutions to many of the problems faced in modern life, as well as the production of food, packaging, water resources, and their respective applications (Baruah and Dutta 2009; Lodhia et al. 2010; Prasad 2014; Bera and Belhaj 2016; Prasad et al. 2017; Sangeetha et al. 2017).

1.2 Nanomaterials

A nanomaterial is an object that has at least one dimension in the nanometer scale. They can be arranged according to their dimensions, as shown in Table 1.1.

An important aspect to be distinguished is the "nano" dimension when compared with other systems. Nanomaterials are larger than individual atoms but smaller than bacteria and cells. In order for a material be considered a functional material, some of its properties (optical, magnetic, electrical, etc.) can be changed in a controlled fashion by external stimuli, making these materials useful in technological products, machines, devices, and so on. Functional materials have been very significant in human life, and the different eras of civilization were marked by the domination of these materials by man, such as the stone, bronze, and iron ages, proving that the changes were essential for survival (Sengupta and Sarkar 2015). Nowadays, with the modern world in constant transformation, in a globalization scenario, and due to technological advances, new areas of knowledge, "nanoscience and nanotechnology," were born (Ramsden 2011). This area consists of the study of materials with dimensions in the nanometer range and has extrapolated the limits of traditional academia and industry (Sengupta and Sarkar 2015). As a multidisciplinary technology, involving the areas of physics, chemistry, biology, and medicine, the field of nanotechnology applications is vast, with great emphasis on nanoelectronics, nanobiotechnology, and nanomaterials (Ramsden 2011).

Nanoscience and nanotechnology contemplate the nanometric universe in which the physical dimensions are represented by a unit equivalent to one billionth of a

Table 1.1 Classification and examples of nanomaterials according to their dimensions

Dimension	Examples
All 3 dimensions <100 nm	Nanoparticles, quantum dots, nanocapsules
2 dimensions <100 nm	Nanotubes, nanofibers, nanowires
1 dimension <100 nm	Thin films, layers, coatings

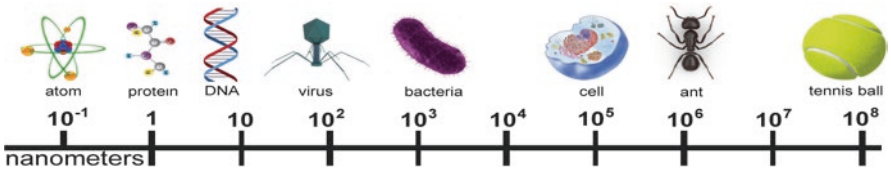

Fig. 1.1 The nanoscale. Some different species and their respective scales

meter (1 nm = 10^{-9} m). The prefix "nano" means "dwarf." As examples of this size, we have 1 nm as almost equal to ten lined atoms; a carbon nanotube has 10 nm in diameter; the size of a DNA molecule is in the 100 nm range and is a little bit smaller than the size of a virus; red blood cell diameter is of the order of 10,000 nm; the diameter of a human hair is close to 100,000 nm. All these examples are illustrated in Fig. 1.1.

Some properties of materials such as optical, magnetic, and catalysis are strongly dependent on the size of the particles that compose them. That means when the dimensions of the particles that built up a given material are below the critical size, its properties become distinguished. Furthermore, below this critical size, the shape of nanoparticles also influences the properties of the material. These considerations are very interesting as they open the way for the discovery of new materials, with new properties and possibilities of applications. In this sense, we can define the nanomaterials as the materials that have at least one dimension in the nanometric size range, below the critical size accomplished to change some of their properties (Sengupta and Sarkar 2015).

Nanometric particles can be incorporated into reflective glass lenses and rear-view mirrors of automotive vehicles to eliminate glare and dazzle under heavy illumination. When these nanoparticles are associated with polymers or resins, they are called nanocomposites and can be used for packaging (e.g., food packaging), offering greater protection against humidity, chemical vapors, and solvents. The use of nanocomposites in bottles' fabrication gives them greater resistance and lightness, in addition to decreasing the amount of plastic. This can reduce costs and minimize environmental impact. Nanocomposites are also commonly used in automotive and aeronautical industries, replacing heavy metal components that are difficult to handle by lighter, more fictile and resistant materials (Ramsden 2011).

In the cosmetics area, the use of nanoparticles enables to efficiently control the creams' degree of diffusion and helps in the removal of dirt and grease or in keeping the skin moist. In addition, the nanoscale gives a larger surface area to the nanoparticles and nanomaterials, i.e., they supply a larger amount of atoms or active site at the surface, increasing the activity of catalysis or adsorption (Ramsden 2011).

Magnetic nanoparticles suspended in a liquid medium create a ferrofluid, which is able to respond to a magnetic field. Its applications include magnetic microsensors, magnetic sealants, magnetic recording, and catalysis, among others (Knobel et al. 2008). In the biomedical field, these magnetic nanoparticles can be modified using appropriate chemical receptors that "stick" to toxins or specific substances of

our body, allowing their removal or recognition, by the application of an external magnetic field, for example (Ramsden 2011). These biomedical applications of magnetic nanoparticles have received increasing prestige in the last years and have been used in in vivo and in vitro applications. In vivo applications can be separated into therapeutic, which involves treatment by hyperthermia and drug delivery, and diagnostic, such as nuclear magnetic resonance imaging (Prasad 2016). In vitro applications are generally used for diagnosis, magnetic separation, and magneto-relaxation, for example (Tartaj et al. 2003).

1.3 Synthesis of Nanomaterials

The synthesis of nanomaterials can be conveniently split into two fundamental categories, top-down and bottom-up methods. The top-down method refers to obtaining materials from a bulk structure, while the bottom-up method essentially includes the process where atoms, molecules, or clusters are assembled to give rise to nanomaterials (Cao 2004).

Physical processes such as mechanical milling and laser ablation techniques are examples of top-down methods. In the milling process, the precursor materials, usually metals and oxides, are mixed in the desired proportions and placed in a milling vessel charged with grinding balls. The displacement of the vessel-ball system causes impact forces to act on the material causing the shear and solder of the particles. The result is a homogeneous reduction and dispersion of the particles. In most cases, when milling process is employed, a wide particle size distribution is obtained, and significant discrepancies in the shape and geometry of the particles can also occur. Further, the nanoparticles can incorporate amounts of impurities coming from the milling vessel. In this way, the milling process is widely used in the production of metal and ceramic materials where the size distribution, shape, and impurities have no significance for the desired applications (Cao 2004).

In the laser ablation technique, a high-power laser beam, usually a pulsed laser, is focused on the surface of a solid target. This can be done in vacuum, gas, or liquid. As the gaseous or liquid medium does not attenuate the energy of the laser, the intensity of light at the surface of the solid is enough to disturb the material out of its equilibrium. When the laser beam reaches the surface of the material, a localized explosion occurs, and the nucleation and growth of the ablated species take place assembling the micro-/nanoparticles (Yan and Chrisey 2012).

Bottom-up methods usually employ chemical synthesis routes. Among these routes, the most commonly considered are coprecipitation, microemulsion, and hydrothermal treatments. The synthesis by coprecipitation comprises the formation of nanoparticles by means of a homogeneous nucleation. The procedure concerns the formation of a supersaturated solution of the precursor salts followed by precipitation due the addition of a base. The size, shape, and composition of the nanoparticles are dependent on factors such as the salt type, molar ratio between salts, pH, and ionic strength of the medium. It is a simple and fast process that, once the

synthesis conditions are fixed, produces quality and totally reproducible nanoparticles (Lu et al. 2007). However, some coating or stabilizing agents are necessary to decrease the agglomeration of the synthesized nanoparticles. This procedure allows the synthesis of metallic nanoparticles, such as Au, Ag, Cu, and Ni, as well as oxides such as Fe_3O_4 and $MnFe_2O_4$. In addition, simple oxides such as NiO and ZnO can also be obtained (Cushing et al. 2004).

Microemulsion is an optically isotropic and thermodynamically stable dispersion of two immiscible liquids where the microdomains of either or both liquids are stabilized by an interfacial film of surfactant molecules. In a water-in-oil (W/O) microemulsion, an aqueous phase is dispersed as microdroplets, surrounded by a monolayer of surfactant molecules, in a continuous phase of hydrocarbons. The size of the established reverse micelle depends on the water to surfactant molar ratio. By mixing two identical W/O microemulsions containing the desired reagents, the microdrops will be constantly colliding, merging, and breaking again, and in the end, the precipitate draws up in the micelle. By adding a solvent, such as acetone or alcohol, the precipitate can be extracted by filtration or centrifugation. In conclusion, microemulsions act as nanoreactors for the synthesis of nanoparticles. Metallic cobalt and cobalt/platinum alloys and cobalt, nickel, copper, and manganese ferrite nanoparticles have already been synthesized using the microemulsion technique (Lu et al. 2007).

In sealed containers, solvents may be raised at temperatures above their boiling point by increasing their autogenous pressure resulting from heating. Chemical reactions performed under these conditions are called the solvothermal process or, when water is used as solvent, hydrothermal process. This procedure results in greater solubility and reactivity of the precursors, which are generally metals or oxides. When the process is performed above the temperature and pressure of the critical point of the water, this is called subcritical, i.e., it has characteristics of liquid and gas. Under these conditions, a rupture in the solid and the fluid surface tensions is obtained, as well as an increase in the viscosity of the system. Then, chemical compounds, which would have a low solubility under ambient conditions, can be dissolved easily (Cushing et al. 2004). By using this method, it is possible to obtain Fe_3O_4 and $CoFe_2O_4$ nanoparticles of uniform size, as well as a number of simple oxides' nanoparticles (Lu et al. 2007; Cushing et al. 2004).

1.4 Some Experimental Techniques for the Study of Nanomaterials

A very important factor to the growth of nanoscience and nanotechnology was the development of increasingly sensitive characterization techniques, remarkably microscopy techniques. By using microscopy techniques, it is possible to evaluate the size, morphology, and size distribution, especially when well-defined characteristics in the nanomaterial applications are required. The electron microscope, other

than the optical microscope that uses a light source and glass lenses to illuminate the specimen and produce magnified images, uses a beam of accelerated electrons and electromagnetic lenses to generate images with high resolution based on wavelengths much lower when compared with visible light (El-Nour et al. 2010).

The scanning electron microscope (SEM) is a very powerful tool for obtaining information about the surface of the material. The incident electron beam scans the surface of the sample interacting with the atoms and generating signals, which can provide the chemical composition and surface topology of the material. As the electron beam hits the surface, it causes the emission of elastically and inelastically scattered electrons, secondary electrons, and X-rays. The most common detector in SEM equipment is for secondary electrons. To obtain these signals, the samples need to be conductive; otherwise an ultrathin layer of material that conducts electricity should be provided. Some modern microscopes have a low vacuum (environmental) accessory, where the samples, mainly biological samples, can be analyzed in their natural state without any modification or previous preparation (Lin et al. 2014).

The transmission electron microscope (TEM) is another frequently used equipment for analysis of nanomaterials, providing images and chemical composition with resolution less than 50 nm. In the conventional mode of TEM, an incident electron beam is transmitted through a very thin sample. This beam interacts with the sample generating elastically or inelastically scattered electrons. The final result is a nonuniform distribution of electrons emerging from the sample, which contains all the chemical and structural information of the analyzed sample (Lin et al. 2014; Carter and Williams 2009).

Atomic force microscopy (AFM) is a high-resolution scanning probe microscopy technique. The basic AFM components consist of a microcantilever (very small holder) containing a very thin probe for the sample surface. Typically, the support arm is made of silicon nitride or silicon. In AFM the image construction is based on the change of forces between the tip of the cantilever and the surface of the sample in which the probe comes into contact. The forces that can be analyzed include mechanical contact force, Van der Waals forces, capillary force, chemical bonding force, and electrostatic and magnetic forces. These forces are measured by the deflection of the cantilever, which behaves as a tiny spring, following Hooke's law. A laser focused on the top of the cantilever and later reflected in a diode array detects this deflection. Any tiny positional deviations of the laser spot as a result of cantilever deflection is recorded and converted into a 3-D image.

Although both AFM and other scanning probe microscopy techniques can produce 3-D images, AFM has several advantages. Typical AFM equipment can produce images with a vertical resolution of 0.5 nm. In addition, nanomaterials analyzed by AFM do not require special preparation; AFM works efficiently in an air or liquid environment, facilitating studies of biological macromolecules and living organisms at the nanoscale. It can be used in the investigation of size, shape, structure, sorption, dispersion, and aggregation of nanomaterials. The different scanning mode employed in the AFM study includes noncontact mode (static mode), contact mode, and intermittent mode of contact with the sample (dynamic mode). In

addition to the size and shape probing of nanomaterials under physiological conditions, the AFM is able to characterize the dynamics between nanomaterials in biological situations, as well as to observe the interaction of the nanomaterials with the substrate. Also, AFM is of great importance because of its ability to form biomaterial images without causing appreciable damage to various types of native surfaces.

The dynamic light scattering (DLS) is one of the most widely used techniques in determining the size distribution of nanoparticles, molecules, or polymers of micrometric scale up to a few nanometers in solution or suspension using a monochromatic light, such as a laser. The principle of DLS technique is to monitor the fluctuation of the light intensity due to an elastic scattering, such as Rayleigh scattering. When a laser is used as the light source, the fluctuation in the scattering intensity is a function of the time. These fluctuations occur as nanoparticles (or small particles in solution) are not static but are in constant and random motion (Brownian motion), and so the distance between the dispersers in the solution is constantly changing over time. This diffuse light undergoes constructive or destructive interference around the particles, and through mathematical calculations using the Stokes-Einstein equation (and Doppler considerations), it is possible to associate this variation of light scattering intensity as a function of the time to the diameter of the particles dispersed in the solution.

The advantages of DLS technique include the short time for data acquisition (minutes), precision in determining the hydrodynamic size of monodisperse samples, and the ability to measure in diluted samples. The DLS is a low-cost equipment and provides a good reproducibility in analyzing.

1.5 Fungi and Nanobiocomposites

The fungi kingdom is a versatile and heterogeneous group that includes eukaryotic organisms with distinct biotechnological applications. Estimation for this kingdom gives 1.5 million species, but only about 5% of the fungal species are already known (Hawksworth 2001).

The fungi can be divided into yeast forms, which are single and small cells, and hyphal, which present tubular and polarized cells and grow continuously (Sievers et al. 1999). The hyphae have 2–10 μm in diameter, reaching several centimeters in length. They may be septate or coenocytic, with multinuclear sections divided by walls (septa) that are connected through pores. The bifurcation and ramification of these hyphae cause the formation of interconnected networks called a mycelium (Mcconnaughey 2014).

Related to the fungi's structure and composition, they are composed by the cytoplasm with several organelles and, superficially, the presence of matrices that are connected to each other: the extracellular or capsular matrix, the cell wall, and the plasma membrane. The extracellular matrix has a mucilaginous substance that helps adhesion and extracellular enzymes (Moore-Landecker 1996). The plasma

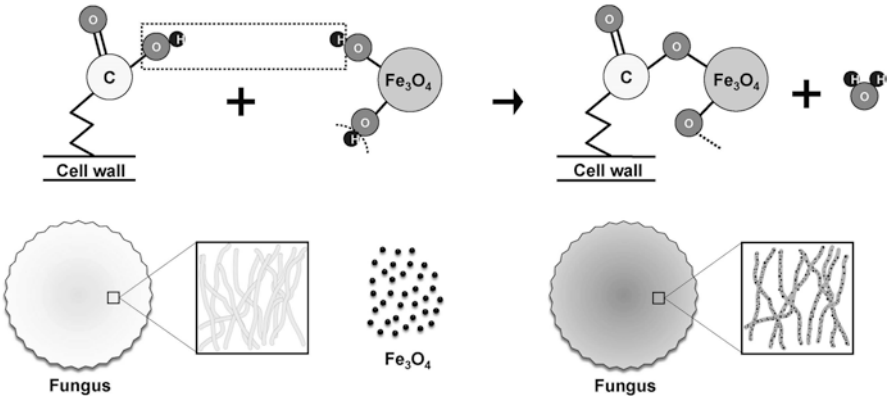

Fig. 1.2 Diagram of the reaction between the fungi and the Fe_3O_4 nanoparticles during the formation of the nanobiocomposites. (Adapted from Li et al. 2015)

membrane consists of layers of phospholipids with proteins and esters (Deacon 1997), presenting enzymes that measure cell surface processes, such as H^+-ATPase (acts in the transport of substances) and chitin synthetase.

The fungal cell wall is a dynamic polymer matrix outside the plasma membrane composed of polysaccharides, proteins, or lipids, which protects the cell from stress and toxic substances, osmotic pressure preventing its lysis and determining the morphological structure of the fungus. Its general characteristic is the presence of chitin (homopolymer with β-1,4 bonds of microcrystalline N-acetyl-glucosamine). Glycoproteins, mannoproteins, and several other polymers, glycosaminoglycans ((1-3)-β-glucan, (1-6)-β-glucan, (1-3)-α-glucan), are present, forming fibrillar structures that contribute to structural support and stiffness. The wall proteins are united by covalent bonds to the polysaccharides and act on the adhesion, recognition, modification, and nutrition of the wall. There may be pigments that contribute to the defense of protoplasm (Wessels 1993; Bueno and Silva 2014).

The functional groups present in the cell wall components of the fungi (-OH, $-NH_2$, -COOH, -SH) can interact with metallic nanoparticles, forming nanobiocomposites. This opens a vast field of studies in bioremediation of contaminated areas, since fungi have many biological activities and applications and nanoparticles have a promising biotechnological potential. The nanoparticles can be dispersed evenly over the fungi wall, interacting with the chitin, proteins, and glucans existing therein (Li et al. 2015; Prasad 2016; Siddhanta et al. 2017).

The nanobiocomposites can be prepared by fungus plus Fe_3O_4 nanoparticles dehydration reaction, in which the carboxyl groups of chitin present within the fungus cell wall can be combined with hydrogen atoms of the carboxyl groups on the surface of the nanoparticles (Fig. 1.2). Li et al. (2015), who performed Fourier transform infrared spectroscopy (FTIR) analyses of the chemical bond changes during the nanobiocomposite formation process of the black *Aspergillus* fungus and Fe_3O_4 nanoparticles (fungus- Fe_3O_4 bio-nanocomposites), demonstrated this.

In a study of an assisted self-organization model of gold nanoparticles on the *Aspergillus niger* mycelium to obtain gold microwires, Sabah et al. (2012) verified that during the growth process, the gradual pH change of the colloids occurred, from 3 to 7, indicating the consumption of glutamic acid present in the colloid by the fungus and the successive aggregation of the nanoparticles in the hyphae. The agglomeration of the nanoparticles in the fungus cell wall occurs due to the Brownian motion that forces the nanoparticles to move and gather toward the growing hyphae, organizing itself in subsequent layers. As the surfaces of hyphae and gold nanoparticles are hydrophobic, what keeps this layered structure stable is the interacting forces between the nanoparticles themselves and between their surfaces and the surface of the hyphae, and these forces depend on the free energy between the solution and surfaces.

The induction of instability in the aqueous solution, by the consumption of stabilizing nutrients of nanoparticles, also causes the hydrophobic gold to accumulate on the fungal hyphae, forming the first layer. The subsequent layers settle on the former due to the agglomeration induced by the continued exhaustion of the stabilizing ions (Rehman et al. 2011).

Bigall et al. (2008), using fungi such as *Trichoderma viride*, *Penicillium citreonigrum*, *Neurospora crassa*, *Bjerkandera adusta*, and others, showed the growth capacity of these lineages in solutions of noble metal nanoparticles, such as gold, silver, platinum, and palladium, in colloidal medium stabilized with citrate. Furthermore, nanoparticles deposited on fungal surfaces result in hybrid systems with optical properties similar to the respective nanoparticle solutions. It was found that, when observed by scanning electron microscopy, the morphology of these nanobiocomposites showed different affinity for each type of metal. These differences in affinity were confirmed by spectroscopic energy dispersive X-ray (EDX) analysis, in which the authors observed that the gold content in the samples varied up to the factor of 60, evidencing differences in the mycelial surfaces or in the metabolic characteristics. This difference in the deposition of the nanoparticles, for example, the gold and silver nanoparticles, on the fungal surface could also contribute to studies of identification and organization of the mycelial structure.

Studies demonstrate that fungi can have increased bioremediation capacity when interacting with metallic nanoparticles due to their chemical residues, especially while interacting with magnetic iron oxide nanoparticles. In fact, nanoparticles cover the mycelial surface of the fungus and increase its action, such as the degradation ability using *Phanerochaete chrysosporium* fungus and Fe_3O_4 nanoparticles (Huang et al. 2015).

Cell immobilization technology can increase the fungi's adsorption capacity and resistance to heavy metals such as lead. Therefore, the connection of biotechnology and nanotechnology has been increasingly employed for the creation of adsorbents by the combination of fungi, such as *P. chrysosporium*, and magnetic nanoparticles of iron oxide fixed by calcium alginate (Xu et al. 2012, 2013).

As previously described, the formation of fungal nanobiocomposites using metallic nanoparticles, especially magnetite nanoparticles, has been the subject of

many studies. This is why biomass substrate has advantages such as mass transferability, low cost, dispersibility, and the scale of action of the nanoparticles. In addition, iron oxide nanoparticles can freely self-assemble over the fungus, following the formation of their mycelium, without the need for special chemical conditions and methods, such as the use of chelating agents and cross-linking agents (Ding et al. 2015).

Fungi can also be used as reducing agents for the formation of nanoparticle films. As an example, Salvadori et al. (2014) used the dead biomass of the filamentous fungus *Aspergillus aculeatus* for the synthesis of nanoparticles of nickel oxide in the film form. In fact, this biomass presents optimal conditions to absorb and produce nanoparticles by the biosorption of nickel by the fungus. Therefore, this is a nanotechnological innovation that is not harmful to the environment, pointing to sustainable strategies for the biosynthesis of metal oxide nanoparticles.

1.6 Applications of Nanocomposites in Nanobiotechnology

1.6.1 Applications in Environmental Area

As already addressed in the previous sections, nanotechnology has been extensively studied and employed in the environmental area. This "new" science field, together with other areas of knowledge, can greatly contribute for new discoveries and applications in the environmental scope. As it could be seen, nanotechnology offers the prospect of great advances to improve the quality of life and help to preserve the environment (Kango et al. 2013). This science has evolved into a multidisciplinary field, revolutionizing the basic sciences (applied physics, chemistry, mechanics, biological and electrical engineering, robotics, and medicine) and their respective applications (Lodhia et al. 2010).

At the same time, with the increase of environmental problems, one of the main concerns of biotechnology is the decontamination of environments, a process that can be carried out through bioremediation, aiming at the decontamination of environments by the use of microorganisms such as fungi or their enzymes to degrade toxic substances into nontoxic substances. The biological process of bioremediation is an ecologically adequate and effective alternative for the treatment of environments contaminated with organic molecules of difficult degradation and toxic metals (Alexander 1999; Prasad 2018).

Considering the low cost and facility to obtain, they produced high yields of biomass that can be manipulated genetically and morphologically. Several species have been used to remove contaminants from the environment (Kaushik and Malik 2009; Fu and Wang 2011). One of the advantages of the fungal process according to Ryan et al. (2005) is the enzyme-mediated activity that is produced during all phases of the fungal life cycle and is present even at low concentrations of pollutants. In relation to producing extracellular enzymes, the fungal mycelia have an additive advantage over single-cell organisms by solubilizing the insoluble substrates. Fungi

have a greater physical and enzymatic contact with the environment, due to the increased cell-to-surface ratio. Because of its extracellular nature, a fungal enzyme is also advantageous in tolerating high concentrations of toxicants (Kaushik and Malik 2009).

The textile industry occupies a prominent place when it comes to environmental pollution, a fact that is due to the large volumes of water used in the processes and the complexity of the waste disposed of by the industries, and during the dyeing process, around 15% of the dye employed is discharged into effluents (Mollea et al. 2005). Dyes and their degradation products present in industrial effluents are reported to be highly carcinogenic and mutagenic (Kaur et al. 2014). Its presence in water effluents, even at very low concentrations, is extremely harmful and undesirable (Saharan et al. 2014). Fortunately, fungi promote the removal of dyes mainly through the mechanisms of biosorption, bioaccumulation, and biodegradation (Kaushik and Malik 2009; Prasad 2017, 2018).

Another environmental problem concerns the heavy metals, which are often released to the surface and groundwater. These metals have been a major concern for many years due to their increased discharge, damage to the environment, acute toxicity, non-biodegradable nature, and tendency to bioaccumulate, which are results of different activities such as industrial and agricultural activities and mining (Gupta et al. 2010; Feng et al. 2010; Xu et al. 2012). Lead is one of the most representative toxins of heavy metals that have toxicological and neurotoxic effects on the liver, kidneys, brain, and central nervous system (Southichak et al. 2006; Xu et al. 2012).

The removal of heavy metals is a huge challenge and has become one of the most serious environmental problems because of the lack of cost-effective treatment alternatives and their recalcitrance and persistence in the environment. Thus, the search for increasingly stringent wastewater treatment has created a growing interest in the progress of conventional treatment processes (Gupta et al. 2010). In the process of removal of heavy metals, fungi have the advantage of binding heavy metals due to a wealth of functional groups (Cheng et al. 2015).

Also, nanomaterials have a great potential for the removal of contaminants, corroborating with the search for more efficient methods, with lower cost, to decontaminate water without further stressing the environment or endangering human health by the treatment itself (Malato et al. 2009).

Several papers found in the literature report that the study of magnetic nanoparticles for biological applications uses iron oxides (Huang et al. 2015; Ding et al. 2015; Su 2017). Iron oxides such as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), and hematite (α-Fe_2O_3) nanoparticles have been used for the separation and removal of organic and inorganic contaminants (Saharan et al. 2014) because they have low cytotoxicity and are well tolerated by biological organisms (Yang et al. 2006; Hafeli and Chastellain 2006). According to Xu et al. (2012), iron oxide nanomaterials are efficient biosorbents for the removal of heavy metals and organic pollutants. In addition, many papers demonstrate the use of modified magnetic nanoparticles in the removal of various dyes (Saharan et al. 2014; Kaur et al. 2014; Tan et al. 2015).

Therefore, the union of nanotechnology with different types of particles and organisms, such as fungi, can provide sustainable ecological alternatives for bioremediation. Rispail et al. (2014) performed a study and confirmed that the nanomaterials can interact with hyphae of the fungus *Fusarium oxysporum*. In addition, different assays showed a low toxicity profile for the different types of nanomaterials for the fungus.

In this sense, the nanobiotechnology can help with the problems due to anthropic action that have reached catastrophic dimensions, which can be observed through changes in soil, air, and water quality (Kunz et al. 2002). Another application concerns contaminated areas with dyes, heavy metals, or radioisotopes which can cause many problems, such as damage to health and the environment and compromised soil and water quality. In this sense, materials such as nanocatalysts, nanobiocomposites, and bioactive nanoparticles have been increasingly used in the development of processes and methods for remediation and monitoring of systems contaminated with chemical wastes (Tratnyek and Johnson 2006; Karn et al. 2009).

Studies presented by Xu et al. (2012) using the *P. chrysosporium* fungus associated with magnetite nanoparticles showed that this fungus had the capacity to absorb heavy metals. By adsorption in batches, the adsorption capacity of Pb ions from the aqueous solution was investigated. In order to obtain the results for analysis, the FTIR method and the intraparticle diffusion analysis were used. The reuse of the resulting adsorbents was estimated. The researchers concluded that the prepared adsorbents presented promising prospects for application in the treatment of wastewater containing heavy metals, with great adsorption capacity and favorable stability.

In recent years, studies with adsorption processes combined with magnetic separation and other techniques have been widely used in processing wastewater treatment containing several pollutants (Mahdavian and Mirrahimi 2010). Several environmental studies with nanoparticles of iron oxide magnetite are gaining space in the area of environmental remediation (Su 2017; Yuan et al. 2009). Magnetic nanoparticles are superior to other types of nanoparticles due to their superparamagnetic behavior (Su 2017), allowing them to separate easily from wastewater (Ding et al. 2015; Jiang et al. 2014).

Another major environmental contamination problem is about the phenol, which is toxic either by ingestion or by contact or inhalation, even at low concentrations. Acute exposure to phenol causes disorders of the central nervous system, leading to collapse and coma (Huang et al. 2015; Nair et al. 2008). This pollutant has been found in large quantities in wastewater from oil refineries, coal gasification plants, and phenol resins industry and is most often found in polluted waters, rivers, and industrial effluents, where studies involving microorganisms and nanoparticles are used to the removal of this toxic contaminant (Martínkova et al. 2009).

In the process of bioremediation, magnetic nanoparticles, allied with microorganisms, are increasingly used, forming unique systems that can be separated or removed from solutions using an external magnetic field (e.g., using a magnetic filter, permanent magnet, or electromagnet). This possibility is very important for biological applications due to the fact that the absolute majority of the biological

materials have diamagnetic properties, which allows the selective and efficient separation of magnetically modified materials.

Huang et al. (2015) investigated the degradation of phenol in wastewater under the action of Fe_3O_4 nanoparticles and Pc fungus (BKMF-1767, *P. chrysosporium*), which causes white rot, together with its secretory oxalate, under the conditions of solar light (three-band fluorescent lamp YZ08-T5, the light intensity of about 200 lux) and dark state. In addition, the different amounts of Fe_3O_4 nanoparticles, the initial concentrations of phenol, and the production of oxalate were discussed. So, it was found that two major ligninolytic enzymes, LiP and MnP, secreted by Pc (BKMF-1767), were followed by their binding with the phenol degradation. Thus, their studies have led to a novel method of treating phenol wastewater and may provide useful references to promote a more efficient treatment.

The search for alternative and effective methods in the adsorption/reduction or complete elimination of environments contaminated by radionuclides is constant. Several authors have proposed sustainable alternatives, such as nanomaterials and bio-nanocomposites in a single system. The availability of fungal mycelia pellet as a biomass transporter allows thr obtaining of new biocomposites for the treatment of wastewater. Nanomaterials are being used in the removal of heavy metals and radionuclides owing to the high surface to volume ratio and the fast reaction kinetics (Ding et al. 2015).

As an example of successful application of a bio-nanocomposite, Li et al. (2015), with the objective of removing radioactive uranium oxide ion $(UO_2)^{2+}$ from water, used the *Aspergillus* fungus with Fe_3O_4 nanoparticles. They verified that the fungus-Fe_3O_4 bio-nanocomposite presented higher adsorption capacity of radioactive $(UO_2)^{2+}$ ions, with a 55% increase in relation to the Fe_3O_4 nanoparticles and 170% in relation to the fungus. The use of a biological system to produce a magnetic adsorbent revealed benefits, since the nanoparticles were magnetic and were adhered to the fungus providing the possibility of removal of the fungus from the water through the use of a magnet (Li et al. 2015).

Ding et al. (2015) performed another example of decontamination process using a biological method composed by bio-nanocomposites of fungus (*Penicillium* sp.) and nanoparticles Fe_3O_4 for the removal of radionuclides Sr (II), Th (IV), and e U (VI). According to the authors, this system was inexpensive; nano-Fe_3O_4 particles could be adsorbed and entrapped on the mycelia pellet core during the growth of *Penicillium* sp. The new bio-nanocomposites of fungus-Fe_3O_4 could grow uniformly on the surface of the fungus without aggregation and are more environmentally friendly and cost-effective than the other described templates. The authors report that the fungus-Fe_3O_4 possesses an advantage in adsorption capacity compared to *Penicillium* sp., nano-Fe_3O_4, and other adsorbents, and maximum sorption capacities of the fungus-Fe_3O_4 went to Sr (II) and U (VI) at pH 5,0 and Th (IV) at pH 3,0.

So, we can say that fungi are well studied due their ability to degrade or transform substances, and decomposing organisms and recyclers. However, when allied to magnetic nanoparticles, they form unique systems that may offer outstanding mechanisms that are normally not found when only fungus or nanoparticles are used. These nanobiocomposites, as we have already observed in several examples, can act

in a promising manner in the remediation of environments contaminated with different pollutants and with the advantage of being removed from the environment by means of a magnetic separation process. The development of low-cost, fast, and large-scale techniques is essential for use in remediation environments.

1.6.2 Applications in Agriculture: Pest Control

For decades agriculture has been suffering because of pesticide use; pesticides cause adverse effects on soil and water, and accumulate throughout the food chain. The use of some of these pesticides has resulted in hormonal problems, increased risk of cancer, inadequate performance of the immune system, and abnormalities in different species of marine and terrestrial animals (Prasad et al. 2014, 2017). In addition, the number of weeds and insects that have become resistant to these products has increased (Rai and Ingle 2012), causing losses in plantations and leading to losses of billions of dollars (Gordon and Waterhouse 2007). Another problem that the agricultural sector is facing is related to climate change, urbanization, and unsustainable use of natural resources. These situations are further exacerbated by the increasing demand for food that will be required to maintain estimated population growth from the current level of about six to nine million by 2050 (Chen and Yad 2011).

Because of so many problems due to the use of pesticides in agriculture, nanotechnology has the potential to transform dramatically this economic segment, since it can improve the quality of life through its applications. As an example, the use of this technology has been promoting sustainable agriculture and improving the quality of food, thus reducing the risks to the community (Rai and Ingle 2012; Prasad et al. 2017). Nanotechnology has shown several applications in agriculture (Fig. 1.3). Prasad et al. (2014) showed that advances in this area have been reducing the negative impacts of pollution due to excessive use of traditional pesticides and fertilizers.

The use of nanotechnology cooperatively with genetics has also helped agriculture, since the use of nanoparticles linked to nucleic acids allows DNA structures to be programmable. Their structural properties can present specific applications,

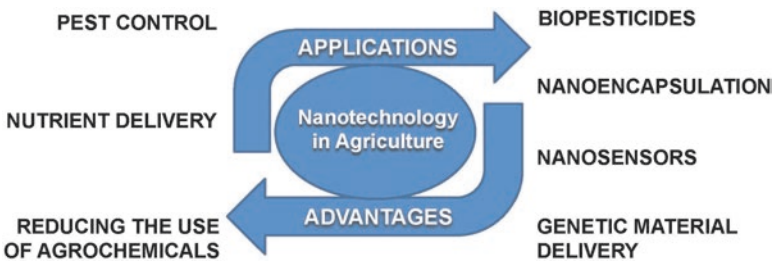

Fig. 1.3 Advantages and applications of nanotechnology in the agriculture

offering unknown paths to that of nucleic acids (Mohri et al. 2014). The union of nanoparticles with genetic material may be a modern form to transfer DNA to insects. According to Khandelwal et al. (2016), this result demonstrates that nanotechnology offers a nontoxic and viable solution to transfer genetic material. It also offers an easy method to manage insects by avoiding a process of giving rise to transgenic plants.

One of the approaches for the use of nanotechnology in the agricultural field is the so-called nanosensors. They are able to recognize nutrients or water levels, improving harvest protection with insecticides, fungicides, or herbicides (Khare et al. 2014). They can detect pathogens at low levels and degrade chemical substances (Baruah and Dutta 2009). Using these nanosensors, it is possible to find out solutions to degrade persistent chemicals.

Nanoencapsulation is an additional way of applying nanotechnology in agriculture. It is currently the most promising technology for screening host plants against pest insects. Nanoparticles can be labeled with chemicals and herbicides reaching specific parts of a plant, for example, the cell wall, cuticle, or a particular tissue, and decreasing the side effects (Khare et al. 2014; Nair et al. 2010; Bhattacharyya et al. 2016). The method can supply significant impacts on "smart" delivery of essential amount to the plant (Ghormade et al. 2011). Viral nanocapsules are described as a powerful tool for the biotechnology field, because the viral particles can transport a nucleic acid that inhibits the growth or interrupts the metabolic pathways of weeds (Pérez-de-Luque and Diego 2009).

On the control of plant diseases, Prasad et al. (2014) report that some of the nanoparticles classified as controllers of plant diseases are composed of carbon, silver, silica, and aluminosilicates. In such a situation, nanotechnology has amazed the scientific community due to the high quantity of materials. The use of silver particles as antimicrobial agents has become common as the synthesis technology advances, making the production more economical. As an example, the *Xanthomonas perforans* bacteria are responsible for the bacterial stain of the tomato; the disease causes several damages to the vegetable. Ocsoy et al. (2013) used silver nanoparticles as a treatment and obtained a significant reduction of the disease.

Moreover, silver nanoparticles have been used for the control of a neotropical cockroach, *Blaberus discoidalis*. This study revealed that the central nervous system of the cockroach suffered alterations in the motor function due to the presence of silver nanoparticles. Soni and Prakash (2012), aiming to destroy mosquito larvae (*Aedes aegypti*), produced silver and gold nanoparticles from the fungus *Chrysosporium tropicum.* The obtained results showed the treatment was more effective when gold nanoparticles were used. Larvae mortality was enhanced, which increased about three times when compared to the silver nanoparticles treatment. As a conclusion, insect treatments using nanoparticles increase the mortality when compared with regular insecticides. Furthermore, the insect mortality increased with the increase of the amount of nanoparticles applied for the treatment.

1.7 Concluding Remarks and Future Prospects

In this chapter the use of nanotechnology in environmental applications is attracting great attention of scientific community in basic science, and social and economic levels were presented. As the main subject, the use of nanobiocomposites in the environment is based on the study and control of phenomena and materials at atomic, molecular, and macromolecular scales where physicochemical properties differ significantly from those at a larger scale. Thus, this is not a one-subject scientific field but a coupling of existing disciplines including chemistry, physics, biology, biotechnology, medicine, neurology, information technology, and engineering, resulting in new multidisciplinary scientific approaches.

In particular, the coupling between biology/biotechnology and nanotechnology gives rise a new scientific area named nanobiotechnology. This new area is based on the engineering of modified biological systems for the assembly of nanostructures. In this sense, as a prospect for the future, the nanobiotechnology can present solutions for open issues in medicine and environmental, agricultural, and other related biotechnological areas.

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Chapter 2
Medical and Cosmetic Applications of Fungal Nanotechnology: Production, Characterization, and Bioactivity

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Abstract Nowadays, nanotechnology is widely applied for the development of highly efficient products in the pharmaceutical and cosmetic industries. Converting bioactive materials to nanoscale not only increases their biocompatibility but also increases their effectiveness, even when lower doses are used. Metal nanoparticles can be synthesized by fungal cells both intra- and extracellularly. Stabilization of the physical and chemical properties of various noble metal nanoparticles produced by fungi can be achieved through controlling the size, surface morphology, and surface chemistry of the nanoparticles. Intracellular synthesis provides smaller nanoparticles with well defined dimensions, but contributes to difficulty in downstream processing activity as compared with synthesis by extracellular methods. Recently, the production of nanoparticles from fungi has received extensive attention, owing to the capacity of fungi to produce nanoparticles extracellularly, a process that is more reliable and

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ecofriendly than intracellular methods, with relatively simple downstream processing. Fungi secrete extracellular enzymes for their survival and they control metal cation transportation to maintain intracellular homeostasis; when more protein is excreted nanoparticle synthesis is increased. To maximize nanoparticle synthesis, the rate of their synthesis can be increased through optimization of the total fungal cell mass and bioprocessing parameters, such as time of exposure, temperature, and pH. This will facilitate increased productivity in the fungal synthesis of nanoparticles for applications in the pharmaceutical and cosmetic industries.

Keywords Cosmetics · Nanotechnology · Antibacterial · Antiviral · Fungi · Algae · Mushroom · Drug delivery · Cancer therapy · Wound healing

2.1 Introduction

Nanotechnology presents a new trend for the creation and control of nanometer-sized features in materials and devices, leading to the development of new products in the food, cosmetic, personal care, pharmaceutical, medical device, agricultural, and environmental industries (Prasad et al. 2014, 2017b). However, a great emergent issue in nanotechnology is the applicability of the technology for the production of reliable and cost-effective synthesized nanoparticles (NPs) or nanocomposites of less than 100 nm (Thakkar et al. 2010). Naturally, bulk materials have consistent physical properties regardless of their size (Husein and Siddiqi 2014). However, when in the form of nanoscale particles they undergo some changes in their electric, magnetic, optical, and chemical properties (Castro et al. 2014; Chan and Mat Don 2013), owing to changes in the shape and size of the particles (Chan and Mat Don 2013). Silver and gold are among the most studied metals because of their potential use in medical and cosmeceutical products (Schröfel et al. 2014). Although regarded as inert materials, silver nanoparticles (Ag-NPs) function well with antimicrobial compounds by inducing the production of reactive oxygen species, such as hydrogen peroxide, to enhance antimicrobial activity (Deepek et al. 2011). Thus, they can act as antimicrobial agents to reduce infections with pathogens during surgery and to combat the problem of microbial resistance to antibiotics. Recent research has shown that Ag-NPs also exhibit anti-inflammatory, anti-angiogenic, and anti-permeability activities, and thus they have become useful for health-care industries (Zare et al. 2012; Schröfel et al. 2014; Park et al. 2016). Gold nanoparticles (Au-NPs) exhibit potential anticancer and antimicrobial properties, as well usefulness in the treatment of a wide range of skin diseases (Schröfel et al. 2014; Zare et al. 2012; Li et al. 2011; Srivastava and Constanti 2012). Advances in this technology have led to the development of Au-NPs with unique optical, electrical, and photothermal properties with high stability that support their application in health care and medical diagnostics (Shedbalkar et al. 2014; Yadav et al. 2015). Apart from Ag-NPs and Au-NPs, other metal nanoparticles, such as those of platinum, magnetite (Fe_3O_4), Zinc oxide (ZnO), and cadmium salts (Cd salts) have been synthesized using different green technologies (Ding et al. 2015; Mirzaei and Darroudi 2017; Bharde et al. 2006; Velusamy et al. 2016).

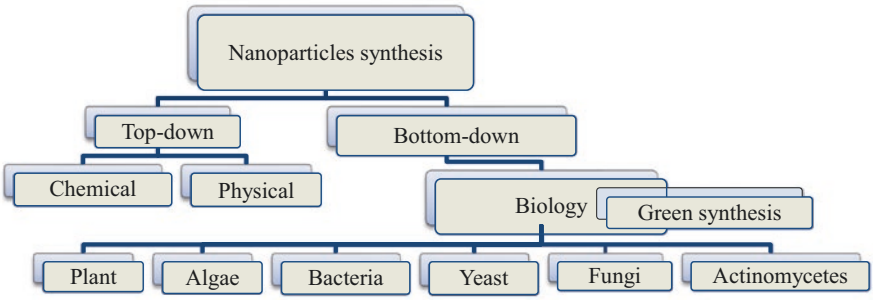

Fig. 2.1 Synthesis of metallic nanoparticles

The production of NPs was previously done by conventional physicochemical methods. However, these methods have several negative impacts, in that they release residues that are highly toxic for humans and cause severe environmental problems for the disposal of this dangerous waste. In addition, these methods involved the use of high tech instruments and high energy consumption, limiting the use of this technology on a commercial scale (Kitching et al. 2014; Pantidos and Horsfall 2014). Recently, novel approaches have been developed for the production of NPs based on using biological systems such as plants and microorganisms; this is known as wet technology. This method has many advantages, such as its low cost, energy-efficiency, and production of nontoxic products, when compared with the physicochemical approach. The most important aspect of this wet technology is that the characteristics of NPs are well defined and their properties can be customized through controlling their particle size, shape, composition, and morphology; this governs their chemical stability and optical properties, such as plasmon resonance (Mock et al. 2002); their antimicrobial activities (Wani and Ahmad 2013); and their catalytic properties (Zhou et al. 2010).

2.2 Green Synthesis of Metal Nanoparticles

Currently, since noble metal nanoparticles are widely applied in areas related to human health, there is a growing need to develop the sustainable production of NPs that avoids the use of harmful organic chemical substances (Shams et al. 2013). The disadvantages of physicochemical NP synthesis are mainly the low production rate, structural particle deformation, and inhibition of particle growth. The diversity and importance of nanotechnology for us has led to the development of versatile methods to synthesize Ag-NPs with well defined and controlled properties. Generally, there are two main approaches to the synthesis of nanomaterials: “top-down” and “bottom-up” (Fig. 2.1). The “bottom-up” approach involves a homogeneous system wherein catalytic activity to synthesize nanoscale materials can be controlled by catalytic agents such as reducing agents and enzymes, with bioprocessing conditions involving stabilizers and appropriate temperatures, exposure times, and pH

with the relevant metal ion (Oza et al. 2012; Iravani 2014; Das et al. 2014). The physicochemical properties, surface structure, and morphological characteristics of the produced NPs depend on the precursor concentrations and reaction conditions (Mason et al. 2012). In contrast, the synthesis of NPs by the "top-down" approach basically works with the material in bulk form, and size reduction to the nanoscale is achieved by specialized physical or chemical, or both, treatments. However, the application of physical treatments such as lithography, thermal decomposition, laser ablation, mechanical milling, etching, and sputtering thermal decomposition of silver compounds; as well as radiation-assisted, electrochemical, sonochemical, and microwave-assisted processes is not preferred because of the high energy consumption involved (Varshney et al. 2009; Abou El-Nour et al. 2010; Elzatahry et al. 2012). Therefore, chemical reduction is the most common synthetic pathway for metal NP synthesis (Pal et al. 2011; El-Faham et al. 2014). In this way, negatively charged Ag-NPs can be obtained from a process using sodium citrate acting as a reducing agent, while positively charged Ag-NPs can be produced from reactions with branched polyethyleneimine, formaldehyde, alkali metals in ammonia, inorganic and organic borohydrides, ascorbic acid, free radicals, monoalcohols, polyols, acetonitrile, hydrazine, citrate, or ethylene diamine tetra acetic acid (Varshney et al. 2009). As reported by Thakkar et al. (2010), the major drawback of chemical and physical synthesis is an imperfect surface structure that can contribute to the physical properties and surface chemistry of the nanoscale materials in terms of the surface area-to-volume ratio.

In general, many of the reducing agents, solvents, and additives used in the reduction process when NPs are synthesized by "top-down" approaches often pose severe environmental and biological risks and the NPs are not suitable for human application. Therefore, it is important to develop ecofriendly non-toxic precipitation processes, especially for the large-scale production of NPs. The physicochemical approaches are also characterized by a low production rate, structural particle deformation, and inhibition of particle growth (Varshney et al. 2009). Considering the rather limited choices of water-soluble precursor compounds in the "bottom-up" approach, most attempts to produce NPs through green synthesis by microorganisms focus on selecting capping and reducing agents which is the metabolites synthesis by the microbes and the cell mass itself. The main reason for this focus is to be able to synthesize homogeneous NPs with uniform shapes and sizes, a feature that contributes to the optical properties of the NPs. This is important for applications in biological and chemical fields, as well as for optical, medical, and electronic devices (Suresh et al. 2010; Gurunathan et al. 2014; Prasad 2014).

2.3 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 capping 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). First, the microorganism chosen for the synthesis of the NPs should be of the generally-regarded-as-safe (GRAS) category according to the United States Food and Drug Administration (FDA) and should belong to Risk group level 1 as defined by the World Health Organization (WHO) (Fernández et al. 2016). Therefore, it is recommended that the synthesis of NPs should be performed with GRAS microorganisms to avoid any toxic metabolites. Thus, research has focused on microbes classified as GRAS, such as lactic acid bacteria, as well as edible mushrooms, for application in the nutraceutical and food industries (Sen et al. 2013a, b; Al-Bahrani et al. 2017; Visha et al. 2015; Markus et al. 2016). The extracellular synthesis of NPs using biomass or cell-free filtrate, owing to its simpler and cheaper downstream processing, seems to be preferable to intracellular synthesis (Abo-State and Partila 2015). The intracellular synthesis of NPs requires additional steps, such as ultrasound treatment or reactions with suitable detergents to optimize the biosynthesis and release of the NPs (Kalimuthu et al. 2008; Das et al. 2014). Castro et al. (2014) reported the extracellular synthesis of Au-NPs of different shapes, including spherical, triangular, hexagonal, decahedral, and pyramidal, by *Botrytis cinerea*. *Aspergillus oryzae* var. *viridis* has been reported to synthesize spherical Au-NPs both extracellularly and intracellularly with particle sizes between 10 and 60 nm (Binupriya et al. 2010; Siddiqi and Husen 2016). However, the greatest advantages of biosynthesis by fungae are that the synthesis of stable NPs is done in a single step and the NP sizes and shapes are controlled; there is also a lack of complex chemicals, and a lack of toxic contaminants (Singh et al. 2016).

The biosynthesis of metal NPs by microbes can be controlled in terms of shape, size, particle dispersity, and stability (Sarangadharan and Nallusamy 2015). The smaller the size of the NPs the better, in terms of conductivity, chemical stability, and catalytic and antibacterial activity. Therefore, the main features of NPs that are good antimicrobial agents are small size and uniform shape. The advantage of the synthesis of small particles with uniform dispersion is that this improves the effective surface area of of the NPs, reducing their stability in solution and their surface

reactivity (Klaine et al. 2008; Wigginton et al. 2010; Sarangadharan and Nallusamy 2015). Several approaches can be used to control NP size during the production process; namely, optimizing the concentration of the metal solution and the pH at the beginning of the process. Some studies have also reported the influence of the microbe growth phase and the role of light during the synthesis of NPs. Sarangadharan and Nallusamy (2015) reported on the production of smaller size Ag-NPs, ranging from 3 to 130 nm, when *Bacillus licheniformis* was exposed to 1 mM $AgNO_3$ solution, and the size of the NPs increased with increased concentrations of $AgNO_3$ solution. The size distribution and dispersal of the NPs contribute to the stability of the Ag-NP surface plasmon band, which avoids aggregation of particles in aqueous solution, was also affected by the concentration of the $AgNO_3$ solution. However, the size and stability of metal NPs also depends on the species and strain of microbe and the biosynthesis capacity of the capping agents used (Ahmad et al. 2003a, b).

2.3.1 Bacteria

The biosynthesis of NPs by microbes provides uniformity in both size and morphology of the NPs when compared with physicochemical methods. It has been reported that, with the extracellular production of Ag-NPs, when Ag+ ions were added to the supernatant the color changed to brown and the color intensity increased with the period of incubation, owing to the reduction of Ag+ to Ag^0 (Kushwaha et al. 2015). Several bacterial strains, such as *Bacillus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Escherichia coli*, *Geobacillus ferrireducens, Klebsiella aeruginosa, Actinomycetes,* and *Aeromonas* sp. were employed successfully for the synthesis of metal NPs. As studied by Zhang et al. (2011a, b), under controlled conditions at 28 °C and ambient pressure, *Pseudomonas alkaliphila* synthesized monoclinic selenium NPs with a well defined shape.

The advantages of using bacteria for the production of NPs, compared with other biological routes, are their ease of handling, short cultivation period, and the ease of high cell density production with an optimized cultivation strategy. Therefore it is important to study the optimum bioprocessing conditions for the biosynthesis of NPs. He et al. (2007) showed the critical effect of the initial pH of the bioprocessing process on Au-NP size and morphology during biosynthesis by *Rhodopseudomonas capsulata*. They also reported that the non-pathogenic *E. coli* MC4100 and *Desulfovibrio desulfuricans* ATCC 29577, isolated from jewelry waste, showed gold bioremediation capacity when added to 2 mM $HAuCl_4$ solution. At acidic pH, single spherical Au-NPs (less than 10-nm diameter) were produced, but for pH values between 7.0 and 9.0, a mixture of smaller (~10 nm) and bigger (~50 nm) well defined triangles, hexagons, and rods was formed (Iravani 2014).

Much research has also shown the capacity of *Bacillus* sp. to produce Au-NPs and Ag-NPs of different sizes and shapes (Gopinath and Velusamy 2013). The study of Das et al. (2014) demonstrated that *Bacillus* sp. isolated from soil heavily contaminated with metal synthesized NPs both intracellularly and extracellularly, with

the size of the Ag-NPs ranging from 42 to 92 nm. Pugazhenthiran et al. (2009) reported the isolation of a silver-resistant airborne *Bacillus sp*. that could synthesize small Ag-NPs of 10–15 nm in size in the periplasmic space of the bacterial cells (i.e., between the outer and inner cell membranes). Other research showed that *Bacillus subtilis* synthesized Au-NPs both intracellularly and extracellularly after 1-day addition of chloroaurate ion, while Ag-NPs were biosynthesized only extracellularly after 7-day exposure to silver ions, with the NPs being smaller than 10 nm (Reddy et al. 2010). The supernatant from *Bacillus licheniformis* may act as both reducing and capping agents in Ag-NP synthesis. This supernatant, which is rich in enzymes, proteins, and amino acids, plays an active role in the reduction of silver ions (Sarangadharan and Nallusamy 2015). The widely accepted mechanism for the synthesis of Ag-NPs is via reduction mediated by the nitrate reductase enzyme (Park et al. 2016; Sawle et al. 2008). Nicotinamide adenine dinucleotide (NADH) and NADH-dependent nitrate reductase enzyme are known as cofactors in the bioreduction of Ag^{+} to Ag^{0} in metal NPs (Sarangadharan and Nallusamy 2015). Fourier transform infrared spectroscopy (FTIR) analysis has been used to determine the functional chemical groups in dried AgNPs samples (Gopinath and Velusamy 2013; Sarangadharan and Nallusamy 2015). FTIR analysis has shown the reducing/capping protein that accounts for both the bioreduction and stabilization of AgNPs by *Bacillus* sp. FTIR spectral analysis of protein in the cell-free supernatant of *Bacillus megaterium* (NCIM 2326) revealed the presence of stretching vibration of Ag-NPs at 3392, 2142, 1642, and 718 in the region of 450–4000 cm^{-1}. The bond stretching were identified as C–H, C=O, C–C and O=C; this arises owing to carbonyl stretch in the amide linkage of the protein, which strongly binds silver to form a coat that covers and stabilizes the Ag-NPs (Saravanan et al. 2011).

2.3.2 Yeasts

Yeasts such as *Candida glabrata* (Velusamy et al. 2016), *Saccharomyces cerevisiae* (Sen et al. 2011), *Cryptococcus laurentii*, and *Rhodotorula glutinis* (Fernández et al. 2016), and *Magnusiomyces ingens* (Zhang et al. 2016a, b) have been reported to produce metallic NPs. The NPs produced by yeasts have good potential for the bulk production of NPs by a simple and ecofriendly method (Yadav et al. 2015). Synthesized Ag^{0} and Au^{0} nanoparticles exhibited excellent catalytic activities for the reduction of nitrophenols (4-NP) (i.e., 4-nitrophenol, 3-nitrophenol, and 2-nitrophenol) to aminophenol (4-AP) in the presence of $NaBH_4$ (Zhang et al. 2016a, b; Fernández et al. 2016); this was related to the particle size and the functional groups on the surfaces of the NPs that were responsible for the catalytic activity (Mishra and Singh 2015). Cell-free supernatant of *Magnusiomyces ingens* LH-F1 showed capacity for the rapid synthesis of Au-NPs within 4–6 h when incubated with Au^{3+}. The produced Au-NPs had various shapes, including spherical, triangular, and hexagonal structures (Zhang et al. 2016a, b). FTIR analyses and sodium dodecyl sulfate-polyacrylamide gel electrophoresis suggested that

protein-containing amide and carboxyl groups could be adsorbed on the surfaces of Au-NPs, and these groups could be involved in the reduction of Au^{3+} and the stabilization of Au-NPs. Similar observations were made in the synthesis of NPs with the cell-free supernatants of *Cryptococcus laurentii* and *Rhodotorula glutinis* (Fernández et al. 2016). The production of Ag-NPs was evidenced by the appearance of a color change from pale yellow to dark brown in the reaction flasks. In this study, the larger nitrate NPs had an important role in catalytic reduction, and the smaller particles generally exhibited higher activities (Zhou et al. 2010).

Zhang et al. (2016a, b) observed that different cell concentrations, as well as initial gold ion concentrations, controlled the sizes and shapes of the synthesized NPs. No synthesis of Au-NPs was observed when the concentration of $HAuCl_4$ at the beginning of the activation was over the threshold limit for the synthesis of NPs (Zhang et al. 2016a, b). On the other hand, with the same accepted concentration of the gold salt for maximum reduction to Au^0 and increasing cell numbers, the particle size was decreased and this contributed to a difference in the average size of the NPs. Cell numbers and gold salt concentrations were also used as critical variables to control NP size (Pimprikar et al. 2009). To conclude, optimization of the processing conditions, such as the incubation period, the ratio of metal ions and cell mass, temperature, pH, and aeration may influence the size, distribution, and shape of NPs, as well as the overall productivity of NP synthesis (Singh et al. 2016).

2.3.3 Algae

The synthesis of pure metallic Ag-NPs and Au-NPs by the reduction of Ag^+ and Au^+ ions was reported using powder or extracts of the seaweed *Sargassum longifolium* (Rajeshkumar et al. 2014), and powder or extracts of *Enteromorpha flexuosa* (Abdel-Raouf et al. 2013), *Padina pavonica* (Isaac and Renitta 2015), *Scenedesmus abundans* (Aziz et al. 2014), *Chlorella pyrenoidusa* (Oza et al. 2012), *Sargassum tenerrimum*, and *Turbinaria conoides* (Ramakrishna et al. 2016). The production of extracellular Au-NPs using *P. pavonica* was achieved within 24-h exposure to the metal ion solution. The resulting NPs were spherical, with size ranging from 30- to 100-nm diameter, and they exhibited high antibacterial activity against *Bacillus subtilis*, but less bactericidal activity when tested against *E. coli* (Isaac and Renitta 2015).

Abdel-Raouf et al. (2013) showed the synthesis of highly stable Au-NPs by *Galaxaura elongata* using the cell powder and ethanolic algal extract. The NPs produced were spherical, along with a few rods and triangular, truncated triangular, and hexagonal-shaped NPs with a size range of 3.85–77.13 nm and very smooth edges. Interestingly, in this study, Abdel-Raouf et al. (2013) observed that chemical constituents of the algal extract, such as andrographolide, alloaromadendrene oxide, glutamic acid, hexadecanoic acid, oleic acid, 11-eicosenoic acid, stearic acid, gallic acid, epigallocatechin, catechin, and epicatechin gallate acted as reducing, stabiliz-

ing, and capping agents. Furthermore, the NPs synthesized by *G. elongata* powder were found to be highly effective as antimicrobial agents against *E. coli* and *Klebsiella pneumoniae*. Recently, the biophysical synthesis of Ag-NPs was successfully achieved by a combination of Calothrix algae with ultrasound irradiation, which process was also efficient for the rapid synthesis of Au-NPs with a truncated conical shape, with sizes in the range of 30–120 nm (Kumar et al. 2016). This synthetic protocol was low cost, ecofriendly, and active for catalytic conversion in reducing 4-NP to 4-AP via a one-step reduction process.

Brown seaweed mediated a single-step process for the synthesis of Ag-NPs when an extract of *Sargassum longifolium* was used (Rajeshkumar et al. 2014). The production of Ag-NPs was improved when the biosynthesis processing conditions, such as incubation time and pH, were further optimized. These authors reported that the increased ultraviolet-visible (UV-vis) absorbance they observed, as a function of increased NP synthesis, was owing to an increase in the pH level. The synthesized Ag-NPs were characterized by scanning electron microscope (SEM), showing that their shape was spherical. FTIR analysis confirmed the presence of biomolecules responsible for the reduction of Ag ions and capping of the particles. The algal-mediated Ag-NPs showed high activity against pathogenic fungi, such as *Aspergillus fumigatus*, *Candida albicans*, and *Fusarium* sp., as well as *S. longifolium* (Rajeshkumar et al. 2014).

2.3.4 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 capping 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).

After the reaction of *Fusarium oxysporum* cell-free supernatant with silver ions following 72-h catalytic activity, the intense yellow color of the aqueous solution showed that the reduction of the metal ions had taken place extracellularly (Ahmad et al. 2003a). The color change was caused by the excitation of surface plasmon vibrations in the NPs. However, the production of metal NPs with a uniform particle size distribution was made possible by using cell-free extract of *Trichoderma asperellum* (Mukherjee et al. 2012). Ahmad et al. (2005) reported that the cell mass of *Trichothecium* sp. synthesized Au-NPs extracellularly under static conditions. Recently, Balakumaran et al. (2015) found that *Guignardia mangiferae*, an endophytic fungus isolated from the leaves of medicinal plants, extracellularly synthesized well dispersed and extremely stable spherical AgNPs, 5–30 nm in size, under optimized reaction conditions within 12 h of the beginning of the process. In this study, the optimum antibacterial activity was achieved when the Ag-NPs were synthesized at pH 7. Other research has shown that the extracellular broth of *A. oryzae* var. viridis could be used for the extra- and intracellular synthesis of spherical Au-NPs with a size range of 10–60 nm (Patra et al. 2015). Castro Longoria et al. (2011) and Zhang et al. (2011a, b) also reported the potential synthesis of Au-NPs using *Neurospora crassa* and *Aureobasidium pullulans.* However, the NPs were produced inside the cells and needed additional purification steps to release the intracellular NPs, unlike the NPs produced by *Penicillium* sp., which could be produced in extra- and intracellular forms (Du et al. 2011). Recently, white rot fungi have been used extensively in the remediation of toxic metal damage, based on their capacity to reduce a wide range of environmentally hazardous compounds to metal ions, by oxidative enzymatic mechanisms. Chan and Mat Don (2013) isolated five species of white rot fungi and studied their potential application in NP biosynthesis. It was interesting that the synthesis of Ag-NPs by *Pycnoporus sanguineus* produced a yield of about 98.9%. The bioreduction of Ag-NPs involved the absorption of metal ions onto the cell surface of *P. sanguineus* by functional groups on the cell wall, and the NPs were indirectly reduced to metal ions by reducing sugars from the polysaccharide hydrolysates of the biomass. In that study, the Ag-NPs produced were spherical, with an average diameter of 52.8–103.3 nm (Vigneshwaran et al. 2006; Chan and Mat Don 2013).

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.

Table 2.1 Synthesis of nanoparticles (NPs) by microorganisms

Microorganisms	Localization	NPs	Shapes	Size (nm)	References
Lower fungi					
Botrytis cinerea	Extracellular	Au	Spherical	1–100	Castro et al. (2014)
			Triangular Hexagonal		
			Decahedral Pyramidal		
Trichoderma reesei		Ag	Spherical	5–50	Vahabi et al. (2011)
Hormoconis resinae		Ag	Cubic	20–80	Varshney et al. (2009)
Fusarium semitectum		Au	Spherical	18–50	Sawle et al. (2008)
		Au-Ag		10–35	
Aspergillus clavatus		Au	Triangular	24.4 ± 11	Verma et al. (2011)
			Spherical		
			Hexagonal		
A. fumigatus		ZnO	Spherical	1.2–6.8	Raliya and Tarafdar (2013)
			Hexagonal		
A. oryzae var. viridis		Au	Spherical	10–60	Binupriya et al. 2010
A. sydowii		Au	Spherical	8.7–15.6	Vala (2015)
A. terreus		Ag	Spherical	1–20	Li et al. (2012)
A. tubingensis		$Ca_3P_2O_8$	Spherical	28.2	Tarafdar et al. (2012)
Aureobasidium pullulans		Au	Spherical	29 ± 6	Zhang et al. (2011a, b)
Cylindrocladium floridanum		Au	Spherical	19.05	Sanghi and Verma (2009)
		Au	Spherical	5–35	
Hormoconis resinae		Au	Spherical	3–20	Mishra et al. (2010)
Penicillium brevicompactum		Au	Spherical	10–60	Mishra et al. (2011)
			Triangular Hexagonal		
P. fellutanum		Ag	Spherical	5–25	Kathiresan et al. (2009)
P. nagiovense		Ag	Spherical	25 ± 2.8	Maliszewska et al. (2014)
Penicillium sp.		Au	Spherical	30–50	Du et al. (2011)
Phanerochaete chrysosporium		Au	Spherical	10–100	Sanghi et al. (2011)
Rhizopus oryzae		Au	Spherical	16–25	Das et al. (2012a, b)
T. koningii		Au	Spherical	10–14	Maliszewska (2013)
T. viride		Ag	Spherical	5–40	Fayaz et al. (2010)
Neurospora crassa	Intracellular	Au	Spherical	3–100	Castro-Longoria et al. (2011)
Hormoconis resinae		Ag	Spherical	20–80	Varshney et al. (2009)

(continued)

Table 2.1 (continued)

Microorganisms	Localization	NPs	Shapes	Size (nm)	References
Fusarium semitectum		Au	Spherical	10–35	Sawle et al. (2008)
		Au-Ag			
Mushrooms					
Pleurotus florida	Aqueous extract	Ag	Face-centered cubic	2.445 ± 1.08	Sen et al. (2013a)
P. florida		Au	Spherical	8	Sen et al. (2013b)
P. ostreatus		Ag	Spherical	10–40	Al-Bahrani et al. (2017)
Agaricus bisporus		Ag	Spherical	5–50	Manzoor-ul-Haq et al. (2015)
Ganoderma sp.		Au	Spherical	20	Gurunathan et al. (2014)
Tricholoma matsutake		Ag	Spherical	10–50	Anthony et al. (2014)
Ganoderma sp.		Ag	Spherical	2	Ekar et al. (2015)
Tricholoma matsutake		Ag	Spherical	20–50	Manzoor-ul-Haq et al. (2015)
Cordyceps militaris		Ag	Spherical	15	Wang et al. (2016)
Schizophyllum commune	Extracellular	Ag	Spherical	54–99	Arun et al. (2014)
	Intracellular				
Flammulina velutipes	Intracellular	Au	Cylindrical tubes	20	Narayanan et al. (2015)
Yeasts					
Candida glabrata	Extracellular	Cd	Hexagonal	20–29 Å	Velusamy et al. (2016)
	Intracellular				
Saccharomyces cerevisiae		Au	Spherical	15–20	Sen et al. (2011)
Cryptococcus laurentii	Extracellular	Ag	Spherical	–	Fernández et al. (2016)
Rhodotorula glutinis		Ag	Spherical	–	Fernández et al. (2016)
Magnusiomyces ingens		Au	Spherical	80	Zhang et al. (2016a)
			Triangular		
			Hexagonal		
Yarrowia lipolytica	Intracellular	Au	Spherical	9–27	Pimprikar et al. (2009)
			Triangular		
			Hexagonal		
Bacteria					
Rhodopseudomonas capsulata	Extracellular	Au	Spherical	10–20	He et al. (2007)
Escherichia coli		Ag	Spherical	20–50	Kushwaha et al. (2015)

(continued)

Table 2.1 (continued)

Microorganisms	Localization	NPs	Shapes	Size (nm)	References
Pseudomonas aeruginosa		Ag	Spherical	50–100	Abo-State and Partila (2015)
Bacillus licheniformis		Ag	Spherical	10–120	Sarangadharan and Nallusamy (2015)
Bacillus strain CS 11		Ag	Spherical	42–92	Das et al. (2014)
Bacillus megaterium		Ag	Spherical	80–99	Saravanan et al. 2011
Bacillus marisflavi		Ag	Spherical	40	Anthony et al. 2014
Actinomycetes	Intracellular	Au	Spherical	18–20	Balagurunathan et al. 2011
Streptomyces virodogens			Rod		
Streptomyces naganishii (MA7)		Ag	Spherical	5–10	Shanmugasundaram et al. 2013
Streptomyces sp.		Ag	Spherical	70	Udaya Prakash et al. 2014
Streptoverticillium sp.		Ag	Spherical	8	Udaya Prakash et al. 2014
Streptomyces griseus	Extracellular	Au	Spherical	50	Derakhshan et al. 2012
Streptomyces glaucus		Ag	Spherical	4–25	Zinicovscaia et al. (2011)
Streptomyces hygroscopicus		Au	Spherical	10–20	Sadhasivam et al. (2012)
Streptomyces albogriseolus		Ag	Spherical	16.25	Samundeeswari et al. (2012)
Streptomyces albidoflavus		Ag	Spherical	10–40	Shetty and Kumar (2012)
Streptomyces rochei		Ag	Spherical	22–85	Abd-Elnaby et al. (2016)
Lactobacillus sp.	Extracellular	Gd_2O3	Spherical	10–20	Jha et al. (2010)
Lactobacillus sp.		Ti	Spherical	40–60	Prasad et al. 2007
Lactobacillus fermentum		Ag	Spherical	13.75	Omidi et al. (2014)
Lactobacillus sp.		Ag	Spherical	2–20	Ranganath et al. (2012)
Lactobacillus casei sub sp. *Casei*		Ag	Spherical	25–100	Korbekandi et al. (2012)
Lactobacillus acidophilus		Ag	Spherical	4–50	Rajesh et al. (2014)
Lactobacillus kimchicus	Intracellular	Au	Spherical	5–30	Markus et al. (2016)
Lactobacillus mindenis		Ag_2O	Spherical	2–20	Dhoondia and Chakraborty (2012)

(continued)

Table 2.1 (continued)

Microorganisms	Localization	NPs	Shapes	Size (nm)	References
Lactobacillus acidophilus	Extracellular	Se	Spherical	15–50	Visha et al. (2015)
	Intracellular				
Algae					
Enteromorpha flexuosa (Wulfen) J. Agardh.	Aqueous extracts	Ag	Circular	2–32	Yousefzadi et al. (2014)
Padina pavonica		Au	Spherical	30–100	Isaac and Renitta (2015)
Chlorella pyrenoidusa		Au	Spherical	25–30	Oza et al. (2012)
Calothrix algae		Au	Truncated conical	30–120	Kumar et al. (2016)
Stoechospermum marginatum		Au	Spherical	40–85	Rajathi et al. (2012)
Sargassum longifolium		Ag	Spherical	40–85	Rajeshkumar et al. (2014)
Turbinaria conoides		Au	Spherical	5–45	Ramakrishna et al. (2016)
Sargassum tenerrimum		Au	Spherical	27–35	Ramakrishna et al. (2016)

2.3.4.1 Edible and Medicinal Mushrooms

The preparation of metal NPs by edible medicinal mushrooms has been widely applied as a clean alternative approach for NP biosynthesis. Manzoor-ul-Haq et al. (2015) reported on the potential use of *Agaricus bisporus*, *Helvella lacunosa*, *Ganoderma applanatum*, *Pleurotus florida*, and *Fomes fomentarius* as biofactories for AgNPs. They showed that, among different types studied, *Agaricus bisporus* was considered as the most potent mushroom for the synthesis of Ag-NPs. Bioactive compounds, such as enzymes, proteins, polysaccharides, and nucleic acids, are extracted from the basidiocarps of mushrooms, which are a potential source of many essential nutrients, as well as therapeutic bioactive compounds that are considered to have immune-modulator, anti-tumor, antidiabetic, and antioxidant properties (Maftoun et al. 2015). Mushroom extracts also contains highly diverse bioactive molecules such as amino acids, fatty acids, vitamins, minerals, and polysaccharides. It is also known that almost 75% of mushroom extracts are rich in proteins that are necessary for the NP biosynthesis process (Anthony et al. 2014). It was also reported that mushroom extracts contain large numbers of volatile organic compounds, such as octanones, octanols, and benzaldehyde, which can act as reducing agents for the reduction of metal ions to the corresponding metal NPs (Philip 2009). The study of Antony et al. (2014) has shown that protein and enzymes extracted from *Tricholoma matsutake* play an important role in the reduction of metal ions by the oxidation of benzaldehyde (aldehyde groups) to carboxylic acids, as FITR analysis detected a band shift of the hydroxyl and carbonyl groups and the loss of existing carbonyls, and the appearance of a new carbonyl peak. The FTIR spectrum of Ag-NPs

synthesized using *T. matsutake* extract showed the broad spectrum of the IR peak at 3400 cm^{-1}, suggesting the binding of silver ions with hydroxyl groups. In addition, detection of the band spectrum at 1640 and 1550 cm^{-1} as the stretching vibrations of the primary and secondary amines, respectively, confirmed the presence of proteins for the synthesis and stabilization of Ag-NPs (Anthony et al. 2014).

Many selected mushroom strains show the capacity to synthesize metal NPs by both extracellular and intracellular synthesis mechanisms. The employment of *Pleurotus* sp. can contribute to the non-toxic production of NPs, since *Pleurotus* sp. is well known as an edible mushroom with GRAS status according to the FDA. Recent research by Al-Bahrani et al. (2017) showed that an aqueous extract of the edible mushroom *P. ostreatus* acted as a reducing and stabilizing agent for the biosynthesis of spherical Ag-NPs and this was an efficient and ecofriendly system for NP synthesis; the size and morphology of NPs, analyzed by transmission electron microscopy (TEM), demonstrated only spherical Ag-NPs, with a size of 10–50 nm. Other research reported that *P. florida* exhibited high capacity for the synthesis of Au-NPs by reducing chloroauric acid ($HAuCl_4$) with a glucan that acts as both a reducing and a stabilizing agent (Sen et al. 2013b). *P. florida* showed size-controlled synthesis with well distributed Au-NPs in a process that depended on the concentration of $HAuCl_4$ in the solution. The resulting Au NP-glucan bioconjugates function as efficient heterogeneous catalysts in the catalytic conversion of reducing 4-NP to 4-AP, in the presence of sodium borohydride, via a one-step reduction process (Sen et al. 2013b).

In addition, the intracellular synthesis of Au-NPs has been reported by using the mushroom *Flammulina velutipes* (Narayanan et al. 2015). The incubation of this mushroom in chloroaurate solution resulted in the synthesis and immobilization of stable Au NPs inside the mushroom mycelia; these AuNPs exhibited heterologous catalytic potential to reduce the organic pollutants methylene blue and 4-NP. Other recent research, by Wang et al. (2016), reported on the high capacity of *Cordyceps militaris* to produce spherical Ag-NPs, using mushroom cell filtration. The resulting NPs were highly crystalline and had a diameter of about 15 nm. The Ag-NPs were relatively stable and exhibited antibacterial activities against clinically pathogenic bacteria (Wang et al. 2016). The synthesis of Ag-NPs of uniform size and typical dispersal by *Ganoderma* sp. was reported by Ekar et al. (2015). The morphology of the spherical particles with an average size of 2 nm was visualized in micrographs of TEM images, showing that *Ganoderma* sp. extract also contains capping and catalytic/reducing agents that have high capacity to biosynthesize highly stable Ag-NPs. NPs of small size are needed, as a small NP is efficient and reliable for improving NP efficiency and biocompatibility (Kim et al. 2008).

2.4 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, biosorption,

ion-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).

2.4.1 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 of Ag-NPs and Au-NPs, respectively (Ahmad et al. 2003a, b; Kumar et al. 2007). Additionally, hydrogenases (Gilbert et al. 2003), flavin adenine dinucleotide (FAD)-dependent glutathione reductase (Scott et al. 2008), and nitrate reductases (Vahabi et al. 2011) have been found to participate in the fungal biosynthesis of metal NPs. However, reports have confirmed that the reductase enzyme system requires an electron shuttle for metal reduction (Durán et al. 2011). Quinones (anthraquinones and naphthopquinones) and their quinine derivatives have been found to participate in the reduction process. Moreover, metalloproteins, i.e., phytochelatin and metallothionein, were found to be overexpressed when fungal cells were subjected to heavy metal ion toxicity, and these metalloproteins helped in the reduction process by complexing the metal ions through their reducing and binding properties (Cobbett and Goldsbrough 2002; Park et al. 2016).

The extracellular fungal biosynthesis of NPs can be mediated through protein molecules embedded in mycelial membranes. It has been proposed that surface-bound proteins present in the mycelial cells of *R. oryzae* and *Coriolus versicolor* bind with gold and silver ions, leading to the reduction and stabilization, respectively, of Au-NPs and Ag-NPs (Das et al. 2012a, b; Sanghi and Verma 2009). The formation of these bonds (i.e., protein-metal bonds) has been attributed to the electrostatic reactions between protein-free amine or cysteine residues and enzyme carboxylate groups in the fungal cells. Accordingly, a redox state is created, leading to the precipitation and formation of metal NPs (Park et al. 2016).

A wide range of extracellular fungal products have also been found to contribute to the biosynthesis of metal NPs, in which these NPs were produced to overcome

the toxic effects of the metal ions, and these ions were precipitated in the form of NPs. *Curvularia lunata* was reported to produce extracellular mucilage materials in response to Cu(II), Pb(II), and Zn(II) toxicity (Paraszkiewicz and Dlugonski 2009). Further, Ni(II) and Cd(II) toxicity was found to trigger pullulan production by *Aureobasidium pullulans*, and glomalin glycoprotein was reported to sequester Cu(II) in cultures of *Glomus* (Cornejo et al. 2008).

2.4.2 *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 (Breierová 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). Oxygen can be reduced intracellularly upon cell exposure to heavy metals, leading to the formation of reactive oxygen species (ROS), e.g., hydrogen peroxide, which can then produce highly active and toxic free hydrogen radicals. The fungal cellular machinery uses different enzyme systems, such as antioxidative systems, to stop these reactions (Morano et al. 2012). Fungal antioxidative systems include catalases and superoxide dismutases (Culotta et al. 2006), methionine sulfoxide reductase (Le et al. 2009), thioredoxins (Trotter and Grant 2005), peroxiredoxins (Park et al. 2000), glutathione (Grant et al. 1996), and glutathione peroxidases and transferases (Michiels et al. 1994; Sheehan et al. 2001).

Fungal cells use different membrane channels and transporter proteins to transport required substrates (C- and N-sources), micronutrients, and ions inside the cells. However, toxic metals can take advantage of these channel systems and enter the cytoplasmic space. Primarily, cells can block or even eliminate such transport systems to avoid the entrance of the toxic metal ions into the cytoplasm (Támas et al. 2005). However, this can affect the cellular machinery, and some metal ions will enter the cells by using multiple transport systems. In such cases, the cellular enzymes will react with the metal ions and precipitate nanomaterials.

With changing growth conditions, the intracellular fungal biosynthesis of metal NPs is mediated by the same enzymes and proteins as those responsible for the extracellular biosynthesis of NPs. *Trichothecium* sp. was found to produce extracellular NPs when cells were grown in static cultures. On the other hand, on growth in submerged culture, the biosynthesis was switched to intracellular mechanisms. This switch was obtained by applying different intracellular mechanisms to biosynthesize metal NPs (Ahmad et al. 2005). This change in biosynthesis was attributed to the finding that static cultures promote fungal cells to excrete their enzymes and proteins extracellularly, while these enzymes were not released from the cells in a submerged culture system (Mohanpuria et al. 2008).

2.5 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.

Table 2.2 Different techniques used to characterize biosynthesized nanoparticles

Category	Techniques	Properties determined
Microscopy	Optical microscope, confocal microscope, scanning electron microscope (SEM), transmission electron microscope (TEM), atomic force microscope (AFM), scanning near-field optical microscope (SNOM)	Morphology, structure, size, composition
Spectroscopy	UV-vis-IR, Fourier transform infra-red (FTIR), atomic absorption, electron spin (paramagnetic) resonance (ER or EPR), nuclear magnetic resonance (NMR), Raman spectroscopy, electron spectroscopy for chemical analysis (ESCA), luminescence techniques, X-ray photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES)	Chemical analysis (bonding, charge transfer), energy gaps, level of impurities, band formation, transition probabilities
Diffraction	X-ray diffraction, electron diffraction, neutron diffraction, small angle X-ray scattering (SAXS), small angle neutron scattering (SANS), dynamic light scattering (DLS)	Particle shape, average particle size, structure
Magnetic properties	Magneto resistivity, superconducting quantum interference device (SQUID), vibrating sample magnetometer (VSM)	Behavior of synthesized material in external magnetic fields
Mechanical properties	Hardness, elasticity	Material properties

2.5.1 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) (Durán et al. 2005). The presence of nanomaterials within fungal mycelia has been confirmed using SEM combined with energy diffraction analysis (Vigneshwaran et al. 2007).

2.5.2 Spectroscopic Techniques

2.5.2.1 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 (Durán 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.

2.5.2.2 Fluorescence and FTIR

Fluorescence and FTIR are generally employed to evaluate the binding properties of the cellular proteins with the biosynthesized NPs (Durán 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.

2.5.2.3 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).

2.5.2.4 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).

2.6 Advantages of Fungal Biosynthesis Compared with Bacterial Biosynthesis

Generally, in terms of procedures, capacities, and costs, biological production processes for metal NPs are more efficient than chemical processes (Durán et al. 2011). Also, chemical processes require toxic solvents and further treatment steps (Dorcheh and Vahabi 2016). Moreover, biologically synthesized NPs have great potential owing to their unique optical, chemical, and electronic characteristics (Mohanpuria et al. 2008).

Fungi have potential characteristics that favor this group of microbes over bacteria and plants in the biosynthesis of NPs. Fungi are more developed than bacteria in terms of cellular organization and metabolic activities (Jha and Prasad 2016), and, compared with bacteria, fungi have fewer cultivation requirements, higher growth rates, and higher maximal yields in terms of the initial raw material ratio (Castro-Longoria et al. 2011). Fungi mostly produce extracellular NPs; therefore, the recovery of the synthesized NPs is much easier and cheaper than the recovery of the NPs synthesized from bacteria (Bäuerlein 2000). Additionally, the waste from the pro-

duction medium and the fungal biomass can be degraded biologically and can serve as organic fertilizers (Mansoori 2010). Concerning metal NPs, fungi have been used to produce NPs that have well defined dimensions and a very good degree of monodispersion (Mukherjee et al. 2001b).

In the large-scale production of metal NPs, fungi are characterized by their fast growth rates, easy downstream processes, easy biomass handling, and their production of large amounts of the enzymes and extracellular proteins required for NP biosynthesis (Vahabi and Dorcheh 2014; Narayanan and Sakthivel 2010; Prasad et al. 2016, Prasad 2016, 2017). On the other hand, although there is much research on the use of safe edible mushrooms, most of the studied fungi are human or plant pathogens, which renders them unsuitable for use in large-scale processes (Vahabi and Dorcheh 2014).

2.7 Applications of Metal Nanoparticles

All biogenic NPs synthesized by edible mushrooms and lower fungi are of special interest, based on their high biocompatibility (Kitching et al. 2015; Gurunathan et al. 2014). Nowadays, researchers involved in this field explore the applications of metal NPs in targeted drug delivery, such as in the delivery of proteins, peptides, DNA, and plasmids; metal NPs are also being investigated for alternative cancer treatments and for gene therapy; for use as biosensors; and for their antibacterial and antifungal activity (Siddiqi and Husen 2016; Wanigasekara and Witharana 2016; Prasad et al. 2016). Therefore, metal NPs synthesized by fungi are finding many applications in the medical and cosmeceutical industries.

2.7.1 Applications of Metal Nanoparticles in Medical Fields

2.7.1.1 Drug Delivery

Over the past decade, NPs have been explored and identified as carriers for drug delivery (Gref et al. 1994; Prasad et al. 2017a). New drug delivery systems based on nanotechnology have been applied in the treatment of human diseases, such as cancer, diabetes, microbial infections, and in gene therapy (Surendiran et al. 2009). The benefits of these treatments are that the drug is targeted to diseased cells, and its safety profile is enhanced by the reduced toxic side effects to normal cells (Wanigasekara and Witharana 2016). In general, NPs can be conjugated with different types of drugs to deliver bioactive compounds to the target site by various methods, such as the use of nanotubes, liposomes, quantum dots, nanopores, and dendrimers (Surendiran et al. 2009). For example, because of their safety in terms of toxicity and immunocompatibility, Au-NPs are suitable for the preparation of drug delivery scaffolds. Nanomaterials synthesized by a biological approach can be employed as alternative drugs for the treatment of diabetes mellitus. Au-NPs showed

good therapeutic effects by reducing the levels of liver enzymes such as alanine transaminase and alkaline phosphatase, and reducing uric acid in a diabetic mouse model (Daisy and Saipriya 2012). Au-NPs synthesized by *Trichoderma viride* with vancomycin were bound to the microbial surface by ionic interaction, and effectively suppressed the growth of vancomycin-resistant *Staphylococcus aureus* at a low concentration, of 8 μg/mL. The cell death of *S. aureus* was proven by TEM analysis, showing that the vancomycin-bound Au-NP had penetrated the bacterial membrane (Fayaz et al. 2011). In another study, Sun et al. (2012) loaded doxorubicin into bacterial magnetosomes by using covalent attachment, and these magnetosomes supressed tumor growth by 86.8%. Brown et al. (2010) reported that Au-NPs functionalized with a thiolated polyethylene glycol monolayer capped with a carboxylate group successfully enhanced the delivery of the anticancer drug oxaliplatin.

2.7.1.2 Cancer Therapy

Cancer is the leading cause of death worldwide. Chemotherapy has led to good results, but in many cases cells developed resistance to the chemotherapy agents. Therefore, scientists have made many attempts to develop methods that are biocompatible and cost-effective for the treatment of cancer patients and that reduce the side effects of the chemicals used. Studies have shown that biogenic Ag-NPs can induce apoptotic pathways in vitro through the generation of free oxygen radicals (Ortega et al. 2015). Accordingly, increased interest has been shown in regard to Ag-NPs for the diagnosis and treatment of human cancer (Zhang et al. 2016a, b; Ortega et al. 2015), and thus these molecules are considered as potential antitumor and anti-proliferative agents; they are also considered to be antiangiogenic. Biosynthesized Ag-NPs produced by the yeast *Cryptococcus laurentii* (BNM 0525) showed significant antitumor activity in the breast cancer cell lines MCF7 and T47D (Ortega et al. 2015). The cyototoxicity of Ag-NPs against breast cancer cells was investigated by Gurunathan and co-workers (2013), who obtained the Ag-NPs from an extract of *G. Neo-japonicum* mycelia. Their study revealed that after 24-h exposure to solutions of Ag-NPs at concentrations of 1 to 10 μg/mL, breast cancer cell growth was inhibited and membrane leakage was induced. Arun et al. (2014) investigated the anticancer activity of Ag-NPs produced in shaken broth cultures; an MTT cytotoxicity assay showed cell deaths of 27.2% to 64% in human laryngeal carcinoma cells (HEP-2) at Ag-NP concentrations of 10 to 100 μg/mL.

2.7.1.3 Wound Healing

Robert Burrell was the first person to develop a commercial nano silver particle product to be used clinically; it was used in the treatment of various wounds, such as burns, ulcers, and epidermal necrolysis (Chaloupka et al. 2010). This approach to wound healing treatment was also taken by Huang et al. (2007), who used a wound

dressing loaded with NPs that reduced healing time and inhibited bacterial growth to a greater extent than standard silver sulfadiazine, without harmful effects on the treated patients. Sundaramoorthi et al. (2009) found that Ag-NPs synthesized using *Aspergillus niger* were promising agents for wound healing, acting against pathogenic bacteria, and modulating the cytokines involved in wound healing. In an in-vivo study of Ag-NPs produced by *Fusarium oxysporum*, this biogenic silver formulation, together with enoxaparin, enhanced wound healing without adverse effects (Marcato et al. 2015). The benefits gained were reduced time required for the differentiation of fibroblasts into hyperactive cells (myofibroblasts) involved in the generation of contraction forces in the wound, and a shorter time for the inflammatory process compared with that seen with standard wound dressings (Marcato et al. 2015)

2.7.1.4 Antibacterial Activity

In recent years, with epidemics and the increasing resistance of microorganisms to many generally used antibiotics, NPs have been considered as potential alternatives to commonly used dosage forms. Ag-NPs synthesized from the fungus *Aspergillus niger*showed good inhibitory activity against Gram-positive bacteria such as *S. aureus* and Gram-negative bacteria such as *E. coli.* Sudhakar et al. (2014) produced Ag-NPs by using the edible mushroom, *Agaricus bisporus*, as a bioreductant. The produced nanometal particle exhibited antimicrobial activity against human pathogens such as *E. coli*, *Proteus vulgaris*, and *Klebsiella* spp. Durán and co-workers (2007) reported that extracellular Ag-NPs secreted by *Fusarium* sp. killed *S. aureus* and thus were of use in the treatment of textile fabrics. Fayaz et al. (2009) reported that Ag-NPs synthesized by *T. viride* had potential to inhibit *E. coli* growth, with a minimal inhibitory concentration (MIC) of 30 μg/mL. *Aspergillus clavatus* has been used as a biofactory for the production of Ag-NPs, with antimicrobial activity shown against *C. albicans*, *P. fluorescens*, and *E. coli* (Verma et al. 2010). Ottoni et al. (2017) reported that, among 20 fungal strains screened, two types of each of *Rhizopus* sp., *Trichoderma* sp., and *Aspergillus* sp. could be used as potential Ag-NP biofactories. The produced Ag-NPs acted as antibacterial agents, with the capacity to inhibit the growth of microbes such as *E. coli*, *S. aureus*, and *P. aeruginosa*. In another study, Govindappa et al. (2016) reported that the Ag-NPs produced by *Penicillium* strongly inhibited the growth of *E. coli* and *P. aeruginosa*, confirmed by the use of SEM. This study also found that the Ag-NPs showed strong antioxidant, anti-inflammatory, and anti-lipoxygenase activity, as well as tyrokinase inhibitory activity, when applied at high concentrations. Another study, by Singh et al. (2017), reported the development of a cheap, rapid, one-step technique for Ag-NP synthesis using endophytic fungal supernatant from *Alternaria* sp. and *Raphanus sativus*. Transmission electron microscope and atomic force microscope results established that the synthesized Ag-NPs were of particle size between 4 and 30 nm, and XRD, EDS, and SAED (selected area diffraction) analysis confirmed the crystalline nature and composition of the synthesized Ag-NPs. These Ag-NPs showed antibacterial activity against *E. coli*, *Bacillus subtilis*, *S. aureus*, and *Serratia marcescens* (Singh et al. 2017).

2.7.1.5 Antifungal Activity

In medicine, scientists have made many efforts to develop antimicrobial agents through the discovery of new bioactive agents and new formulations of products that can be used for the clinical treatment of diseases caused by pathogenic bacteria and fungi. Consequently, Ag-NPs have been shown to have great potential as antimicrobial agents, and they are of proven use in the formulation of clinical products for preventing secondary hospital infections (Rodrigues et al. 2013; Duran et al. 2010; Gade et al. 2008; Aziz et al. 2016). It has been shown that *Aspergillus tubingensis* and *Bionectria ochroleuca* synthesized Ag-NPs that had antifungal activity and these Ag-NPs could be used in hospital infections caused by *Candida* sp., when applied at concentrations of 0.11–1.75 μg/mL (Rodrigues et al. 2013); this study also reported that *A. tubingensis* synthesized Ag-NPs extracellularly, with a high yield. Another study, by Ishida et al. (2013), reported a green chemistry approach (integrated microbial and nanotechnology) to obtain NPs produced by *Fusarium oxysporum.* The produced particles showed high antifungal activity and inhibited the growth of human fungal pathogens such as *Candida* spp. and *Cryptococcus neoformans*. These NPs showed ability to damage the cell walls and cytoplasmic membranes of fungal cells (Ishida et al. 2013). Further, Ag-NPs produced by *Schizophyllum commune* exhibited excellent antifungal activity against dermatophytic fungal pathogens such as *Trichophyton simii*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum* (Arun et al. 2014). Xue et al. (2016) studied the antifungal activity of Ag-NPs produced by *Arthroderma fulvum* against ten fungal pathogens, involving *Candida* spp., *Aspergillus* spp., and *Fusarium* spp. The results clearly demonstrated that the Ag-NPs exhibited significant antifungal activities against all tested pathogenic fungi when applied at concentrations ranging from 0.125 to 4.00 μg/mL.

2.7.1.6 Antiviral Activity

As well as the effects exhibited by nano metals as antifungal and antibacterial agents, they also exhibit antiviral properties. It has been reported that Ag-NPs synthesized by *Aspergillus fumigatus* showed antiviral activity against HIV-1. Other research, done by Narasimha et al. (2012), reported the antiviral properties of Ag-NPs synthesized from *Aspergillus* sp., noting that at concentrations of 30 to 180 ppm, the Ag-NPs reduced the number of viral plaques, whereas at higher concentrations, of 210 to 240 ppm, the Ag-NPs showed total inhibition of the viral particles in the bacterial host, leading to complete inactivation of viral replication. Narasimha et al. (2011) and Elechiguerra et al. (2005) reported that Ag-NPs of 1–10 nm in size could bind to HIV-1 and prevent viral attachment to the host cell surface.

2.7.2 *Applications of Metal Nanoparticles in Cosmetics*

Cosmetics have been defined by United States Federal Food, Drug, and Cosmetic Act as "articles intended to be applied to human body by being rubbed, poured, sprinkled, or sprayed for cleansing, beautifying, promoting attractiveness, or altering the appearance" (U.S. Food and Drug Administration 2016). The term 'cosmeceutical' is used to descibe a cosmetic product for which there are specific therapeutic claims. Cosmeceutical products have shown strong growth on the global market in recent years, with up to approximately $US42.4 billion forecast for 2018, up from $US31.84 billion in 2016 (GBI Research; RNCOS E-Sevices 2016). In cosmeceutical technologies, nanotechnology has been the most effective approach in the cosmetic arena by introducing smaller particles (<100 nm) that can penetrate the skin and be easily absorbed, reaching the targeted tissue easily (Lohani et al. 2014). Thus, nanoparticles are commonly used in the cosmetics industry, being employed in different products and formulations.

Nanotechnology is now widely used in cosmetics and dermatological products, such as soaps, anti-wrinkle creams, perfumes, toothpastes, lipsticks, moisturizers, sunscreens, hair care products, skin cleansers, and nail care products (Lohani et al. 2014). According to Lohani et al. (2014), NPs are generally classified into eight product classes in terms of their size and functionality; these are liposomes, nanocapsules, solid lipid nanoparticles, nanocrystals, dendrimers, cubosomes, niosomes, and nanogold and nanosilver. Recently, considerable attention has focused on ecofriendly new technologies for the production of metal nanoparticles such as gold, silver, and platinum (Rai et al. 2010). The technology is called ecofriendly because the agents used, such as bacteria, fungi, yeasts, and plants, are the biofactories for the NPs (Rai et al. 2008).

2.7.2.1 Silver Nanoparticles as Preservatives in Cosmetics

In the formulation and production of cosmetics, preservatives are essential components to prevent primary microbial contamination, and they are also important to prevent secondary microbial contamination after manufacture, when the consumer opens and closes the containers during daily use (Kokura et al. 2010). Phenoxyethanol and parabens are commonly used in cosmetics; however, these antibacterial compounds not only temporarily irritate the skin but they can also increase skin sensitivity to UV light (Handa et al. 2006; Ishiwatari et al. 2007). Therefore, for many years researchers have been looking to replace these chemicals with safe alternatives. Ag-NPs are now commonly used as preservatives, based on their antimicrobial properties (Gajbhiye and Sakharwade 2016). These NPs are extensively used in cosmetics such as deodorants, face packs, and anti-aging creams. (Lohani et al. 2014). Gajbhiye and Sakharwade (2016) also reported that, owing to the antibacterial activity of Ag-NPs, these agents are now incorporated as preservatives in toothpastes and shampoos.

Penicillium is an endophytic fungal genus that is used to synthesize Ag-NPs. Phytochemicals identified in *Penicillium* extracts include tannins, saponins, terpenoids, and flavonoids. These substances can act as reducing and capping agents in the conversion of silver particles into NPs (Govindappa et al. 2016). It has also been reported that capping agents, such as the amide and carbonyl groups detected in Ag-NPs of 10–60 nm biosynthesized from *Fusarium semitectum*, are stable for 6–8 weeks. Capping agents are important to avoid the agglomeration of NPs and they can also give stability to the product (Rai et al. 2009). These stable properties can contribute to the appearance of cosmetic products and they can also improve the sensory properties of the product, because they support the product's homogeneous appearance and prevent sedimentation of the product for more than 1 year (Kokura et al. 2010). In cosmetic products, nano zinc oxide and titanium dioxide can give better feel and spreadability to cosmetic formulations. Other than that, they can also exhibit better sun protection than their non-nano equivalents. Similarly, nano silver can increase the antimicrobial properties of the molecule compared with the application of silver in the original state (Raj et al. 2012).

2.7.2.2 Antimicrobials in Cosmetics

Penicillium spp. silver NPs (PAg-NPs) have strong antibacterial properties with a high capacity to inhibit *E. coli* and *P. aeruginosa* growth at 100-µl culture filtrate/1 ml pathogen broth (Govindappa et al. 2016). Other studies of Ag-NPs have reported that these NPs also showed potential antimicrobial effects against *E. coli, B. subtilis, V. cholera, P. aeruginosa,* and *S. aureus* (Cho et al. 2005; Morones et al. 2005). When applied to fungal cells, Ag-NPs can disturb the fungal envelope structure and lead to significant damage to fungal cells, including many strains of Candida spp., such as *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis*, and *C. krusei* (Kim et al. 2008; Mehnert and Mader 2001).

Antimicrobial activity was also reported by Kokura et al. (2010), where Ag-NPs, at the low concentration of 1.0 ppm, exhibited antimicrobial properties in mixed bacterial (*E. coli, P. aeruginosa, S. aureus*) and fungal (*C. albicans, A. niger, P. citrium, A. pullulans*) extracts present in filtered kitchen and drainage wastewater . Ttitanium oxide (TiO_2) NPs have been used in sunscreen cosmetics (Rai et al. 2010), as well as in whitening creams, morning and night creams, and skin milks (AzoNano 2013). It was reported that TiO_2 NPs produced by *Aspergillus flavus* inhibited *E. coli* growth when applied at a concentration of 40 µg ml^{-1} (Rajakumar et al. 2012).

2.7.2.3 Antioxidants and Anti-inflammatory Agents in Cosmetics

Recently, nanoparticles have been included in many of the cosmetic products due to their advantages. They improve sensory properties and stability of products, give better feeling, and enhance better sun protection (Gajbhiye and Sakharwade 2016). PAg-NPs exhibited strong antioxidant activity, proven by DDPH

(1,1-diphenyl-2-picrylhydrazyl) and FRAP (Ferric Reducing Ability of Plasma) tests. They also showed anti-inflammatory activities, and therefore, they have been considered as additives in cosmetic products in order to benefit from their expected functions. Furthermore, the Ag-NPs proved to significantly increase wound healing properties of some cosmetic products. The anti-inflammatory effect of PAg-NPs is exhibited by their role in increasing membrane stabilization. There are only few reports available regarding the anti-inflammatory activities of endophytic fungal Ag-NPs (Joel and Bhimba 2012; Ruma et al. 2013; Pretsch et al. 2014; Naz et al. 2014). However, the main concern was the risk of NPs toxicity owing to their nano-size and expected penetration through the skin. Recently, it has been reported that about 0.002-0.02 ppm of AgNPs could penetrate the skin, which did not show any toxicity at these levels (Gajbhiye and Sakharwade 2016). It has been claimed that at these levels, AgNPs are flushed away from the blood stream, showing no toxicity.

2.7.3 Other Applications

Metal nanoparticles synthesized by fungi have great potential to be used as sensors for optical and electronic devices. Fayaz et al. (2010) found that *Trichoderma viride* synthesized Ag-NPs that were successfully used in biosensor and bio-imaging applications. These Ag-NPs were used for blue orange light emission at wavelengths of 320–520 nm and full characterizations were carried out by EDX (Energy Dispersive X-ray) and XRD analyses. Zheng et al. (2010) described the synthesis of Au-Ag alloy nanoparticles by yeast; the application of these Ag-NPs as a novel vanillin sensor showed that they were five times more sensitive than other methods. This study revealed the high potential of Ag-NPs as sensors in the quantitative determination of vanillin production from the vanilla bean and vanilla tea. Thibault et al. (2008) showed that Au-NPs enhanced the enzyme activity of glucose oxidase (GOx) as an indicator for the determination of glucose content in commercial glucose injections. The action of this Au-NP-GOx-based biosensor is based on the highly sensitive detection exhibited by Au-NPs (Thibault et al. 2008).

2.8 Potential Hazards of Metal Nanoparticles

Generally, all NPs are potentially hazardous to humans because of their small size and the nature of the particules. Nanoparticles have the potential to cause a wide range of respiratory, gastrointestinal, and cardiovascular system pathologies. Nanoparticles can enter the central nervous system by two routes: first, through axons of the olfactory pathway and, second, through the systemic circulation. Ag-NPs can cause in-vivo cytotoxicity to human peripheral blood mononuclear cells (Shin et al. 2007) and human alveolar epithelial cells (Soto et al. 2007) and they have also shown cytotoxicity in a macrophage cell line (Hussain et al. 2006).

In regard to in-vivo toxicity, Kim et al. (2008) reported that Ag-NPs have the potential to accumulate inside human organs such as the liver, lungs, kidneys, stomach, testes, and brain. When human mesenchymal stem cells were exposed to Ag-NPs at a concentration of 10 µg/ml for 1, 3 and 24 h, cytotoxic and genotoxic effects were shown in these cells (Hackenberg et al. 2011). The biological effects included DNA damage, functional impairment, and cell death. Lee et al. (2007) reported that Ag-NPs at a size less than 12 nm disturbed the early development of fish embryos, damaged DNA, caused chromosomal abnormalities, and induced proliferation in zebrafish cell lines. Coradeghini et al. (2013) showed that Au-NPs penetrated the blood brain barrier and accumulated in neural tissue fibroblasts, these authors also showed that Au-NPs of 5 nm in size exhibited cytotoxic effects in Balb/3T3 mouse fibroblasts (Coradeghini et al. 2013). Zinc oxide NPs are widely used in cosmetics and in antimicrobial coatings for food containers; humans are exposed to these NPs daily through dermal, inhalation, and oral routes. Exposure to zinc oxide NPs after the oral consumption of up to 300 mg/kg for 14 days caused liver cellular injury, apoptosis, and DNA damage in a rat model (Sharma et al. 2012). Therefore, safety assessments should be considered before products containing NPs are submitted for approval

2.9 Global Market for Nanoparticle Products

Nanoparticles generally consist of nanometer-scale materials, in the range of 1–100 nm, which can be produced through biosynthesis or physicochemical processes. More than 1000 nanoparticle technology-based products are now available on the market. Some of the leading manufacturers who market products involving nanoparticle technology include Nanophase Technologies Corporation, Romeoville, IL, USA, Altair Nanotechnologies, Reno, NV, USA, Unidym, Sunnyvale, CA, USA, Nanosys, Milpitas, CA, USA, PEN, Miami, FL, USA, Advanced Diamond Technologies, Romeoville, IL, USA, and Bruker Nano GmbH, Karlsruhe, Germany. Nanotechnology plays a significant role in various industries, such as the healthcare, biomedical diagnostics, food and beverage, textile, and agriculture industries. A BBC Research Report noted that the global market for nanotechnology products was valued at $US22.9 billion in 2013 and had increased to about $US26 billion in 2014. This market is expected to reach about $US64.2 billion by 2019, a compound annual growth rate (CAGR) of 19.8% from 2014 to 2019. According to a new study by Grand View Research 2015, the global market trend for Ag-NPs is expected to reach $US2.54 billion by 2022, and the market trend for Au-NPs was more than $US1.30 billion for 2014, with a CAGR of over 25% forecast by 2022. In 2014, the largest application of NP technologies was in the health-care industry, accounting for more than 30% of the Ag-NP global market. As reported by Grand View Research, the global NP technology market is dominated by North America and the European region, with their increasing demand for the technology and its products, in line with the fast growth of research and development in those countries. The

United States is the world leader in the NP market and in research innovation. Recently there has been an increase in research and development spending in biotechnology industries by Asian companies, particularly in India and China, and this is expected to strengthen the growth of the global NP technology market in Asia. Asian manufacturers are expected to increase their research and development expenses in order to gain a competitive edge in the global NP technology market over the coming few years. More research and development spending by companies is expected to increase the development of new NP production approaches using safe microorganisms. In addition, more research will underpin the development of novel NP-based products, especially in the medical and cosmeceutical industries.

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Chapter 3
Fungal Nanoparticles: A Novel Tool for a Green Biotechnology?

Shadia M. Abdel-Aziz, Ram Prasad, Ahmed A. Hamed, and Mohamed Abdelraof

Abstract Bio-nanotechnology is regarded as one of the key technologies of the twenty-first century. In bio-nanotechnology, green methods or green chemistry is employed with the biological systems to fabricate nanostructures. Microorganisms have a promising role in biosynthesis of nanoparticles, especially fungi that secrete enzymes and proteins as reducing agents which can be used for synthesis of metal nanoparticles from metal salts with great potential. In recent years, various approaches have been made to maximize the yield of nanoparticles of varying shape, size, and stability. Increased surface and shape of nanoparticles are responsible for their different chemical, optical, mechanical, and magnetic properties. Use of bio-nanotechnology for synthesis of nanoparticles is a rapidly developing and emerging field. However, nanoparticle biocompatibility must be tested to access their safety before use in different fields. Prior to the clinical use, in vivo evaluation of nanoparticles should demonstrate a high degree of biocompatibility, with minimal negative effects on cell viability, immune function, and blood components. Safety of using nanoparticles in food industry, medicine, pharmaceutical, and agriculture fields should be evaluated to assure human health. The extremely small size of nanomaterials makes them more readily taken up by living tissue and possibly dangerous to humans.

Keywords Nanocomposites · Food industry · Nano-emulsion · Cytotoxicity · Antimicrobial · Nano-coating

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3.1 Introduction

Nanotechnology is a rapidly growing field of science that has become an integral part of biotechnology and regarded as one of the key technologies of the twenty-first century. Nanotechnology has great potential in biological and medical applications such as gene and drug delivery, bio-sensing, diagnostics, and tissue engineering (Prasad 2014; Prasad et al. 2014, 2017). The field of nanotechnology has received much attention in many fields, and application of microorganisms in the green synthesis of inorganic nanoparticles has been extensively studied (Prasad et al. 2016; Shakeel et al. 2016; Khalid et al. 2017). Innovative biosynthesized nanoparticles are used in different areas such as medical applications (e.g., diagnostics and tissue engineering), pharmaceutical fields, feed (e.g., vitamins), packaging, agro-food production (i.e., processing of pesticides, fertilizers, food additives), cosmetics (Raj et al. 2012), textiles (Yetisen et al. 2016), and nano-bioremediation (Yadav et al. 2017). The vast majority of manufactured nanomaterials are available in different shapes and sizes. It is expected that their use will significantly increase in the next decade. Currently, nanoparticles are produced in the hundreds of thousands of tons and used in a variety of products, including electronics, automobiles, aerospace, sporting goods, household, and hygiene (Pulit-Prociak and Banach 2016). Production of nanoparticles and nanomaterials may be carried out by three types of methods: (1) chemical (e.g., chemical vapor deposition, chemical reduction), (2) physical (e.g., physical vapor deposition, production of thin films), and (3) biological (production of nanoparticles by microorganisms) (Pulit-Prociak and Banach 2016; Prasad et al. 2016). The physicochemical methods for production of metallic nanoparticles, in general, and of gold nanoparticles, in particular, rely either on a top-down or bottom-up approach (Rudramurthy et al. 2016).

Biosynthesis of nanoparticles is extensively produced from bacteria (Khandel and Kumar 2016; Chaudhari et al. 2016), fungi (Prasad et al. 2016; Karimi and Khabat 2016; Khwaja and Azamal 2016; Guilger et al. 2017; Mohana and Sumathi 2017; Ottoni et al. 2017), yeast (Pantidos and Louise 2014, Amin et al. 2015; Boroumand Moghaddam et al. 2015), algae (Derek et al. 2017), and plants as well (Prasad 2014; Pantidos and Louise 2014; Mohanta et al. 2017; Protima and Rauwel 2017). It is recognized that microorganisms grow fast, secrete extracellular reducing enzymes, and synthesize inorganic nanoparticles such as gold, silver, calcium, silicon, iron, and lead. Among the many possible bio-resources, fungi represent excellent nanoparticles and produce nanoparticles faster than some chemical synthesis methods. Downstream processing and handling of fungal biomass can be much simpler than the processes needed for chemical synthesis (Mandal et al. 2006). Exploration of fungi in nano-biotechnology has attracted more attention for production of metallic nanoparticles due to their toleration and metal uptake and accumulation capability (Volesky and Holan 1995; Sastry et al. 2003). Fungi have a number of advantages for nanoparticle synthesis compared with other microorganisms (Castro-Longoria et al. 2011). Particularly fungi (*a*) are relatively easy to be isolated and cultured, (*b*) secrete large amounts of extracellular enzymes, (*c*) tolerate higher

metal concentrations than bacteria and secrete abundant extracellular redox proteins that reduce soluble metal ions to their insoluble form, and (*d*) harbor untapped biological diversity and may provide novel metal reductases for metal detoxification and bioreduction (Mandal et al. 2006; Kitching et al. 2015; Prasad 2016, 2017; Prasad et al. 2016). Production of nanomaterials, biologically, is inexpensive, undemanding, effective, energy-saving, and environment-friendly. Shape and size of biologically synthesized nanoparticles by various fungal species, including *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Mucor*, *Trichoderma*, and *Pleurotus*, are studied (Rai et al. 2014; Xue et al. 2016; Aziz et al. 2016; Prasad 2016, 2017; Khalid et al. 2017; Ottoni et al. 2017). This chapter is an up-to-date review that deals with fungal nanotechnology as a new science for green synthesis of nanoparticles. Benefits and health risks of nanoparticles in different areas are highlighted, with a special attention to the nano-toxicology and regulatory recommendations related to the use of nanoparticles.

3.2 Nanoparticles and Nanotechnology

Nanoparticles can be defined as natural substances or manufactured materials containing particles in a free state or as aggregates. Nanoparticles are also called nanomaterials, nanocrystals, or nano-powders. Nanoparticles are intentionally produced or engineered to have specific properties, wherein one or more dimensions are typically between 1 and 100 nm (Pulit-Prociak and Banach 2016). Nano-biotechnology, bio-nanotechnology, and nano-biology are terms that refer to employ biological systems to produce bio-nanoparticles, which exhibit benefits for improving biocompatibility. Nanoparticles can be synthesized in numerous shapes (e.g., spherical, triangular, rods) from various metal ions (Rai et al. 2009). Nanoparticles have high surface area-to-volume ratio, nanometer regime, and unique properties, which makes them highly applicable. Myco-nanotechnology (myco = fungi, nanotechnology = creation and exploitation of materials in the size range of 1–100 nm) is defined as the fabrication of nanoparticles by fungi and their subsequent application, particularly in medicine (Rai et al. 2009). Myco-nanotechnology is the interface between mycology and nanotechnology and is an exciting new applied science that may have considerable potential, partly due to the wide range and diversity of fungi (Gupta et al. 2012). According to the definition, nanoparticles occur naturally or can be prepared intentionally. The latter group is divided into the following categories (The global market 2015).

1. Nonmetallic inorganic nanoparticles (TiO_2, SiO_2, ZnO, $Al(OH)_3$, Fe_2O_3, Fe_3O_4, CeO_2, ZrO_2, CaO, ITO, ATO)
2. Metals and metal alloys (Au, Ag, Pt, Pd, Cu, Fe, Ni, Co, Al, Mn, Mo)
3. Nanomaterials based on carbon (fullerenes, carbon nanotubes, carbon nanofibers, graphene)

4. Nanopolymers and dendrimers (polymeric nanoparticles, polymer nanotubes, nanowires and nanorods, nanocellulose, nanostructured polymer films)
5. Quantum dots (cadmium telluride, cadmium selenide, quantum dots free of cadmium)

3.2.1 History

Although, in general, nanoparticles are considered a discovery of modern science, they actually have a very long history. Nanoparticles were used by artisans as far back as the ninth century in Mesopotamia for generating a glittering effect on the surface of pots (Reiss and Hutten 2010). The first scientific description regarding the properties of nanoparticles was provided by Michael Faraday in his famous paper "Experimental relations of gold to light." In 1959, Richard Feynman gave a talk on nanotechnology describing molecular machines built with atomic precision, entitled "There's plenty of space at the bottom" (Feynman 1991).

The word "nano" is derived from the Greek word for dwarf and means "a billionth." A nanometer is a billionth of a meter, which is 250 millionth of an inch. The term "nanotechnology" was coined by Prof. Norio Taniguchi, Tokyo Science University, in 1974, to describe the precision manufacture of materials with nanometer tolerances and was unknowingly appropriated by Eric Drexler in his 1986 book *Engines of Creation: The Coming Era of Nanotechnology* (Drexler 1981). By the mid-1970s, Asilomar guidelines were developed by biotechnologists seeking to conduct their early work in ways both safe and publicly acceptable. It was not until 1990 that the first journal and funding of nanotech projects in Japan were begun. The first textbook about nanoparticles was published in 1992 (Markus and James 1995).

The first Feynman Prize in Nanotechnology was awarded in 1993 for modeling a hydrogen abstraction tool useful in nanotechnology (Peterson 2004). The first nano-bio-conference was in 1996, and the first nanomedicine book was published in 1999 (Peterson 2004). First report on nanotech industry, first nanotech industry conference, and first policy conference on advanced nanotech were accomplished from 2001 to 2004 (Markus and James 1995). Foresight in 1999 published draft guidelines for safe development of molecular nanotechnology, including specific recommendations for environmental protection such as requiring artificial rather than natural fuel sources (Foresight Guidelines, Freitas 1999).

3.2.2 Properties

Properties and behavior of materials at the nanoscale differ significantly when compared to microscale, i.e., there are two basic factors which cause nanomaterials to behave differently than macromaterials (Gatti and Rivasi 2002). These are surface

effects (properties of surface atoms fraction) and quantum effects (Pulit-Prociak and Banach 2016). These factors affect the chemical reactivity of materials and determine their mechanical, optical, electrical, and magnetic properties (The global market 2015). Nanoparticles show enhanced electrical, optical, and magnetic properties. Compared to microparticles, the fraction of surface atoms in nanoparticle is increased, i.e., in relation to microparticles, nanoparticles are characterized by increased mass of surface particles. The ratio of surface area to mass in nanometric particles is 1000-fold greater than in micrometric particles. The nanometric particles are, thus, characterized by increased chemical reactivity, which is approximately 1000-fold higher compared to micrometric particles (The global market 2015, Khatoon and Ahmad 2012). Favorable modification of the properties of materials by changing their size is possible, enhancing their profitability to be used in a broad spectrum of scientific fields.

3.3 Green Syntheses of Nanoparticles

Nanoparticles can be produced by chemical methods in large quantities with a defined size and shape in a relatively short time. Synthetic techniques being employed for assembly of metallic nanoparticles are, however, quite costly with hazardous effect on the environment. Such process is not only expensive but also produces a toxic chemical that poses health threats. Therefore, biosynthetic approach is adopted to design nanoparticles with unique properties for potential scientific applications (Khan et al. 2017). Biosynthetic methods are clean and cost-effective compared to chemical methods which may lead to presence of some toxic chemicals absorbed on the surface. Green synthesis through microorganisms might overcome these toxicity issues. Biological methods for synthesis of nanoparticles using microorganisms have been suggested as a possible eco-friendly alternative to chemical methods and to be suitable for large-scale synthesis of nanoparticles (Karimi and Khabat 2016). Nanomaterials synthesized by bacteria, yeast, fungi, and algae are characterized by their mechanical strength and chemical properties. Frequently used strains of fungi and yeasts for metal nanoparticle synthesis are shown in Fig. 3.1.

Green synthesis provides advancement over chemical and physical methods as it is cost-effective, environment-friendly, and easily scaled up for large-scale synthesis and in such method there is no need to use high pressure, energy, and temperature and toxic chemicals (Chaudhari et al. 2016). Nanoparticles are biosynthesized when microorganisms grab target ions from their environment and then turn the metal ions into the element metal through enzymes generated by the cell activities (Sunkar and Valli 2013). Putative mechanisms during intracellular synthesis include heavy metal binding to fungal cell wall by proteins or enzymes present on via electrostatic interactions. The metal ions are reduced by enzymes present in cell wall. This leads to aggregation of metal ions and formation of nanoparticles.

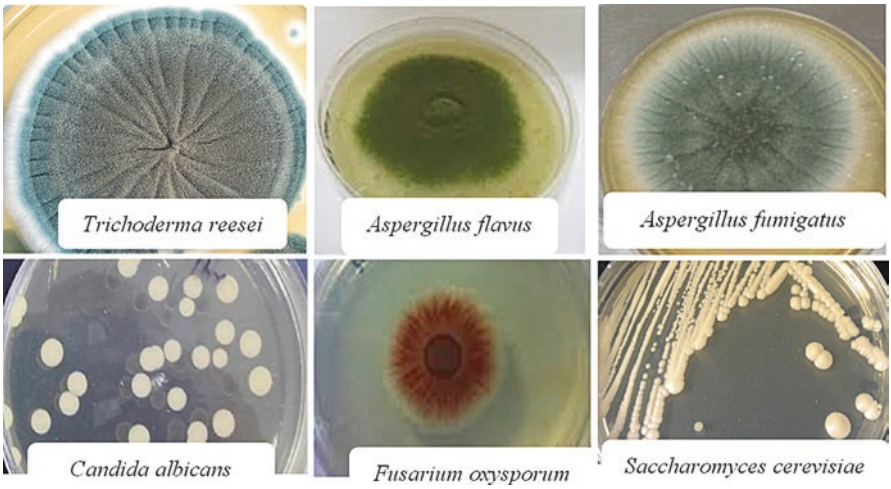

Fig. 3.1 Fungi and yeasts frequently used for metal nanoparticle synthesis

Development of green processes for biosynthesis of nanoparticles by fungi evolves an important branch of nanotechnology (Gupta et al. 2012; Chaudhari et al. 2016; Velusamy et al. 2016). Fungal species can readily synthesize metal nanoparticles both intra- or extracellularly using high levels of secreted proteins and/or enzymes (Rai et al. 2009; Ingale and Chaudhari 2013; Chaudhari et al. 2016). However, extracellular synthesis has advantages over an intracellular one: (*a*) fungal nanoparticles are mostly precipitated outside the cells and do not require lysis of fungal cells; (*b*) fungal biomass can withstand flow pressure and agitation conditions in bioreactors and other chambers; (*c*) it can be easily handled in downstream processing and, therefore, has advantages through processing for recovery and purification of nanoparticles and does not require equipment or long processing techniques; and (*d*) it is extremely cost-effective and less time-consuming (Gade et al. 2008; Ranjan and Joshi 2012; Prasad et al. 2016, 2017). Biosynthetic methods, in a renewable manner, ensure lower environmental impact and increase cost-effectiveness without the need of exogenous chemicals. Furthermore, biosynthetic mechanisms might produce nanoparticles of the desired shape, size, and distribution, given the highly specific interactions of the biomolecular templates and inorganic materials (Kitching et al. 2015).

Fungal endophytes are microorganisms that colonize living, internal tissues of plants without causing any immediate, negative effects (Shukla and Singh 2017). Endophytic fungi have been recognized as important sources of a variety of structurally novel active secondary metabolites with anticancer, antimicrobial, and other biological activities (Ranjan and Joshi 2012; Bose and Uma 2017; Sandhu et al. 2017). Endophytic-mediated synthesis of metal nanoparticles is gaining great importance owing to its simplicity; rapid rate of synthesis of nanoparticles of attractive, diverse morphologies; and eco-friendliness.

3.4 Applications of Nanotechnology

Current interest in metallic nanoparticles is due to their variable chemical, physical, and optical properties as well as the extremely small size which means very large surface area and increased reactivity per equivalent weight (Chaudhry 2016). Nanoscale, where less may be more, and nano-sizing may generate new properties and functionalities (Chaudhry 2016). Advantages of nanotechnology that can be obtained and exploited are as follows: *a*) lower doses and increased bioavailability (quick dissolution, improved penetration and permeation through membranes), (*b*) controlled release and targeted bio-distribution, (*c*) lower dose-dependent toxicity, and (*d*) reduction of the influence of environment on bioavailability (Josef and Katarina 2015). However, it is important to address the ethical, social, and regulatory aspects of nanomedicine to minimize its adverse impacts on the environment and public health (Coles and Frewer 2013). Different applications of nanotechnology are shown in Fig. 3.2. At present, the most significant concerns for application of nanoparticles involve risk assessment, risk management of engineered nanomaterials, and risk communication in clinical trials (David and Tinkle 2007). In the future, education about the benefits and risks of nanomedicine is an urgent role and challenge to gain and maintain public support and ensure health care. Many studies have reported the pros and cons of applying nanotechnology (Gwinn and Val 2006; Johanna et al. 2016). Advantages and disadvantages of nanotechnology applications are represented in Table 3.1.

3.4.1 Antimicrobial Activity

Nanoparticles are proven to have novel antimicrobial effects which offer several advantages such as broad-spectrum activity. Fungi have become one of the main biological candidates for synthesizing nanoparticles because of their metabolic

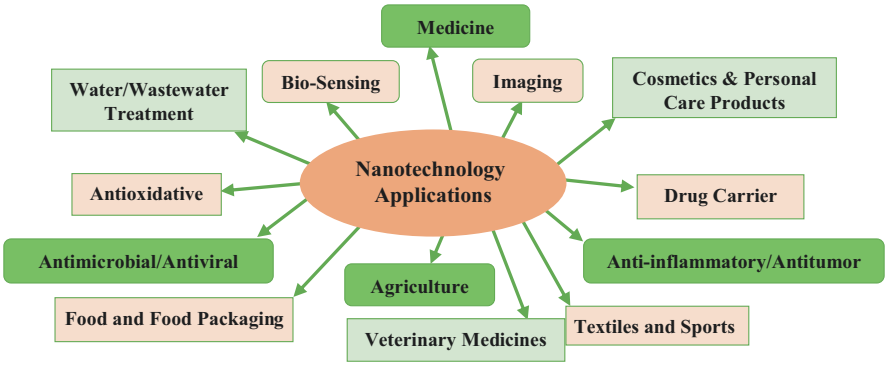

Fig. 3.2 Fields for applications of nanotechnology

Table 3.1 Advantages and disadvantages of nanotechnology applications

Pros	Cons
Nanotechnology can actually revolutionize a lot of electronic products, procedures, and applications	Nanoparticles are very small, and thus, problems can actually arise from the inhalation of these minute particles
Nanotechnology can benefit the energy sector, i.e., effective energy-producing, energy-absorbing, and energy storage products in smaller and more efficient devices	Nanotechnology production could develop unanticipated illness arising from their exposure
Nanotechnology can benefit the manufacturing sector that needs materials like nanotubes, aerogels, and nanoparticles	Inhalation of a sufficient doses can lead to lung ailments, including asbestosis and lung cancer, related to their size and chemistry as well as their ability to remain in lungs for a long time
Nanotechnology can benefit fields such as food, agriculture, cosmetics, and wastewater treatment	Exposure to much nanoparticles can weaken the natural defense systems
Nanotechnology, in the medical world, can help with creating smart drugs that help in faster cure and are without side effects that other traditional drugs have. Nanotechnology in medicine is now focusing on areas like tissue regeneration, bone repair, immunity, cancer, diabetes, and other life-threatening diseases	During the last few decades, there has been a continued increase in the morbidity and mortality attributed to air pollution in industrialized and developing countries

diversity (Rai et al. 2009). The first report of using fungi to synthesize nanoparticles dates back to a letter in *Nature* in 1989, reporting the production of cadmium sulfide nanoparticles by *Candida albicans* (Dameron et al. 1989). Silver nanoparticles are more effective against Gram-negative bacteria due to the different construction of cell walls. Gram-negative bacteria have lipopolysaccharide layer on the outside of the cell wall beneath, where a thin layer of peptidoglycan is located. The lipopolysaccharide layer structure is characterized by a lack of rigidity and strength (Pulit-Prociak and Banach 2016). A negative charge, located on lipopolysaccharides, attracts the positively charged particles of silver nanoparticles. The main component of cell walls of Gram-positive bacteria is a thin layer of peptidoglycan, which creates a rigid three-dimensional structure. Its structure comprises polysaccharide cross-linked short chains of protein molecules (Pulit-Prociak and Banach 2016). The stiffness and geometry of these layers are not conducive to penetration of silver nanoparticles through the cell wall of Gram-positive bacteria. However, some studies have reported that silver nanoparticles are able to penetrate both types of cell wall and enter into the cell, resulting in the uncontrolled tyrosine phosphorylation (Pulit-Prociak and Banach 2016).

Most antimicrobial agents inhibit microbial growth through several mechanisms such as cell wall inhibition and lysis, inhibition of protein synthesis, alteration of cell membranes, inhibition of nucleic acid synthesis, and antimetabolite activity (Yah and Simate 2015). Two main hypotheses can explain the toxic effects of

Table 3.2 Antimicrobial applications of nanotechnology

Field	Effect	Reference
Antibacterial activity	Silver interacts with sulfhydryl groups of proteins and with DNA, altering hydrogen bonding, respiratory processes, DNA unwinding, cell wall synthesis, and cell division, resulting in bacterial death. Induces denaturation and oxidization for cell wall and modulates the phosphotyrosine profile of putative bacterial peptides, which could inhibit protein synthesis and cell growth. Interaction with thiol (-SH) groups in bacteria and production of reactive oxygen species	Rai et al. (2014), Banerjee et al. (2010), Azmath et al. (2016), Ottoni et al. (2017), and Aziz et al. (2015, 2016)
Antifungal activity	Attacking membranes and inhibition of normal budding process in yeasts due to the destruction of the membrane integrity. Affect membrane dynamics and transmembrane pores are formed, thus causing a leakage of cell constituents and eventually cell death. Leakage of ions and other materials as well as dissipating the electrical potential of the membrane. Interaction between nano-Ag and the membrane structure leading to cell death	Kim et al. (2009), Rai et al. (2009), Scorzoni et al. (2017), Xue et al. 2016, and Aziz et al. (2016)
Antiviral activity	Nanoparticles show antiviral target human immunodeficiency virus type-1 (HIV) and inhibit replication of HIV in a dose-dependent manner. Might inhibit postentry stages of infection by blocking other functional HIV-1 proteins or reducing reverse transcription or proviral transcription rates by directly binding to the RNA or DNA molecules	Malik et al. (2017) and Sandhu et al. (2017)

nanoparticles on living organisms. The first is the harmful activities of nanoparticles due to the release of metal ions (Li et al. 2011). The second states that toxicity is induced by formation of free radicals, i.e., reactive oxygen species (Li et al. 2011). These free radicals are able to damage any components of the cell and initiate the production of increasing numbers of reactive oxygen species (Klaine et al. 2008). For example, generated free radicals are able to oxidize the double bonds of fatty acids in cell membranes, resulting in increased permeability of membranes, which contributes to the osmotic stress. The free radicals may also inhibit the activity of enzymes by binding to them and changing the helix of DNA, leading to cell death (Pulit-Prociak and Banach 2016). Formation of larger amounts of reactive oxygen species is induced by the higher surface area of nanoparticles as compared to their larger analogues. One of the most common mechanisms of silver nanoparticles is their natural affinity for bonding with a thiol group that is present in cysteine, which is a building block of the protein in bacterial cell wall (Pulit-Prociak and Banach 2016). Consequently, the enzymatic function of proteins is disturbed, and the chain of cellular respiration is interrupted. The spectrum antimicrobial activity of nanotechnology applications in food science and technology is given in Table 3.2.

3.4.2 Food Industry

Food-biocatalysts and bioprocessing industries face great challenges to develop high-quality and safe foods. Food processing aids to improve texture, flavor, taste, and consistency as well as improved delivery of bioactive compounds and nutrients through the integration of nanocomposites, nano-encapsulation, nano-emulsion, and edible nano-coatings in food technology (Sharma and Singh 2016). However, it is particularly important to ensure and assess the benefits and risks of nano-food products with urgent need for international regulation system for use of nanoparticles (Trujillo et al. 2016). Nanotechnology's success in the food industry depends on the social acceptance and awareness with possible toxicity of nanoparticles to the environment and potential risks to human health as well as the different mechanisms of action of nanoparticles in biological systems (Stark 2011).

Nano-biocatalyst is considered an innovative branch of the nanosciences. Several nanomaterials are currently used in nano-biocatalyst for enzyme immobilization or encapsulation. Such immobilized enzymes could be recovered and reused in a large-scale continuous process, reducing the overall cost of the biocatalytic process (Misson et al. 2015). When immobilized on nanocarriers, enzyme activity significantly increases, and mechanisms of enzyme action are enhanced. Enzyme activity increases when physical adsorption onto nanocarriers, through hydrophobic interactions, is carried out (Trujillo et al. 2016). The food and beverage industry is a focus for nanomaterial applications and strategies. Potential applications of nanotechnology in food include (*a*) nano-sensors for food quality control and smart packaging; (*b*) nanocomposites, nanocoating, and nanofilms for foodstuffs; (*c*) antimicrobial, hygiene coatings, detection of pathogens in food and beverages, self-sanitizing surfaces, and polymeric films for food packaging with high antibacterial properties; and (*d*) nanoscale freshness indicators and nano-emulsions for fat reduction (Magnuson et al. 2011; Aguilera 2014; Prasad et al. 2017).

3.4.2.1 Nanocomposites

Nanocomposites provide advantages in food packaging techniques; prevent growth of bacteria, fungi, or any pathogens; improve barrier abilities; provide resistive packaging; maintain the quality of foods; and increase the shelf life (Sharma and Singh 2016). Incorporation of the nanocomposites within the packaging material has been reported to increase the strength and thermal stability.

In many reports, it has been determined that silver metal nanoparticles can breach into the membranes of the bacterial cells. Antimicrobial properties against *E. coli* and *Staphylococcus aureus* have been reported with lots of nanocomposite systems, comprising polymer and silver nanoparticles (Sharma and Singh 2016). Significant antibacterial activity, shown against the food-borne pathogen by the nanocomposite film, will help to compete with and eradicate the bacterial invaders and to improve the shelf life and food quality (Sharma and Singh 2016).

3.4.2.2 Nano-encapsulation

Nano-encapsulation aids as a strategy to control a delivery system for food ingredients and additives in food processing. Food industry claimed that addition of nanocapsules to processed foods will improve both the availability and delivery of nutrients, thereby enhancing the nutritional status of food (Neethirajan and Jayas 2011). In nanotechnology, during various food processing operations, encapsulation of nanoparticles can deliver a material to the targeted site, enhance the flavor and shelf life during storage, and integrate antimicrobial agents with the nanoparticles in food (Sharma and Singh 2016). Encapsulated protective coating and advanced packaging system prevent spoilage and improve food quality (Sharma and Singh 2016; Souza and Fernando 2016). Nano-encapsulation system presents various benefits together with ease of handling, improved stability, withholding volatile components, controlled moisture release, pH-triggered controlled release, and enhanced bioavailability and efficacy (Sharma and Singh 2016). Nano-encapsulation has been tested for the safe and controlled discharge of favorable live probiotic cells to boost healthy gut function.

3.4.2.3 Nano-emulsion

Operations such as the targeted delivery of lipophilic products (i.e., nutraceuticals, medicines, flavor, antioxidants, and antimicrobial agents) are accomplished by carriers, named nano-emulsions. Due to their comparatively miniature size, nano-emulsion is very stable to gravitational separation (McClements et al. 2009). Bioavailability of the captured components can be enhanced through the non-emulsion-assisted delivery systems due to their relative smaller size as well as more surface area-to-volume ratio (Acosta 2009). Particles that are trapped within the nano-emulsions scatter light waves weakly, which make them suitable for integration with products like carbonated drinks, soups, ketchups, and dips (Sharma and Singh 2016). The highly viscous or gel-like product in food products can be formed from nano-emulsion. The nano-emulsion in many studies is stated as a most prominent way for capturing and transporting antimicrobial agent to the targeted site (Sharma and Singh 2016). Even the decontamination of food packaging equipment and function associated with varieties of food surfaces are accomplished with antimicrobial nano-emulsions (Sekhon 2010).

3.4.2.4 Edible Nano-coatings

Edible films are defined as a thin layer that can be safely consumed and provides a barrier against moisture, oxygen, and solute movement for the food. An edible film can be coated on food or placed between the food and the surrounding environment (Donhowe and Fennema 1993). In particular, safety and physical properties of the edible film-forming materials and coating are carefully examined. However,

technical information is still needed to develop films for food application. Edible films and coatings have received considerable attention in recent years because of their advantages over synthetic films (Bourtoom 2008). Nanoparticles are used on the packaging material cover, incorporated as nano-sensors in the packaging system or as a nano-laminate layer (Sharma and Singh 2016). The edible nano-laminates are applied to fresh fruits and vegetables, confectionary products, and formulation due to their ability to provide barrier against water, lipids, gases, odors, and off-taste (Sharma and Singh 2016). Besides, many nanomaterials provide extensive properties to the packaging material like antimicrobial activity, oxygen-reducing activity, and immobilization of enzymes.

Nanostructured coatings should (*a*) exhibit antimicrobial capability, (*b*) extend the shelf life and enhance food quality, (*c*) reduce packaging wastes, (*d*) integrate with eco-friendly and biodegradable polymer used for active food packaging, and (*e*) not affect the development of beneficial bacteria that are present in the digestive tracts like probiotics (Kuzma et al. 2008). Knowledge and awareness related to nanomaterials, regarding side effects for human health and environmental risk, is scarce (Sharma and Singh 2016). To what extent usage of natural biodegrading material and recycling of nanomaterial is prohibited is not well defined (Kuzma et al. 2008). The British Standards Institution and International and European Committee for Standardization have undertaken all the ethical, social, and demand issues related to nano-safety. Assessment of nanomaterials safety is entirely based on the chemistry and toxicity details (Sharma and Singh 2016). Significantly, accomplishment of nanotechnology in the food and bioprocessing industry is determined by alertness in the society and consumers. Non-degrading food packaging materials such as plastic enhance severe environmental problems. Novel bio-nanotechnology-based materials are being designed to develop cost-effective packaging materials and formulate edible films and eco-friendly biodegradable biopolymers that possess antimicrobial activity to extend shelf life of food (Sharma and Singh 2016).

3.4.3 Nanotechnology in Medicine

Owing to their unique chemical and physical properties and high surface area-to-volume ratio, nanoparticles are found to possess many important biological activities. An important feature is the structural stability of nanoparticles to effectively deliver the drug over a long period of time without degradation occurring before it reaches the cellular target (Rai et al. 2012; Duran and Marcato 2013; Anderson et al. 2016). Nanoparticles for biomedical application should be prepared only with biocompatible chemicals to minimize their toxic effect and increase their safe usage (Kitching et al. 2015). Applications of nanomaterials in medicine include fluorescent biological labels, drug and gene delivery, tumor destruction via heating (hyperthermia), phago-kinetic studies, and separation and purification of biological molecules and cells (Salata 2004; Acosta 2009). Important recent advantages

include tissue engineering, probing of DNA structure, bio-detection of pathogens, detection of proteins, and the drug delivery system (Pedro et al. 2015). For instance, biologic treatments such as insulin and calcitonin that cannot be delivered by conventional methods as an oral treatment have successfully been packaged in hollow nanoparticles that protect it from degradation in the gastrointestinal tract allowing for systemic delivery of the drug and avoiding alternative methods of delivery such as subcutaneous injection (Pridgen et al. 2015). The advantages of using nanoparticles for the drug delivery result from their two main basic properties (Parveen et al. 2016). First, due to their small size, nanoparticles can penetrate through smaller capillaries and are taken up by cells, which allow efficient drug accumulation at the target sites. Second, use of biodegradable materials for nanoparticle preparation allows sustained drug release within the target site over the period of days or even weeks.

Nanoparticles are being advanced as novel and more targeted treatments for difficult to manage diseases such as cancers. One of the main problems in anticancer treatments is the continuous growth of tumor cells resistant to a broad range of anticancer agents (Abraham et al. 2012). Use of engineered nanoparticles offers the ability to transport therapeutics to specific sites of a disease, thus reducing the off-target toxicity of many drugs. This is especially true in the use of chemotherapeutics where off-target reactions cause serious side effects in cancer patients (Anderson et al. 2016). It is crucial to deliver a drug to a desired target site in a controlled manner while not causing additional adverse health effects to the patient. In particular, gold nanoparticles have unique features that make them excellent nanomaterials, enabling the integration of targeting, imaging, therapeutics, and applicability in management of heterogeneous diseases such as cancer (Pedro et al. 2015). However, in contrast to their beneficial effects, use of nanoparticles for drug delivery can raise various risks to human health. The small size is beneficial, but it could have negative effects due to the following: (*a*) some nanoparticles can cause inflammation and fibrosis as a result of causing phagolysosomal membrane permeability and formation of reactive oxygen species; (*b*) the small size indicates a large surface area which could be harmful by exposing more surface molecules to cellular components; (*c*) preparation and stabilization processes of the nanoparticles for drug delivery can cause negative effects, since chemical reducing agents and radiation can stimulate cytotoxicity (Anderson et al. 2016). Some benefits, risks, and toxicity of nanoparticle applications in medicine are represented in Table 3.3.

3.4.4 Nanotechnology in Cosmetics

"Silver and gold nanoparticles" applied as preservatives in cosmetics have been found to be very stable and did not exhibit sedimentation for over 1 year. In cosmetics, nanoparticles exhibit sufficient preservation efficacy against mixed bacteria and mixed fungi and did not penetrate normal human skin (Raj et al. 2012). Nanoparticles

Table 3.3 Medicinal applications of nanoparticles

Field	Benefit	Risk/toxicity	Reference
Wounds and burns	Can induce tissue regeneration and prevent fibrosis of the injured tissue such as surgical incisions, ischemic heart muscle, and severed nerves. Dried nanoparticles, suspensions, aerosols, hydrogels, or incorporated into sheets of biodegradable scaffold materials such as collagen, all can easily be used in wound dressings, restores normal structure of the injured skin before onset of the fibrosis process and scar formation	Toxicity from silver is observed in the form of argyria that is caused by silver ions release from the dressing in large open wounds. Silver nanoparticles show toxic effect on fibroblasts and human lung adenocarcinoma epithelial cell line	Rigo et al. (2013), Galili (2017), Konop et al. (2016), Yang and Hong (2015), and Kamoun et al. (2017)
Cancer	Nanotechnology represents possibility to enhance the diagnosis and treatment of cancer, multifunctional targeted devices capable of bypassing biological barriers to deliver therapeutic agents to the biological target involved in cancer, nano-biosensors for predicting the disease, minimizing the growth of cancer cells, and reducing the cost of treatments	Toxicity is dose-dependent and causes cellular damage in human epidermoid larynx cell line through reactive oxygen species formation	Jacob et al. (2012) and Liu and Jiang (2017)
Dental practice	Silver nano-solution has been used as a caries inhibitor. Fluoride and silver interact synergistically to form fluorapatite showing slower bacterial growth. Silver nanoparticles provide self-cleaning against plaque biofilm	Potential toxicity to the central nervous system	Feng et al. (2015)
Drug delivery	Using nanoparticles, it may be possible to achieve improved delivery of poorly water-soluble drugs by delivering drugs in small particle size and increasing the total surface area of the drugs allowing faster dissolution in blood stream and faster absorption by human body-targeted delivery of drugs in a cell- or tissue-specific manner. Size of nanoparticles opens the potential for crossing the various biological barriers within the body	Risk assessment reveals great carcinogenic potential; acute toxic response can accumulate in the body and over time result in the development of nanopathologies, such as granulomas, lesions (areas of damaged cells or tissue), cancer, or blood clots	Gatti and Rivasi (2002), Gatti (2004), and Srinivas (2015)

can be attached to the cell membrane, penetrate inside the bacteria, and disrupt the membrane structure of bacterial cells (Kokura et al. 2010; Swati and Satish 2016).

Antimicrobial mechanisms of nanoparticles are related to the formation of free radicals. In the field of cosmetics, it is very important to protect the products against

microbial contamination which may occur during production of cosmetics or their storage. Before nanotechnology permanently penetrated the cosmetic industry, organic compounds such as parabens and phenoxyethanol had been used to control unwanted microbial flora (Pulit-Prociak and Banach 2016). Studies revealed the irritant effects of these types of preservatives, especially parabens, in relation to epidermis and cancer (Darbre et al. 2004; Cross and Roberts 2000). The harmful preservatives have been partially replaced by metal nanoparticles, in particular silver nanoparticles (Kokura et al. 2010). By introducing nanoparticles in the structure of materials, it is possible to give products antibacterial and anticorrosion properties and protection against UV radiation, and the resultant structures can be easily cleaned. Careful studies regarding toxicity of applying nanoparticles in cosmetics must be considered. Toxicity of nanoparticles, due to their smaller size, chemical composition, surface structure, solubility, and shape, can easily gain access to the blood stream via skin or inhalation and transport to the various organs and can lead to their dysfunction (Yevgen et al. 2012; Swati and Satish 2016).

3.4.5 Nanotechnology in Agriculture

The current world population of 7.3 billion is expected to reach 8.5 billion by 2030, 9.7 billion in 2050, and 11.2 billion in 2100, according to a new UN DESA report, World Population Prospects (World Population Project). Agriculture is the backbone of most developing countries, and it is widely recognized that global agricultural productivity must increase to feed a rapidly growing world population (Sekhon 2014). Nanotechnology has the potential to positively protect plants, monitor plant growth, detect plant and animal diseases, and increase global food production. Agriculture and food production capacity is faced with many challenges, and agriculture as a source of food is becoming increasingly important (Fraceto et al. 2016; Singhal et al. 2017). Thus, it is necessary to use the modern technologies such as nano-biotechnology in the form of nanopesticides or nanofertilizers, later referred to as nanoagrochemicals (Sekhon 2014; Kah 2015; Prasad et al. 2017; Bhattacharyya et al. 2017). There are major points for incorporating biosynthesized nanoparticles in agriculture (Mishra et al. 2017). These include the following:

- Alleviating the toxicity of nanoparticles by assessment of risk factors (evaluation for fate, transport, behavior, bioavailability, and toxicity).
- Optimizing the permissible level of nanoparticle dose within the safety limits by performing dose-dependent studies.
- Designing the experiments in natural habitat and avoiding in vitro assays for accurate interpretation.
- Most importantly, applying biosynthesized nanoparticles from laboratory to field conditions and using nanomaterials for sustainable agriculture production that allows risk-free environment in the near future to reduce their impact on human health (Arif et al. 2016; Mishra et al. 2017).

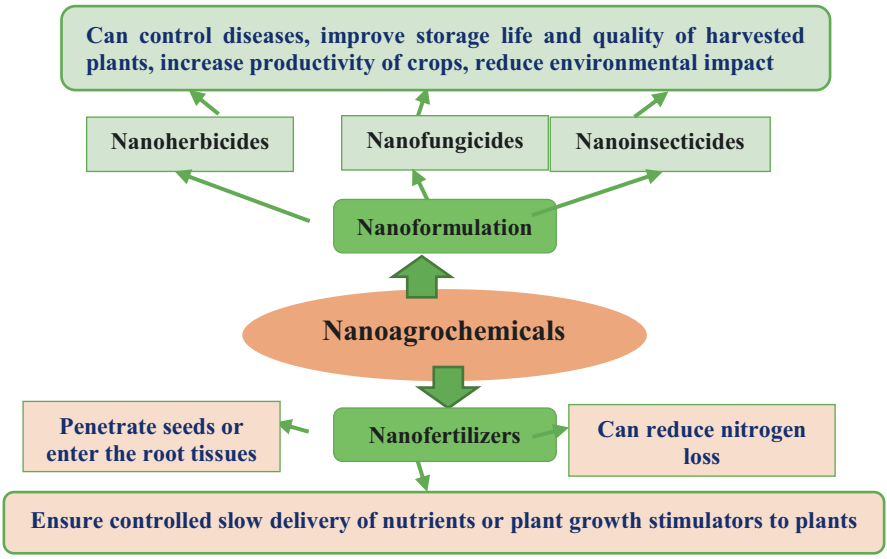

Fig. 3.3 Application of nanoparticles for plant growth stimulation and crop protection

- Costs for escalating production of nanoparticles and devices must be affordable, and safety of nanotechnology must be addressed before its spreading into the environment (Alejandro 2017).
- Nanoparticle-plant interactions, availability of soil microorganism, macro- and micro-nutrient availability, and effect of different abiotic stress factors on plant should be considered (Arif et al. 2016).
- Release and behavior of nanoparticles with the living systems are a major threat that must be analyzed before their wide applications to avoid their harmful impact on human health and the environment (Arif et al. 2016)

Nanoparticles can be applied in plants, through nanoagrochemicals, by (*a*) nanoformulations (i.e., nanoherbicides, nanofungicides, nanoinsecticides) and (*b*) nanofertilizers. Nutrients in nanofertilizers are delivered to crops within nanoparticles, whereby nutrients can be encapsulated inside nanomaterials, coated with thin protective polymer film, and delivered as particles or emulsions of nanoscale dimensions (Josef and Katarína 2015). Nanofertilizers can reduce nitrogen loss due to leaching, emissions, and long-term assimilation by soil microorganisms (Josef and Katarina 2015). Applications of nanoparticles for plant growth stimulation and crop protection are represented in Fig. 3.3.

The ability of some nanoparticles to penetrate seeds or enter the root tissue indicates the possibility to develop new nutrient delivery systems that exploit the nanoscale porous domains on plant surfaces and show sustained release of nutrients (Josef and Katarína 2015). On the other hand, the ability to incorporate genetic materials such as plasmid DNA, RNA, and siRNA into functionalized nanoparticles with little toxicity demonstrates a new era in delivering genes selectively to tissues

and cells (Jin et al. 2009). Nanoparticle-mediated DNA delivery involves coating DNA molecules onto the nanoparticles to be delivered directly into plant cells. After entering into the plant cells, DNA molecules will be released by nanoparticles and integrate into the host genome (Josef and Katarína 2015). Nanoformulations and nanofertilizers are smart delivery systems to plants.

Biotechnology of nanoparticles finds a broad range of uses for plant growth, defense, and nutrients. Nowadays nanomaterials like metal and metal oxide nanoparticles can be used to improve crop yield and alleviate toxicity (Arif et al. 2016). Currently, there is a common assumption that the small size of nanoparticles allows them to easily enter tissues, cells, and organelles and interact with functional biomolecular structures (i.e., DNA, ribosomes) since the actual physical size of an engineered nanostructure is similar to many biological molecules (e.g., antibodies, proteins) and structures (e.g., viruses) (Solanki et al. 2015). Nanofertilizer can be used for delivery to the plants. Uptake, translocation, and fate of nanoparticles in plant system are unknown, resulting in rise of various ethical and safety issues regarding the use of such nanofertilizers in plant productivity.

Public awareness about the advantages and challenges of nanotechnology will lead to better acceptance of this emerging technology. "Nanotechnology" has great potential for sustainable agriculture, improving and enriching food, and enhancing the quality of life. All over the world, nanotechnology has become the future of any country (Prasad et al. 2014). Combination of nanotechnology, food, and pesticides has high potential regarding public concern. Phytotoxicity cannot be neglected because nanotechnology will significantly affect the agricultural ecosystem by the increasing release of nanoparticles into the environment which will subsequently generate nano-wastes (Thul et al. 2013; Kah 2015). Nanoparticles are now introduced at all stages of the food chain, and public awareness about nanoagrochemicals seems generally low. Biological control agents are generally regarded as safe for humans and the environment. However, increased exposure of consumer population to fungal substances (mycotoxins) and fungal nanoparticles can affect the immune system and human body (Kah 2015). Particular attention should be devoted to non-biodegradable materials due to risks of accumulation and persistence in soil, plants, and mammals, which may subsequently result in various pathological processes (Handy et al. 2008; Josef and Katarina 2015).

3.5 Commercialization of Nanoparticles

The market for nanotechnology products is already huge and is predicted to grow rapidly in the coming years. Most nanoparticles are produced in multiton volumes in varying sizes, shapes, and surface coatings. Nanoparticles cover a range of materials such as inorganic metal and metal oxide nanomaterials, carbon-based nanomaterials, and polymeric particulate materials in a variety of forms (The global market 2015). Complete understanding of nano-bio interactions and the challenges regarding chemistry, manufacturing, and controls is required for clinical translation and

commercialization. Today, nanotechnology is used in a broad spectrum of scientific fields such as biotechnology, medicine, pharmacy, ecology, electronics, clothing, agriculture, veterinary medicine, food industry, and cosmetology. Nanomaterials of particular interest include nanoparticles of silver, gold, zinc, selenium, titanium dioxide, and carbon nanotubes (Pulit-Prociak and Banach 2016). According to a statement issued by the European Commission, the global amount of manufactured nanomaterials is close to 11.5 million tons, which is equivalent to their market value reaching 20 billion per year. It is estimated that the current global market for nanomaterials is from 300,000 tons to 1.6 million tons. The Asian region accounts for the largest market share (approx. 34%), followed by North America (approx. 31%) and Europe (approx. 30%) (The global market 2015). Silver nanoparticles market size was over $ 1 billion in 2015 by application (health care, life sciences, textiles, electronics, food, and beverage) with industry analysis report, regional outlook (USA, Canada, Germany, UK, France, Italy, Spain, Poland, Russia, Netherlands, China, India, Japan, South Korea, Australia, Indonesia, Malaysia, Brazil, Argentina, Mexico, Saudi Arabia, UAE, South Africa), growth potential, price trends, competitive market share, and forecast, from 2016 to 2024 (Global Market Insight 2017).

3.6 Cytotoxicity of Nanotechnology

Nano-toxicology is a branch of bio-nanoscience that includes the study of toxicity of nanomaterials. Due to the small size of nanoparticles, nano-toxicological studies are planned to determine whether and to what level these particles may pose a risk to the environment and human health (Salaheldin et al. 2016). In general, toxicity of nanoparticles is determined by their particle size, shape, and biodegradability. The small size of nanoparticles allows them to easily enter tissues, cells, and organelles and interact with functional biomolecular structures (i.e., DNA, ribosomes) (Kah 2015; Pulit-Prociak and Banach 2016). The actual physical size of an engineered nanostructure is similar to many biological molecules (e.g., antibodies, proteins) and structures (e.g., viruses) (Xia et al. 2009).

Based on the particle size and biodegradability, nanoparticles can be classified into four classes: (1) size ˃100 nm and biodegradable, (2) size >100 nm and non-biodegradable, (3) size <100 nm and biodegradable, and (4) size <100 nm and non-biodegradable (Josef and Katarina 2015). Non-biodegradable materials which remain in the body, of course, can accumulate and affect the immune system and represent an increased risk of toxicity (Keck and Muller 2013). Nanotechnology is incorporated into a large variety of goods, such as food, food packaging, sunblock, chemical fertilizers, and animal feed. However, little is currently known about the possible effects of nanotechnology on human or environmental health. Nanoparticles can enter in the human system through several ways: (*a*) via the lungs where a rapid translocation through the blood stream to vital organs is possible, including crossing the blood-brain barrier, (*b*) absorption by the intestinal tract, or (*c*) the skin (Josef and Katarína 2015). Nanoparticles can affect cell membranes and causing DNA

damage, which could be harmful to the human health (Solanki et al. 2015). Nano-size materials change their physical and chemical properties in comparison with bulk materials and can become toxic when they reach nano-size (Josef and Katarína 2015). Therefore, an increased attention must be devoted to the impact of risks associated with their usage:

- Nanoparticles usually aggregate after entering an environment, and therefore, investigation of mechanisms of toxic effects/impacts and toxicity trials should be performed.
- Use of nanotechnology in medicine, more specifically as a drug delivery, spreads rapidly. The pharmaceutical sciences are using nanoparticles to reduce toxicity and side effects of drugs. However, these carrier systems themselves may impose risks to the patient (Jong and Borm 2008).
- Cytotoxicity studies show that nanoparticles can easily penetrate DNA and the cells of the lungs, skin, and digestive system, thereby causing harm to living organisms (Oberdorster et al. 2005). One example of a commonly used but potentially harmful nanoparticle can be found in the beverage industry. Beverage companies have been using plastic bottles made with nanocomposites, which minimize the leakage of carbon dioxide out of the bottle. This increases the shelf life of carbonated beverages without using heavy glass bottles or more expensive aluminum cans (Adam 2012).
- Nanoparticles are now being engineered, thereby increasing the risk of causing irreversible damage to living organisms. It is important to proactively address the ethical, social, and regulatory aspects of engineered nanoparticles in food, medicinal, pharmaceutical, and agricultural fields to minimize the adverse impacts on the environment and public health and to avoid a public backlash (Oberdorster et al. 2005).
- Penetration of nanoparticles into an environment and its specific reactivity can cause dangerous effects. Therefore, there is a need to develop techniques to monitor their potential risks. Understanding the life cycle of nanoparticles in the environment and their chemical stability is an important step in the process of determining their influence on living organisms.

3.7 Regulation of Nanoparticles

Nanoparticles tend to accumulate in various organs, especially in the liver, kidneys, and lungs. Presence and accumulation of silver nanoparticles in these organs may be particularly dangerous and can have negative effects in the future (Pulit-Prociak and Banach 2016). Toxic effects of nanoparticles are mostly connected with the damage of membranes and DNA, generation of reactive oxygen species, and genotoxicity. Some toxicological studies have reported that the effect of nanoparticles can be cytotoxic, genotoxic, neurotoxic, and ecotoxic (Pulit-Prociak and Banach 2016). Based on these facts, regulation requirements for nanotechnology, preparation, and

application are strongly needed, especially in relation to nature, environment, and human health (Josef and Katarina 2015). It is very important to know the whole life cycle of products containing nanomaterials. Full scientific information is not yet available, and developed conclusions are incomplete. It is necessary to perform tests to determine the degree of nano-accumulation in a living matter (Pulit-Prociak and Banach 2016). If alarming data are confirmed, it will be necessary to provide methods for environment protection against a possible threat of nanomaterials that may accumulate in the future.

The most significant ethical issues relating to nanomedicine involve risk assessment, risk management, and risk communication in clinical trials. Before a nanomedicine product can be used in diagnosis and prevention or treatment of disease, extensive clinical testing must be first achieved to explore the toxicological, pharmacological, and immunological properties of different nanomaterials (David et al. 2007). Educating members of society about the benefits and risks of nanomedicine is important to gain and maintain public support. The problem of nanomedicine products could be a significant concern in countries that do not have guaranteed health-care coverage (David et al. 2007). New technologies such as genetically modified foods and nanomedicine are likely to be considered as dangerous or disruptive.

Existing data show how nanomaterials accumulate in the environment, and moreover, every year their number drastically increases (Pulit-Prociak and Banach 2016). For example, the amount of silver nanoparticles derived from various sources in 2005 was equal to 4 tons per year, while in 2008 it increased to 563 tons (Pulit-Prociak and Banach 2016). There are currently several food and beverage products with nanotechnology on the market. Governments and food companies in several countries are investing in hundreds of projects developing nanotechnology in food and agriculture (Bhagat et al. 2015). Nanotechnology can be applied in all aspects of the food chain, both for improving food safety and quality control and as novel food ingredients or additives, which may lead to unforeseen health risks. The Organisation for Economic Co-operation and Development's Working Party on Manufactured Nanomaterials, which is comprised primarily of regulators from various countries, is looking to share information among the countries about regulatory actions and voluntary programs and the data they have or need for discussions relating to regulatory decisions (Josef and Katarina 2015).

3.8 Challenges and Future Prospects

Bio-nanoparticles possess excellent biocompatibility and are, therefore, promising materials for several applications such as coatings, packaging, medicine, construction, electronics, as well as cosmetics, textile, and agriculture (Sohel et al. 2017). There has been increasing interest in the use of fungi for these processes, because fungi may have the potential to provide relatively quick and ecologically clean biofactories for metallic nanoparticles (Sohel et al. 2017). Many industrial areas,

including food, enzyme, and pharmaceutical production and processing, currently use fungal biomass. Downstream handling and processing procedures for fungal material are already well established (Rai et al. 2009; Bose and Uma 2017; Sandhu et al. 2017). In relation to many other uses of fungi, production of metallic nanoparticles is a relatively new development. Fungi is a very effective secretor of extracellular enzymes and able to produce metal nanoparticles and nanostructure. Fungal nanotechnology would appear to be a considerable potential biotechnology to develop in the future (Rai et al. 2009; Li et al. 2017; Palanivel et al. 2017). A number of challenges, however, need to be undertaken before the full evaluation and application of myco-nanotechnology.

Despite the broad range of applications, cytotoxicity of nanoparticles and health risks cannot be neglected (Thul et al. 2013). The interactions of nanomaterials with biological systems may change the properties of nanomaterials and in return affect their biological responses. Therefore, studies on innovative methodologies on the surface chemistry, unique mechanisms of the nano-bio-interface, and relevant biological applications are challenges of great importance (Li et al. 2017). Release and behavior of nanomaterials with the living systems are a major threat that must be analyzed before their wide application to avoid their harmful impact on human health and the environment (Thul et al. 2013).

The future of nanotechnology provides an opportunity for positive economic development through effective and innovative products. However, there is a lack of in vitro validated tests and in vivo toxicity data and limited evaluation of risk assessment in this area (Solanki et al. 2015). Therefore, in this context, the following key points should be considered in the future:

- Nano-risks and nano-safety should be determined to control and monitor the development of such a new technology.
- Permissible levels of nanoparticles dose within safety limits need to be explored and clarified to gain comprehensive knowledge of nanotoxicity.
- A clear overview of soil physicochemical characteristics in the agricultural field, where nanoparticles are to be applied, may help in reducing their risk toward plant and soil biota (Mishra et al. 2017).
- Studies on nanoparticles should discuss what is known concerning their fate, behavior, toxicity, and effects on human health and agroecosystem. Several issues must be addressed to ensure safety of nanoparticles (Klaine et al. 2008; Mishra et al. 2017).
- It is believed that biosynthesized nanoparticles may possess relatively lesser or no toxicity, and hence future researches must precisely focus on their practical utility.
- Safety of nanoparticles regarding the human health is associated with great uncertainty. Governments across the world should form common and strict norms and monitoring, before commercialization and bulk use of nanomaterials (Agrawal and Rathore 2014).

3.9 Conclusion

Bio-nanotechnology involves the use of biological components and nanotechnology to support biotechnological processes on a nanoscale level. Bio-nanoparticles possess excellent biocompatibility and are, therefore, promising materials for several applications such as coatings, packaging, medicine, construction, and electronics as well as cosmetics, textile, and agriculture applications. In recent years, a lot of attention is being paid to bio-nanotechnology due to its amazing applications and beneficial effects. Numerous microorganisms have been shown to have potential for biosynthesis of metallic nanoparticles. There has been increasing interest in the use of fungi for these processes because fungi may have the potential to provide relatively quick and ecologically clean bio-factories for metallic nanoparticles. Fungal nano-biotechnology has scopes for solving some of the major problems faced by humans in today's world. The need for more safe alternatives in large-scale production of nanoparticles resulted in the development of eco-friendly methods. Industrial bio-nanotechnology takes advantage of biological-based approaches to produce nanoparticles using biological renewable resources to reduce hazardous waste production which is the main advantage of nanoparticle biosynthesis. However, as with all powerful tools of science, this rapidly evolving biotechnology needs to be handled with care. Considering the great benefits and risks as well, is bio-nanotechnology a novel green tool of the future?

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Chapter 4
Application of Nanotechnology in Mycoremediation: Current Status and Future Prospects

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Abstract Bioremediation is a growing area of green biotechnology and may be defined as the application of biological methods to the treatment of pollution. Much bioremediation effort has concentrated on organic pollutants, although the materials that are able to be transformed or detoxified by microorganisms include both natural resources and inorganic pollutants, such as toxic metals. The buildup of toxic chemicals and heavy metals in the environment is an ever-increasing and serious problem. These toxic materials threaten humans, animals, and the ecosystem. Despite noticable progresses in the field of bioremediation in recent years, there is a distinct lack of appreciation of the potential roles and participation of fungi in bioremediation. Mycoremediation is the use of fungi to collapse or eliminate toxins from the environment. There are evidences of the role of specific fungi in neutralizing toxic weapons and waste. Research is being done to use mycoremediation in national defense against chemical and biological warfare. This also births the chance to use mycoremediation to help mend war-torn environments. Nanomaterials also display exclusive physical and chemical properties, and they have received much attention from researchers and scientists in dissimilar areas of environmentally friendly sciences, especially in bioremediation. Bioremediation of pollutants by use of existing knowledge is not always effective and efficient in cleaning up the environment. Therefore, nanomaterials may be useful for bioremediation, which

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will not only have less toxic effect on microorganisms but will also increase the microbial activity of the particular waste and toxic materials which will reduce the total time consumption as well as reduce the total cost. This chapter highlights the potential of fungus-originated nanomaterials in mycoremediation of waste and toxic materials.

Keywords Fungal biotechnology · Bioremediation · Mycoremediation · Nanotechnology · Pollution · Heavy metals

4.1 Introduction

Nanotechnology is the knowledge which deals with various methods of metal nanoparticles. Nanoparticle (10^{-9} m) is described as a small piece that works as a whole unit in terms of its transport and properties (Mukherjee et al. 2010; Gholami-Shabani et al. 2016a, b, c). In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Although nanoparticles are considered an invention of modern science, they actually have a long history. Specially, nanoparticles were used by craftsman as far back as the ninth-century Mesopotamia for producing a glittering effect on the external of pot. Nanoparticles used in red glasses in late Bronze Age from di Frattesina (Rovigo, Italy) provided colored outstanding to excitation of the surface plasmon resonance of copper nanoparticles (Angelini et al. 2004; Lee et al. 2013). In protohistoric period, glasses were developed using copper crystals on the top layer via revealing the material to reducing circumstances. Copper and gold nanoparticles were also used during Roman times (Holister et al. 2003; Shankar et al. 2004; Amin et al. 2011). The well-known sample is Roman Lycurgus Cup (Kreuter 2007; Ueda et al. 2014; Bulte and Modo 2017). The color of the cup was greenish yellow, although the glass gave ruby color after transmission of light. Change in color is commonly due to colloidal metal and nanocrystals, a silver-gold alloy dispersed throughout the glassy matrix (Barber and Freestone 1990). Nanoparticles that are synthesized naturally, chemically, and biologically are promising tools for the mankind in different fields like medical, pharmaceutical, genetic engineering, agriculture, industrial, and environmental remediation. From the point of view of remediation, nanoremediation is a developing field of nanotechnology commercially used in 44 cleanup sites around the world. A wide array of nanoparticles are able to green remediate easily the various contaminant without any problems and limitations because this method is very specific for any pollutants without any circumstance.

Growth in the population density, requirement of upgradation in agriculture efficiency, industrial development, and urbanization of human societies are responsible for environmental pollution. Part of knowledge and innovation that will most likely produce the advances of tomorrow is defined as “nanotechnology.” Various technologies have been developed for effectively treating environ-

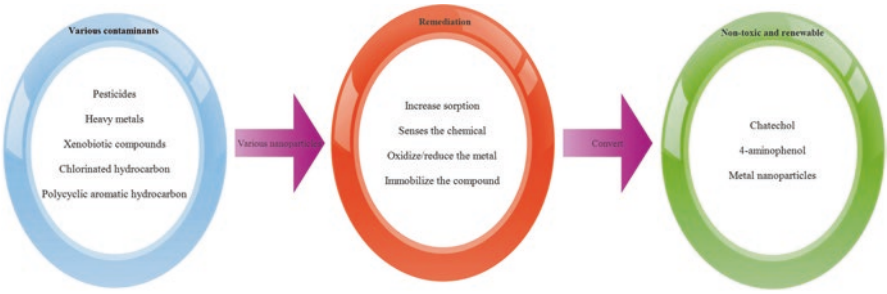

Fig. 4.1 Outcome of metal nanoparticle action on contaminated materials and their potential to reduce the pollutants

mental pollutants. Pollutants which are highly toxic, immanent, and difficult to treat are being current particular challenges. Nanotechnology possibly will offer fast and effective solutions for environmental cleanup (Fig. 4.1). There are different sustainable applications of nanomaterials especially in providing unpolluted water from polluted water resources and find out and cleans up natural and unnatural contaminants include waste and toxic material, "that is named remediation" (Jansen et al. 1994; Pourabadehei and Mulligan 2016). Bioremediation means eliminating the harms using biological materials. It is a process by which various natural agents, such as microorganisms (bacteria, fungi, yeast, algae), and their products are used to reduce the environmental contaminants into a reduced amount of toxic or nontoxic forms (Bai et al. 2009; Khan et al. 2015). The vital advantage of green bioremediation beyond economic aspects is high capability, minimization of biological and chemical toxics, selectivity to exact metals, no extra nutrient requirements, renewal of bio-sorbent, and the chance of metal recovery (Cheng et al. 2004; Purnomo et al. 2014). In green bioremediation, adding of fertilizers for the improvement of bioavailability within the environment is known as biostimulated bioremediation. Most common bioremediation technologies include bioventing, bioreactor, bioleaching, bio-augmentation, bio-stimulation, composting, land agriculture, rhizoremediation (plant and microbe interaction), phytoremediation, and green bioremediation via biosynthesis of nanoparticles (Gholami-Shabani et al. 2013, 2014, 2015, 2016a). Bioremediation of a polluted site naturally works in one of two approaches. First, various parameters such as amount of oxygen, nutrients, and the exact temperature are used to increase the growth of whatever microbes (local microorganisms) might already be living at the contaminated site. Second, in less common cases, particular microorganisms (exogenous microorganisms) are added to reduce the pollutants and toxins. But in both cases once dangerous chemicals are cleaned up and microorganisms have consumed their existing "food," the microorganisms die. Therefore, bioremediation applications are divided mostly into two broad categories: "in situ and ex situ." In situ bioremediation treats the contaminated materials in the location in which it is found, so it is economical. In this case, there is a reduced release of contaminants, pollutants, or toxins to the environment, as it is treated at the place

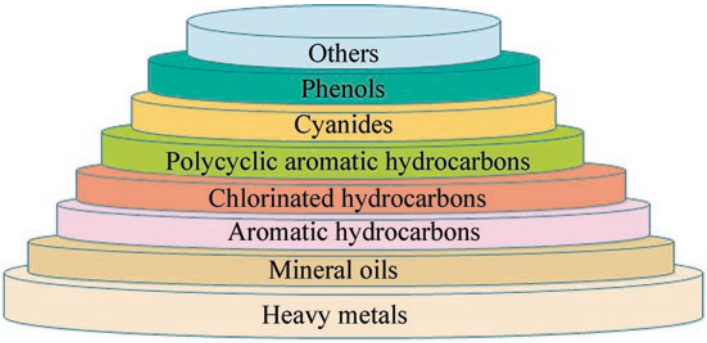

Fig. 4.2 Various contaminants produced in diverse natural resources. Most of the pollution caused by heavy metals

where it is found. Due to confinement of pollutants and toxins, less amount of nanomaterials can treat bulky volume or area. However, it is slower and some time may be challenging to manage, whereas ex situ bioremediation methods require excavation of contaminated material or toxic substances before they can be treated. Ex situ approaches can be faster and easier to control and are used to treat a wider range of contaminants and soil types than that of in situ approaches (Satinder et al. 2006; Gan et al. 2009; Haritash and Kaushik 2009; Onwubuya et al. 2009). Currently preserving ecosystem and biodiversity has become more and more important field of research, as well as a resource management goal. Various industrial chemicals are released daily which are hazardous to the surroundings and cause dangerous effects to the biodiversity by entering the food chain. Consequently, such contaminants persist in soil and water for long time and become a challenge to remediate these compounds (Fig. 4.2). These chemicals include petroleum hydrocarbon, heavy metals, metalloids, phenolic compounds, effluents, radionuclide, and halogenated cleaners from agricultural chemicals, industrial sources, and explosives (Wilson and Jones 1993; Reda 2009; Chen et al. 2015). There are several examples of pollutants which affect human and animal health; chlorinated hydrocarbons simply dissolved in water mostly affect the respiratory tract and cause irritation in eyes. "Phenols" that are irritating have corrosive effect on the skin and become chronic by affecting the central nervous system and kidneys. "Arsenic exposure" causes hyperkeratosis and lung cancer. "Lead exposure" causes blood toxicity. "Chromium exposure" causes epigastric pain, nausea, diarrhea, and chronic ulcer. "Heavy metals" like cadmium induce renal tubular damage and affect bones. With the recent development of nanotechnology, the grouping of nanoparticles and biological process is effective in enhancing measurement accuracy, improving bioremediation efficiency, and broadening biochemical application in environmental investigation. Nanoparticles related bioremediation has low hazard of genetic leakage in the environment and can provide additional functions and characters to the biochemical process (Xie et al. 2016).

4.2 Green Synthesis of Metal Nanoparticles

Metal nanoparticles are synthesized by "green" methods using microbes (Dhanasekar et al. 2015; Huang et al. 2015; Prasad et al. 2016). Biological technology is the commonly accepted approach for green bioremediation because of their nontoxic effect and unpolluted and environmentally friendly approaches (Fig. 4.3). Although there are several approaches for the synthesis of metal nanoparticles like sol-gel technique and chemical synthesis, biological synthesis of metal nanoparticles is most suitable and environmentally friendly (Iravani et al. 2014; Prasad et al. 2016). In green nanotechnology, metal nanoparticles are synthesized by living organisms, microbes, and plants of which microbes (fungi, bacteria, actinomycetes) are used mainly for commercial uses and rapidly decontamination procedures due to their high tolerance and reproduction power. These are generally synthesized from secondary metabolites of intracellular or extracellular metabolism of microorganisms (Malarkodi et al. 2013; Prasad 2014; Prasad et al. 2016). Green synthesis of metal nanoparticles is a kind of bottom-up method where the main reaction that occurs is reduction/oxidation. The microbial enzymes are responsible for reducing properties for reduction of metal compounds into their respective metal nanoparticles (Gholami-Shabani et al. 2016c). The particles synthesized biologically have higher catalytic reactivity and greater specific surface area (Park et al. 2015). Synthesized metal nanoparticles either intracellular or extracellular do not aggregate outstanding to the presence of protein capping agent secreted by specific microorganism. Extracellular biosynthesis has increased a lot of attention because of low-cost necessity and no downstream processing requirements (Gholami-Shabani et al. 2017). The secondary metabolites and extracellular components present in cell-free extract carry out the redox reaction for green synthesis of nanoparticles (Fig. 4.4). By modifying physical and biological parameters, the structure (shape and size) of particles can also be different. These particles can be characterized by UV-visible

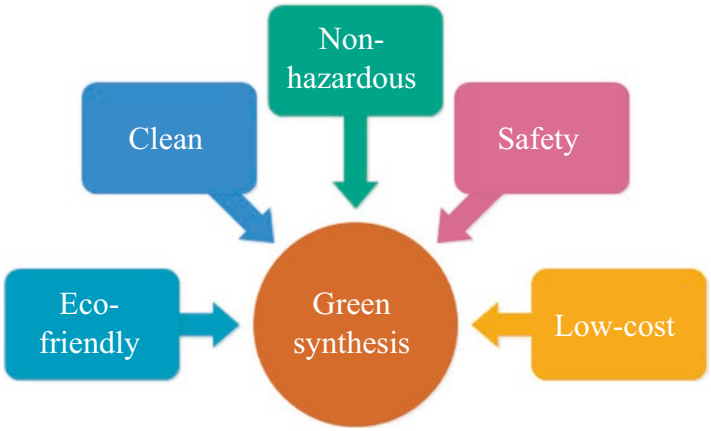

Fig. 4.3 Advantages of green nanotechnology over other approaches

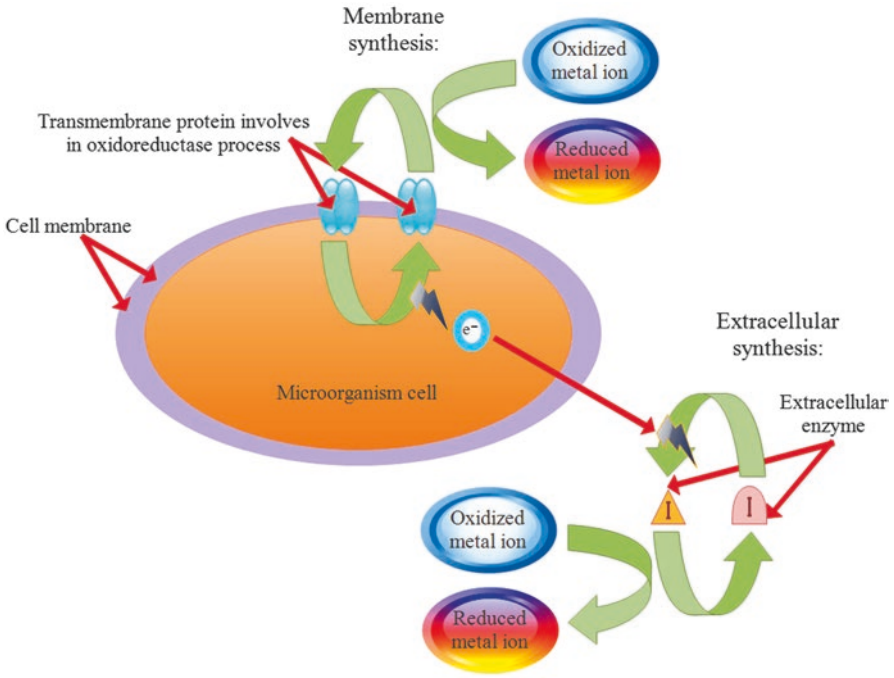

Fig. 4.4 Various approaches of microbial synthesis of metal nanoparticles

spectroscopy, zetasizer, transmission and scanning electron microscopy, X-ray diffraction, and Fourier-transform infrared spectroscopy (Narayanan and Sakthivel 2010; Mukherjee et al. 2012). The potential of metal nanoparticles for the environment can be categorized as green remediation, sensing and detection, sequestration of elements, and contamination control. Field of green remediation by metal nanoparticles is basically groundwater, wastewater, and soil.

4.3 Myconanoparticles

Fungi are an important part of the list of microorganisms used for metal nanoparticle synthesis which are more advantageous than other microorganisms in many approaches. They grow in the form of mycelial mesh which helps them to bear flow pressure and agitation and other conditions to which microorganisms are subjected to in a bioreactor used for large-scale production. The ability of filamentous fungi to grow on readily available and cheap substrates, in addition to their capability to produce a wide range of commercially interesting metabolites, has attracted considerable interest to exploit them as production microorganisms in biotechnology

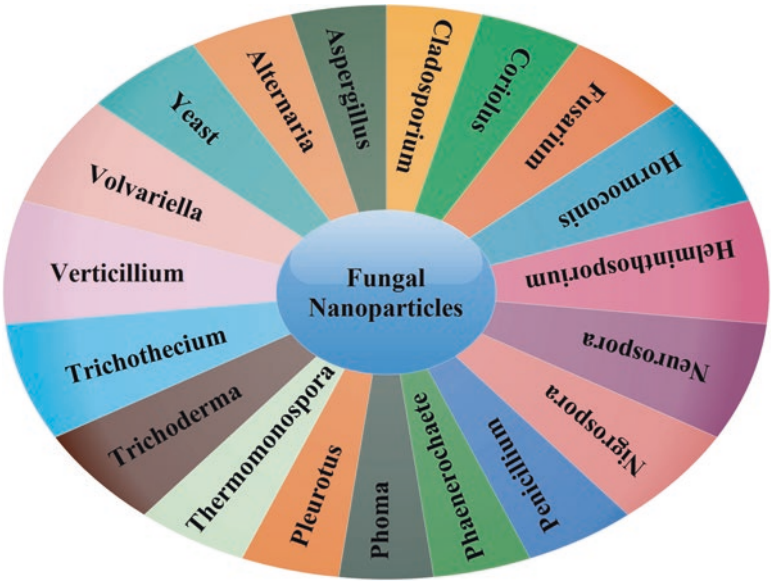

Fig. 4.5 Fungal species used as bionanofactory for synthesis of metal nanoparticles

(Dhanasekar et al. 2015). The mycosynthesis of metal nanoparticles or myconanotechnology (Honary et al. 2012) is the use of fungi in nanotechnology for the synthesis of nanoparticles (Prasad 2016). Several fungal strains have been used as promising resources for metal nanoparticle fabrication, for example, *Aspergillus*, *Fusarium*, *Penicillium*, and *Verticillium*. Different fungal species are proficient candidates for synthesis of metal nanoparticles both intracellularly and extracellularly (Fig. 4.5). Types of myconanomaterials synthesized by fungi are shown in Table 4.1. A wide array of fungi has been examined for their ability to produce metal nanoparticles. Mycosynthesis of silver, gold, gold-silver alloy, platinum, selenium, tellurium, palladium, silica, titanium, quantum dots, zirconium, usnic acid, magnetite, uraninite, and cadmium telluride nanoparticles has also been reported by different researchers. There are several potential mechanisms for such syntheses discussed by investigators, but the exact mechanism has not been explored in many cases. Bacteria are supposed to use an enzyme to metabolize oxygen to stand life. Metal ions cripple the enzyme and stop the metabolization of oxygen. This suffocates the fungi and bacteria, subsequent in death. It has been shown that fungal enzymes interact with metal ions and reduce to form metal nanoparticles (Mukherjee et al. 2002; Gholami-Shabani et al. 2016c).

Table 4.1 List of some fungi that synthesize metal nanoparticles or other metallic nanostructures

	Mode of synthesis	Nanoparticles	References
Alternaria alternata	Extracellular	Silver	Sarkar et al. (2011)
Aspergillus clavatus	Extracellular	Silver	Verma et al. (2010)
Aspergillus flavus	Intracellular	Silver	Vala et al. (2014)
Aspergillus fumigatus	Extracellular	Silver	Ranjbar-Navazi et al. (2010)
Aspergillus fumigatus	Extracellular	Silver	Bhainsa and D'souza (2006)
Aspergillus niger	Extracellular	Silver	Gade et al. (2008)
Aspergillus niger	Extracellular	Silver	Kathiresan et al. (2010)
Aspergillus tamarii	Extracellular	Silver	Kumar et al. (2012)
Aspergillus terreus	Extracellular	Silver	Li et al. (2011)
Aspergillus species	Extracellular	Zinc	Pavani et al. (2012)
Cladosporium cladosporioides	Extracellular	Silver	Balaji et al. (2009)
Coriolus versicolor	Extracellular	Cadmium sulfide	Sanghi and Verma (2009)
Fusarium acuminatum	Extracellular	Silver	Ingle et al. (2008)
Fusarium semitectum	Extracellular	Silver	Basavaraja et al. (2008)
Fusarium solani	Extracellular	Silver	Ingle et al. (2009)
Fusarium oxysporum	Extracellular	Silver	Ahmad et al. (2003a)
Fusarium oxysporum	Extracellular	Zirconia	Bansal et al. (2004)
Fusarium oxysporum	Extracellular	Silica/titanium	Bansal et al. (2005)
Fusarium oxysporum	Extracellular	Magnetite	Bharde et al. (2006)
Fusarium oxysporum	Extracellular	Gold	Mukherjee et al. (2002)
Fusarium oxysporum f.sp. *lycopersici*	Intra-/ extracellular	Platinum	Riddin et al. (2006)
Hormoconis resinae	Extracellular	Gold	Varshney et al. (2009)
Helminthosporium tetramera	Extracellular	Silver	Shelar and Chavan (2014)
Mucor hiemalis	Extracellular	Silver	Aziz et al. (2016)
Neurospora crassa	Extracellular	Gold, silver	Castro-Longoria et al. (2011)
Nigrospora oryzae	Extracellular	Gold	Kar et al. (2014)
Penicillium brevicompactum	Extracellular	Gold	Mishra et al. (2011)
Penicillium citrinum	Extracellular	Silver	Honary et al. (2013)
Penicillium citrinum	Extracellular	Copper oxide	Honary et al. (2012)
Penicillium fellutanum	Extracellular	Silver	Kathiresan et al. (2009)
Penicillium nalgiovense	Extracellular	Silver	Maliszewska et al. (2014)
Penicillium purpurogenum NPMF	Extracellular	Silver	Nayak et al. (2011)
Penicillium glabrum	Extracellular	Silver	Nanda and Majeed (2014)

(continued)

Table 4.1 (continued)

	Mode of synthesis	Nanoparticles	References
Phanerochaete chrysosporium	Extracellular	Silver	Vigneshwaran et al. (2006)
Phoma glomerata	Extracellular	Silver	Birla et al. (2009)
Phoma gardenia	Extracellular	Silver	Rai et al. (2014)
Phoma sp.3.2883	Extracellular	Silver	Chen et al. (2003)
Pleurotus sajor-caju	Extracellular	Silver	Nithya and Ragunathan (2009)
Thermomonospora sp.	Extracellular	Gold	Ahmad et al. (2003b)
Trichoderma asperellum	Extracellular	Silver	Mukherjee et al. (2008)
Trichoderma viride	Extracellular	Silver	Fayaz et al. (2010)
Trichothecium sp.	Intra–/ extracellular	Gold	Ahmad et al. (2005)
Verticillium sp.	Extracellular	Magnetite	Bharde et al. (2006)
Yeast cells	Extracellular	Cadmium telluride	Bao et al. (2010)

4.4 Proficiency of Nanoparticles

Metal nanoparticles have the capability to absorb maximum amount of contaminants and pollutants due to large surface area and high surface energy (Fig. 4.6). They catalyze the reactions in faster rate in comparison to bulk material, thus reducing energy consumption during degradation or helps in stopping produce of pollutants. The nanosized form of particles makes them easy to get into the contaminants, hence promoting in situ remediation rather than ex situ remediation. The capability of the metal nanoparticles to be coated with different ligands and control of surface area to volume ratio by changing the shape of the metal nanoparticles enables the design of sensors with high selectivity, sensitivity, and specificity (Bindhu and Umadevi 2014).

4.5 Approaches of Nanoparticles to Control the Pollution

Control of contaminants from the source of origin is a promising field of nanotechnology. The contamination strategies can be regulated by different methods. In decreasing pollutants at the point of source, the controlling procedure is in situ, while degradation procedure is fundamentally ex situ. Use of a reduced amount of complex products and disposable matters prevents release of waste product and pollutants at manufacturing sites and controls mechanism for the involvement into natural resources. Exclusion of dangerous intermediate and by-product formation reduces energy consumption. Green synthesized metal nanoparticles are

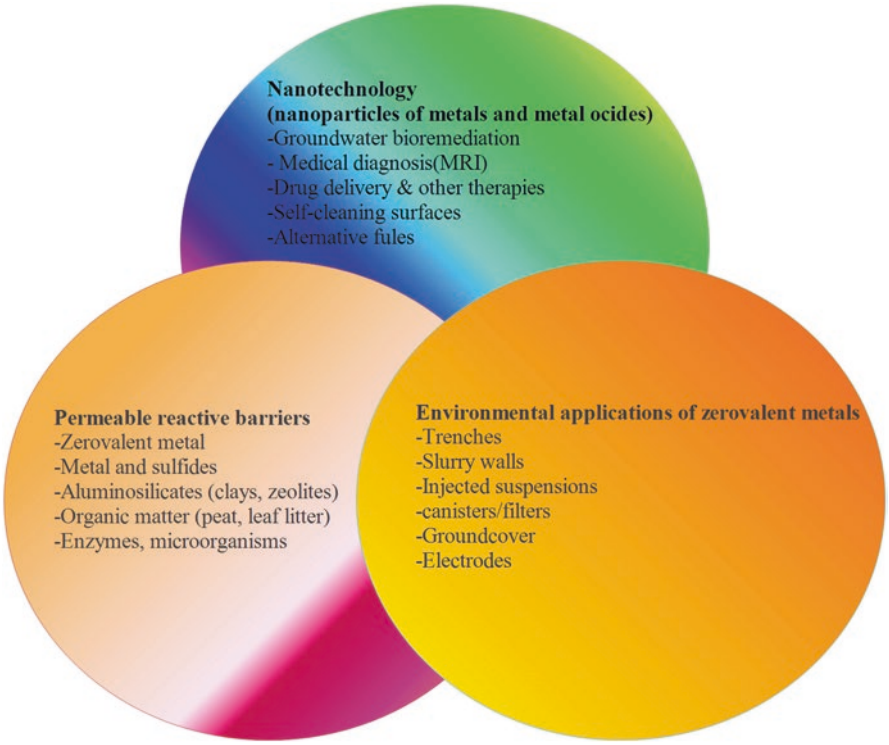

Fig. 4.6 Interconnected relationships between environmental applications of nanoparticles and other established and emerging environmental technologies

proficiently used in bioremediation and biofuel generation. They are used to produce hydrogen via oxidizing the carbon monoxide which can be used as fuel cell and to reduce several organic chemical pollutants including para-nitro phenol. A current study has reported the degradation of para-nitro phenol to amino phenol in 30 min using green synthesized gold nanoparticles by *Trichoderma viride* as heterogeneous catalyst (Narayanan and Sakthivel 2010; Mishra et al. 2014). Along with mycodegradation ability, *Trichoderma viride* also illustrate an efficient mycocontrol activity against human pathogenic bacteria. Titanium dioxide nanoparticles have photocatalytic property that indicates catalytic reaction in the presence of light (Zielińska-Jurek and Hupka 2014). It also acts as an oxidizing agent in the presence of UV light, so it is also used in contamination control. The second method of contamination control is use of environmentally friendly nontoxic materials by the use of metal nanoparticles. It helps in sustainable development of the environment. Cathode ray tubes changed by the carbon nanotubes in computers, preventing the use of heavy metals like lead. Another example is the use of silicon and titanium dioxide in photovoltaic cell formation (Serrano et al. 2009).

4.6 Nanoparticles As a Sensor

Sensing the contaminants in environments is a vital step for controlling the contamination. There are different approaches for sensing the pollutants, but these methods are unable to determine the exact composition and nature of the contaminant under field conditions, and they are also time-consuming. Currently, nanotechnology plays an important role in sensing the contaminants by improving sensors more specific and sensible for environmental checking either by targeting the binding between the contaminant and the recognition element or optimizing the transduction and electronic interface to the sensing layer. Sensor can be used for the sensing of contaminants related to organic contaminants, inorganic contaminants, or biological organisms. Quantum dots are used as a fluorescent labeling system for detection of microorganisms. Zhu et al. (2004) reported that conjugation of antibody to quantum dots can be used for the detection of pathogenic microorganisms, viz., *Cryptosporidium* and *Giardia*, also it is used in detection of *Salmonella*, *Escherichia coli*, and *Staphylococcus* endotoxins. Quantum dots exhibit superior photostability and multiplexing analysis as compared to organic dyes. Apart from this, they are also useful in diagnosis of diseases by giving fluorescent color (Liu et al. 2007). Gold and silver nanoparticles have been used to detect heavy metals like lead, mercury, and cadmium, while silica nanomaterials are used for pesticide detection. Sugunan et al. (2005) functionalized gold nanoparticles with 11-mercaptoundecanoic acid and chitosan separately for the detection of heavy metal ions like lead, mercury, and cadmium. Mercaptoundecanoic acid and chitosan are metal chelating agents. Binding of heavy metal ions to these metal chelators results in aggregation of the metal nanoparticles yielding a shift in wavelength absorption and resulting change in color from red to blue. Though it is not exact for a particular ion, the detection of heavy metal ions in general was very sensitive. Liu and Lu (2004) developed a sensor for Pb detection for those gold nanoparticles which were coated with lead-dependent DNA enzyme. The combination of the exceptional optical properties of gold nanoparticles (AuNPs) with the inherent advantages of microfluidic devices can be used to detect ziram [zinc bis (dimethyldithiocarbamate)], a wide kind fungicide member of the dithiocarbamate (DTC) family of pesticides (Lafleur et al. 2012). SnO_2 and In_2O_3 are two metal oxides interesting for sensing application. SnO_2 shows good sensitivity to reducing gases (e.g., CO) and In_2O_3 to oxidizing ones (e.g., NO_2) depending, among other parameters, on the process of preparation and crystallite size. To investigate the sensing characteristics of the as-synthesized nanocrystals, SnO_2 and In_2O_3 have been deposited without adding of any binder or activation layers on a micro patterned alumina substrate and examined for their ability to detect trace levels of NO_2, CO, and CH_4 in air. Due to their specificity and targeted specificity of enzymes, SnO_2 and In_2O_3 have vast abilities in the areas of chemical conversions, biosensing, and bioremediation (Duran and Esposito 2000). Microbial enzymes can be stabilized by producing single-enzyme nanoparticles containing single-enzyme molecules surrounded by a porous organic/inorganic network of less than a few nanometers thick (Kim et al.

2006). Immobilized enzymes in biopolymers and carbon nanotubes are another approach for developing environmental nanobiosensors. Although their lifetime is less, several genetic modifications have been used for improvement. Tyrosine is used for the electrochemical detection of phenols and pesticides, when it conjugates with the gold nanoparticles. Tyrosinase is an oxidoreductase containing copper that catalyzes two disparate reactions: (1) the *o*-hydroxylation of monophenols (cresolase activity) and (2) the oxidoreduction of *o*-diphenols to *o*-quinones (catecholase activity) (Wu et al. 2003). Tyrosinase offers great potential for the progress of biosensors for the detection of mono- and diphenolic compounds (Karim and Fakhruddin 2012). Another example of single-enzyme-linked metal nanoparticles is catechol-sensitive magnetic core-shell (Fe_3O_4/SiO_2) nanoparticles with laccase enzyme. Catechol itself is a dangerous phenolic compound which affects the nerve center system of human beings, inhibits DNA replication, and leads to chromosomal aberration (Topping et al. 2007). Catechol concentrations can be determined in compost samples by using the laccase sensor (Tang et al. 2008). Zhang et al. (2007) reported the synthesis of Fe_3O_4 magnetic nanoparticles and the immobilization of laccase on the surface of nanoparticles as efficient approach for contamination control.

4.7 Nanoparticles Can Clean Up Environmental Pollutants

Nanoparticulate materials are new but not new. The chemical composition of nanomaterials may be the same as an equivalent material in bulk form, but nanoparticles can show totally new characteristics due to their high surface to volume ratio and because, at their small size, quantum effects come into play (Yan et al. 2013). For example, TiO_2 has a long history of application as a white pigment in high opacity paints for a long time. By contrast, nanoscale TiO_2 particles are transparent to visible light (wavelength 400–800 nm). Aluminum in cans is inoffensive, while nanoscale aluminum is highly explosive and can be used in rocket fuels. Hematite particles with a diameter of 7 nm adsorb Cu ions at lower pH values than particles with a diameter of 25–88 nm, showing one approach in which surface reactivity of iron oxide particles differs with reducing diameter (Madden et al. 2006). Nanomaterials may be significantly more reactive than larger particles because of their much greater surface area per unit of mass which makes them suitable in environmental remediation (Hochella et al. 2008). Zerovalent iron (ZVI) is reported to be used in reactive barriers at various sites all over the world for the elimination of organic and inorganic pollutants (Fu et al. 2014). ZVI efficiently dechlorinates many halogenated hydrocarbon compounds (Wang et al. 2014). The degradation is based on redox reactions, in which iron donates electrons to the pollutants, decreasing them to less toxic compounds. ZVI can also reduce the concentrations of nitrate, selenite, perchlorate, arsenate, arsenite, and chromate (Fu et al. 2014). The reaction rates of nanoscale zerovalent iron (nZVI) are 25–30 times more rapidly than the reaction rates of granular iron in the micrometer to millimeter range, and the

Table 4.2 Classification of bioremediation approaches involving nanoparticles

	In situ	Ex situ
Adsorptive	In situ sequestration of contaminants by adding binding agents, e.g., iron oxides	Extraction of contaminated solution, which is then treated with adsorbents, as in nanofiltration
Reactive	In situ reaction of nanomaterial, e.g., nZVI, with target contaminant	Extraction of contaminated solution, which is then treated with reactants, as in TiO_2 photooxidation

sorption capability is also much higher (O'Carroll et al. 2013). nZVI particles are able to have surface areas per unit of mass up to 30 times greater than larger-size powders of granular iron and can be 10–10,000 times more reactive. When nZVI comes into contact with air, it ignites spontaneously. In contrast, the reactivity of granular iron with oxygen gas is very slow, leading to slow oxidation (oxidizing) of the surface.

4.8 Soil and Groundwater Bioremediation with Metal Nanoparticles

Green remediation approaches can be classified as adsorptive or reactive and as in situ or ex situ (Table 4.2). The use of nanomaterials in all these scenarios has been studied. In soil and groundwater remediation, in situ applications seem to be most promising as they are in general less costly (Hodson 2010). For in situ treatment, it is essential to produce either an in situ reactive zone with relatively fixed nanoparticles or a reactive nanoparticle plume that migrates to polluted zones. For applications in topsoil, metal nanoparticles can be worked into the surface of the polluted soil using conventional agricultural practices. Approaches targeting soil pollution indirectly affect the quality of the groundwater and vice versa. Little work is being carried out on topsoil remediation using metal nanoparticles. Contamination of groundwater and soil with carcinogenic organic substances or/and metals occurs all around the world. Groundwater sources are also frequently polluted with halogenated compounds or pesticides. Landfill leakage, agriculture, and chemical accidents are the main sources of these contaminants. Conventional remediation technologies include in situ thermal treatment, use of reactive barriers and chemical oxidation, ex situ soil washing and pump-and-treat operations, and iron treatment (Hodson 2010). Soil and groundwater remediation is generally very expensive, and conventional approaches are not always successful, or they take a long time for the remediation to become effective. Bezbaruah et al. (2009) state that pump-and-treat methods need several years of process on average, compared to 1–2 years for a usage with nZVI. New and more effective applications are therefore needed. Table 4.3 gives examples of the current use of nanoparticles in remediation.

Table 4.3 Samples of the use of nanoparticles in bioremediation

Process exploited	Nanomaterials used	Target compounds
Photocatalysis	TiO_2	Organic pollutants
Adsorption	Iron oxides, dendrimers	Metals, organic compounds, arsenic
Redox reactions	Nanoscale zerovalent iron (nZVI), nanoscale calcium peroxide	Halogenated organic compounds, metals, nitrate, arsenate, oil

4.8.1 Adsorption by Nanoparticles

In recent years, nanoscale materials have considered for their potential as adsorbents. Iron oxides are able to strongly adsorb metals in soils. Adding nanoscale metal oxides to soils will thus immobilize soil metals (Shipley et al. 2011). A mixture of iron and iron oxide has also been displayed to be effective in phosphate elimination and even more effective than higher cost produces, for example, activated alumina while being active for even longer periods (Shipley et al. 2011). Green rust a very reactive iron oxide can be used to decrease Cr(VI) to Cr(III), which is not soluble and much less toxic than the mutagenic Cr(VI) (Xu et al. 2012). Carbon-based nanomaterials, such as dendrimers and polymers, are also currently being explored for the elimination of metals and organics from soils and groundwater (Wang et al. 2013). Iron oxide minerals also can be used to adsorb arsenic, in addition of metals (Wu et al. 2012). Arsenic in groundwater in the Bengal region of Southeast Asia and elsewhere has been found to be a major danger to the health of millions of people who use these waters for cooking, drinking, and irrigation (Smedley and Kinniburgh 2013). Iron oxide NPs can bind arsenic five to ten times more efficiently than larger particles (Rickerby and Morrison 2007). In laboratory tests, more than 99% of the arsenic in water was bounded by iron oxide (12 nm) nanoparticles. This approach is thus some 2500–25,000 times more efficient than current systems, at least at the laboratory scale. Hua et al. (2012) found that nanoscale magnetite particles can be used efficiently to immobilize phosphate in soil via adsorption. They compared the immobilization efficiency of micro/nanoscale particles stabilized by a coating of carboxymethyl cellulose and found that only nanoscale particles were capable to penetrate the soil column. Non-stabilized "nanomagnetite" could not pass through the soil column under gravity because it quickly agglomerated into microparticles. The coated NPs were more transportable due to their small size and the increased negative charge related with the carboxymethyl groups. So, a large proportion (72%) of the coated nanoparticles could pass through the column due to their charge repulsion with the negatively charged soil particles. Transport over a definite distance is required to achieve a well distribution of the reactive particles in the soil matrix and so an even adsorption capability for phosphate. Much research has already been conducted on nano-sorbents, and although the consequences are promising, there are only a few commercial applications. The problems with using nano-sorbents in soils are like to those encountered with conventional adsorbents (Mueller and Nowack 2010).

Nanoparticles used as adsorbent for the elimination of heavy metals should have high adsorption capacity, be nontoxic, the ability to adsorb contaminants in less concentration (ppb), adsorb pollutants that can be easily eliminated from adsorbent surface and can be recycled for numerous times (Cloete 2010).

4.8.2 Nanoscale Zerovalent Iron (nZVI)

The use of nZVI in groundwater bioremediation is the most widely investigated environmental nanotechnological technique and has considerable potential benefits (Crane and Scott 2012). Field-scale commercial applications of nZVI have already become common in the United States, and also there have already been a few projects in Europe (Mueller and Nowack 2010; Fajardo et al. 2012). A web-based list of the sites where nZVI has been applied is available at http://www.nanotechproject.org/inventories/remediation_map. Competition among suppliers has led to a significant drop in the price of nZVI materials. nZVI can be produced by top-down (milling) or bottom-up (chemical synthesis) processes (Tratnyek and Johnson 2006; Sun et al. 2006). Due to its high reactivity, nZVI must always be handled as slurry. This requires some additional infrastructure above ground because the suspension has to be remixed immediately before its injection. nZVI is injected to form a reactive barrier of iron particles. nZVI is injected in surface-modified form (e.g., coated with surfactants or cellulose/polysaccharides) to create a plume of reactive iron, which destroys any organic pollutants within the aqueous phase. Some studies have displayed that nZVI as a reactive barrier is very efficacious in the reductive degradation of halogenated solvents, such as brominated methanes, chlorinated methanes, trihalomethanes, chlorinated ethenes, chlorinated benzenes, and also polychlorinated hydrocarbons, in groundwater (Shi et al. 2011). nZVI has also been displayed to be effective against pesticides and dyes (Son et al. 2006). Efficient elimination by nZVI of polycyclic aromatic hydrocarbons (PAHs) adsorbed to soils was described at room temperature (Xiu et al. 2010), while under the same conditions only 38% of the polychlorinated biphenyls (PCBs) has been destroyed because of the very strong sorption of PCBs to the soil medium (Varanasi et al. 2007). Tratnyek and Johnson (2006) used nZVI in real-world groundwater bioremediation that has a particle size larger than 100 nm and is thus strictly talking outside the standard definition of nanoparticles size. These authors also stated that the mobility of nZVI will be less than a few meters under nearly all related conditions as nZVI tends to aggregate, generating clusters that may have several micrometers in size and thus be easily removed from the pore water. Companies are consequently functionalizing nZVI particles to stop them from aggregating, such as surfactants or polymers. Other approaches combine the nZVI with carbon platelets or embed the nZVI in oil droplets to simplify particle delivery into the polluted area (Mueller and Nowack 2010). In the United States, it is common to combine the nZVI with other metals, for example, palladium, to grow the reactivity. In Europe, bimetallic particles are not used due to their possible toxicity and the limited additional benefit (Mueller and

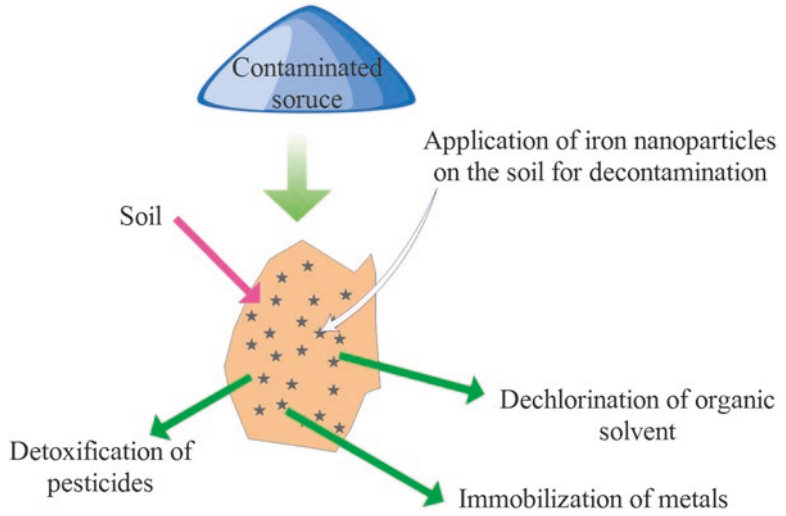

Fig. 4.7 Potential bioremedial scheme for ZVI nanoparticles

Nowack 2010). Tratnyek and Johnson (2006) showed that high reactivity tends to correlate using low selectivity. For this goal, remediation with nZVI may be inefficient because nZVI particles may react with nontarget substances, counting dissolved oxygen, nitrate, sulfate, and water. This also implies that nZVI will have a limited lifetime in porous media and reinjections of nZVI may be necessary, which makes the treatment costlier. On the other hand, the short in situ lifetime of nZVI may be beneficial by preventing unwanted exposure of the environment and humans to nZVI. Injection of nZVI particles caused significant changes in redox chemistry, while other physicochemical and hydrochemical parameters of the groundwater were not negatively affected. The limited penetration depth and lateral dispersion of the nZVI led to the cleanup of only a small part of the test site. Results from other pilot sites have shown that the oxidation reduction potential in a soil decreases significantly following nZVI addition, while the pH increases slightly. In the United States, many of the contaminated sites are on military bases. The US Navy and NASA have used nZVI in remediation for a number of years and have had positive experiences. The use of nZVI has been shown to be successful in the remediation of groundwater in porous soils. However, not much is known about the efficiency of the treatment in soils that are not saturated with water. The main challenge to date is the limited mobility of the particles. The actual efficiency of nZVI remediation depends on the geo- and hydrochemistry of the site. Zerovalent iron (Fe°) nanoparticles illustrate the redox activity to detoxify the organic and inorganic contaminants. Fe0 and bimetallic Fe° particle and Fe°/Pd°, Fe°/Pt°, Fe°/Ag°, Fe°/Ni°, and Fe°/Co° have widely been used in environmental remediation (Fig. 4.7).

4.8.3 Manganese Oxide (MnO) Nanoparticles

Manganese oxide (MnO) NPs illustrate high adsorption capability due to their high Brunauer-Emmett-Teller (BET) surface area and polymorphic structure (Yin et al. 2010). They have been widely used for the elimination of various heavy metals such as arsenic from wastewater (Fu and Wang 2011). Most frequently used modified MnOs contain nanoporous or nanotunnel manganese oxides and hydrous manganese oxide (HMO) (Gupta et al. 2015). HMO was prepared by adding MnSO4·H2O into NaClO solution. Modified HMO has around 100.5 $m^2\ g^{-1}$ BET surface areas (Zaman et al. 2009). Adsorption of different heavy metals such as Pb (II), Cd (II), and Zn (II) on HMOs usually happens due to the inner-sphere formation mechanism that can be defined by ion-exchange process (Gupta et al. 2015). Adsorption of divalent metals on the surface of HMOs occurred in two steps; metal ions adsorb on the external surface of HMOs and then follow by intraparticle diffusion (Parida et al. 1981).

4.8.4 Zinc Oxide (ZnO) Nanoparticles

Zinc oxide (ZnO) has a porous micro-/nanostructure with high BET surface area for the adsorption of heavy metals. Nanoassemblies, nanoplates, microspheres with nanosheets, and hierarchical ZnO nanorods are widely used as nanoadsorbent for the elimination of heavy metals from wastewater (Ge et al. 2012; Kumar et al. 2013). The above-mentioned modified forms of ZnO nanoadsorbent illustrated high elimination efficiency of heavy metals as compared to commercial ZnO. Wang et al. (2010) used ZnO nanoplates and porous nanosheets for the elimination of Cu (II) from wastewater. These modified ZnO nanoadsorbent showed high elimination efficiency of Cu (II) due to their unique micro-/nanostructure as compared to commercial ZnO. Besides, nanoassemblies were used for the elimination of various kinds of heavy metals which includes Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, and As^{3+} (Singh et al. 2013). Microporous nanoassemblies display high affinity for the adsorption of Pb^{2+}, Hg^{2+}, and As^{3+} due to their electropositive nature (Gupta et al. 2015). Kumar et al. (2013) reported the high elimination efficiency of Pb (II) and Cd (II) from wastewater by using mesoporous hierarchical ZnO nanorods.

4.8.5 Magnesium Oxide (MgO) Nanoparticles

Magnesium oxide (MgO) nanoparticles are used for the removal of diverse kinds of heavy metals from polluted water. MgO microspheres are novel structures, which can enhance the adsorption affinity for the elimination of heavy metals (Gupta et al. 2015). To grow the adsorption capacity of MgO, different types of modification

were carried out in NPs morphology to produce nanorods (Engates and Shipley 2011), nanobelts, and fishbone fractal nanostructures induced, by nanowires, nanotubes, nanocubes, and three-dimensional entities (Li et al. 2003; Klug and Dravid 2002). These modified structures were reported to have efficient adsorption of Pb (II) and Cd (II) on flowerlike mesoporous MgO.

4.8.6 Nanoscale Calcium Peroxide

Nanoscale calcium peroxide has lately been used for the cleanup of oil spills (Qian et al. 2013). Some projects have been carried out in New Jersey, USA. Two American companies are using nanosized calcium peroxide as an oxidant in the bioremediation of soils containing different organic contaminants, such as heating oil, gasoline, ethylene glycol, methyl tertiary butyl ether, and solvents. Nanoscale calcium peroxide is claimed to be highly efficient in eliminating aromatics and is also used in enhanced green remediation. The oxygen generated in the reaction of calcium peroxide with water leads to an aerobic environment that supports natural green remediation by aerobic organisms present in the soil.

4.8.7 Nanobioremediation of Soil Pollutants

Soil is a precious natural resource which contributes the major part in economy. Economy in many countries depends on agriculture. Due to anthropogenic activity, land is subjected to pollutants from industries, metal from mines, and household discharges. Soil remediation has been done by excavation followed by incineration landfilling, but this process takes several months and also produces dangerous gases and by-products. Green technology method of the bioremediation does not damage the environment and also decreases the amount of energy used in chemical processes (Tungittiplakorn et al. 2004; Theng and Yuan 2008). Pollutants continuously released from the industries and houses mix with soil present in environment. Plants are also able to bioremediate the soil pollutants by extracting of heavy metals. Plants accumulate the heavy metals by their cellular transport channel and get accumulate into the vacuole, but due to less biomass and bioavailability of the aerial parts, they are not comparable to microbial degradation in pollutant remediation. Native microflora in soil also contributes in remediation of organic and inorganic pollutants in it. Several microflora which are present in polluted sites have a good tolerance capability in comparison to non-polluted soil; these microbes chelate the heavy metal present in higher amount, or sometimes it may convert and accumulate the metal into metal nanoparticle of corresponding salt or ions present in soil. These green synthesized nanoparticles can be extracted from the microbe and used in industrial purpose or can enhance the soil and plant growth activity (Mishra et al. 2014; Gholami-Shabani et al. 2015). Zerovalent iron is usually used for soil remediation.

It has been shown that application of heavily chlorinated pesticides in soil and environment gradually changes the pH of the soil (Sayles et al. 1997). These irons are used in field to reduce the chlorinated compounds. Malathion is a wide spectrum nonsystemic organophosphate insecticides (Singhal and Rogers 2012) used in agriculture. Half-lives of malathion are 1–25 days depending on the soil binding, soil condition, the process enhanced in the existence of sunlight, and moisture/alkaline condition.

4.9 Mycoremediation of Toxic Weapons

Clearly, there is scope for the use of fungi in decomposing in situ intractable, persistent, and highly toxic pollutants, including TNT (2,4,6-trinitrotoluene) and the nerve gases VX and sarin (Rhodes 2014). By inoculating a plot of soil contaminated by diesel oil, with mycelia from oyster mushrooms (*Pleurotus ostreatus)*, it was found that after 4 weeks, 95% of polycyclic aromatic hydrocarbons (PAHs) had been converted to nontoxic compounds. It seems that the naturally present community of microbes acts in concert with the fungi to decompose the pollutants, finally to CO_2 plus H_2O (full mineralization). In 2007, a cargo ship spilled 58,000 gallons of fuel along the San Francisco shoreline (Incardona et al. 2008). Mats woven from human hair (resembling doormats) were used as sponges to soak up the spilled oil. These were then collected and layered with oyster mushrooms and straw: the mushrooms broke down the oil, and after several weeks the ensuing soil was clean enough to be used for wayside landscaping. Wood-degrading fungi are extremely effective in decomposing toxic aromatic contaminants from petroleum and also chlorinated persistent pesticides (Rhodes 2013). Mycofiltration is a similar way, in which mycelia are used as a filter to remove toxic materials and microorganisms from water in the soil. Stamets (2005) has suggested that there should be "mycological response teams," who would employ fungi to recycle and rebuild healthy soil in the area following any pollution incident (oil spill, chemical leak, radiation egress, e.g., at Fukushima). It has been suggested that edible mushrooms might be grown for the purposes of mycoremediation, and the visions of whether they would be safe to eat afterwards are considered (Kulshreshtha et al. 2014). Naturally this depends on the exact nature of the contaminants, consequently that heavy metals are likely be a problem (if they are absorbed and concentrated into the mushroom), while some organic soil contaminants might be decomposed without so imparting toxicity. In the latter case, the benefit is proposed that land that is contaminated and unfit for agriculture could be both cleaned and made to yield a nutritious food crop.

4.10 Conclusions

In an ever-increasing field of development, environment pollution has become a major concern. To combat with pollution, nanotechnology has emerged as a powerful tool to make the environment clean. It can act as sensor to detect pollutants and controls the release of pollutant and has the potential to remediate in situ and ex situ. Zerovalent iron, silver, gold, titanium, quantum dots, and carbon nanotubes have showed their potential as efficient sensors and remediators. Gold and silver nanoparticles act very well as heterogeneous catalysts for degrading environmental pollutants such as para-nitro phenol. With the use of microbial systems to synthesize nanoparticles, they are giving an added advantage of clean and green nanotechnology in environmental cleanup. Though the field is rapidly increasing, the exact mechanism of biosynthesis and bioremediation through metal nanoparticles remains unknown which needs to be more explored. Metal nanoparticles will be used as a transporter for supportable delivery of pesticides and chemical fertilizers for efficient, less, and equal distribution in soil. Soil microflora can be used to synthesize metal nanoparticles and further can be used as green remediators to elucidate the role of microbes in biosorption of heavy metals from contaminated area and extract for industrial purpose. Microbes can be used as a factory for various purposes to explore the nanoremediation along with fertility of the soil and maintaining the balance in ecosystem. Nanoparticles can also limit the use of pesticides by biosynthesizing the nanoparticles by native microbes which is emerging as a new technology for mankind to protect their crop. The nascent field of green nanotechnology needs to be bloomed up to make the earth greener and clean with the rapid advancement of eco-friendly microbial synthesis procedures.

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Chapter 5
Fungal Nanotechnology: A New Approach Toward Efficient Biotechnology Application

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

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

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5.1 Introduction

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

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

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

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

Table 5.1 Synthesis of different nanoparticles (NPs) by fungi

Fungi	NPs	Size (nm)	Shape	Pathway of biosynthesis	Application	Reference
Aspergillus terreus HA1N	AgNPs	10–18	Spherical	Extracellular	Antifungal activity	Ammar and El-Desouky (2016)
Aspergillus niger	AgNPs	20	Spherical	Extracellular	–	Ghazwani (2015)
Aspergillus niger	AgNPs	43–63	Spherical	Extracellular and intracellular	Antibacterial activity	Vanaja et al. (2015)
Aspergillus niger PFR6	AgNPs	8.7	Spherical	Extracellular	–	Devi (2015)
Aspergillus sp.	AgNPs	–	–	Extracellular	Antimicrobial activity	Prabavathy and Vaishnavie (2015)
Aspergillus tamarii PFL2	AgNPs	3.5	Spherical	Extracellular	–	Devi (2015)
Penicillium expansum HA2N	AgNPs	14–25	Spherical	Extracellular	Antifungal activity	Ammar and El-Desouky (2016)
Penicillium chrysogenum	AgNPs	30–150	Spherical	Extracellular and on the hyphal surface	–	Mohammadi and Salouti (2015)
Penicillium expansum	AgNPs	50–200	Spherical	Extracellular and on the hyphal surface	–	Mohammadi and Salouti (2015)
Penicillium spinulosum (OC-11)	AgNPs	25	Spherical	Extracellular	Promoted wound healing antibacterial activity	Wen et al. (2016)
Penicillium decumbens (MTCC-2494)	AgNPs	30–60	Spherical	Extracellular	Antimicrobial activity	AbdelRahim et al. (2017)
					Anticancer activity against human lung cancer cell line	
Penicillium ochrochloron PFR8	AgNPs	7.7	Spherical	Extracellular	–	Devi (2015)
Fusarium oxysporum	AgNPs	34–44	Spherical	Extracellular	Antibacterial activity	Hamedi et al. (2016)
Fusarium oxysporum	AgNPs	5	Spherical	Extracellular	–	Ghazwani (2015)

(continued)

Table 5.1 (continued)

Fungi	NPs	Size (nm)	Shape	Pathway of biosynthesis	Application	Reference
Rhizopus stolonifer	AgNPs	2.86	Spherical	Extracellular	–	Khalid AbdelRahim et al. (2017)
Arthroderma fulvum	AgNPs	15.5	Spherical	Extracellular	Antifungal activity	Xue et al. (2016)
Lentinus edodes, *Pleurotus ostreatus*, *Ganoderma lucidum*, *Grifola frondosa*	AgNPs	5–20	Irregularly spheres	Extracellular	–	Vetchinkina et al. (2017)
Alternaria solani	AgNPs	25	Spherical	Extracellular	–	Ghazwani (2015)
Mucor hiemalis	AgNPs	5–15	Spherical	Extracellular	Antibacterial and antifungal activities	Aziz et al. (2016)
Guignardia mangiferae	AgNPs	5–30	Spherical	Extracellular	Antimicrobial activity	Balakumaran et al. (2015)
					Cytotoxicity against human cancer cells	
Curvularia lunata	AgNPs	10–50	Spherical	Extracellular	Antibacterial activity	Ramalingmam et al. (2015)
Pestalotiopsis microspora VJ1/VS1	AgNPs	2–10	Spherical	Extracellular	Antioxidant activity	Netala et al. (2016)
					Cytotoxicity activity	
Sclerotinia sclerotiorum MTCC 8785	AgNPs	10	Spherical	Extracellular	Antibacterial activity	Saxena et al. (2016)
Rhizopus oryzae	AuNPs	16–43	Spherical	Extracellular (purified surface proteins)	Hemocompatibility	Kitching et al. (2016)
			Flower			
Aspergillus aculeatus	AuNPs	60–140	Microtubules	Depositing AuNPs on the surface of fungi biotemplates	–	Kubo et al. (2016)
Aspergillus sydowii	AuNPs	8.7–15.6	Spherical	Intracellular and extracellular	–	Vala (2015)

Fungi	NPs	Size (nm)	Shape	Pathway of biosynthesis	Application	Reference
Cladosporium oxysporum AJP03	AuNPs	72.32	Quasi-spherical	Extracellular	Degradation of rhodamine B	Bhargava et al. (2016)
Penicillium brasilianum	AuNPs	80–500	Microtubules	Depositing AuNPs on the surface of fungi biotemplates	–	Kubo et al. (2016)
Lentinus edodes, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Grifola frondosa*	AuNPs,	5–50	Spherical	Intracellular or on the surface of the hyphae	–	Vetchinkina et al. (2017)
Alternaria sp.	AuNPs	7–18	Spherical, square, pentagonal, hexagonal	Extracellular		Dhanasekar et al. (2015)
Pycnoporus sanguineus	AuNPs	10–500	Spherical, triangular, pentagonal	Intracellular	Degradation of 4-nitroaniline	Shi (2015)
Penicillium sp.	Fungus-Fe_3O_4	<50	Spherical	Magnetic core and a mycelia layer	–	Ding et al. (2015)
Lentinus edodes, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Grifola frondosa*	Silicon NPs	50–250	Spherical	On the hyphal surface	–	Vetchinkina et al. (2017)
L. edodes and G. frondosa	SeNPs	20–200	Spherical	Intracellular or on the hyphal surface	–	Vetchinkina et al. (2017)
Fusarium oxysporum	Tb_2O_3 NPs	10	Spherical	Extracellular	Cytotoxicity activity	Iram et al. (2016)

5.2 Biosynthesis of NPs by Fungi

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

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

5.2.1 Silver Nanoparticles

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

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

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

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

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

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

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

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

5.2.2 Gold Nanoparticles

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

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

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

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

The synthesis of NPs not only is made by extracellular metabolites but also by proteins bound to the cell surface which showed a significant biomineralization activity. In fact, several studies have shown that cell surface proteins of *Rhizopus oryzae* play a crucial role in biomineralization of Au (III) to produce gold NPs. Cell surface proteins are able to reduce Au^{3+} to later produce the nucleation and growing of Au crystals (Kitching et al. 2016). Other proteins act as capping agents, thereby controlling the size of the gold NPs (Kitching et al. 2016; Das et al. 2009).

5.2.3 Magnetic Nanoparticles

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

Bharde et al. (2006) focused their studies in the biosynthesis of magnetic NPs. The authors observed that when *Fusarium oxysporum* and *Verticillium* sp. were exposed to an aqueous solution of a 2:1 molar mixture of $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$, extracellular magnetite was synthesized. The proteins secreted by *Fusarium oxysporum* and *Verticillium* sp. hydrolyzed iron precursors extracellularly to form iron oxide prevailing in the magnetite (Fe_3O_4) at room temperature. Particularly, the fungi were capable of hydrolyzing metal ion precursors under acidic conditions. Protein analysis suggests the induction of two proteins of molecular weights of 55 and 13 kDa, which might be responsible for the hydrolysis of magnetite precursors and/or the capping of magnetite particles. The magnetite biosynthesis mentioned above presents a simple and aerobic route for the magnetic nanomaterial synthesis.

New techniques for the synthesis of magnetic NPs have been evaluated. One of them consisted in the growing up of magnetite NPs (nano-Fe_3O_4) on the mycelium

of fungus *Penicillium* sp. (Ding et al. 2015). Given a large number of functional groups naturally present on the mycelium surface, the assembly of nano-Fe_3O_4 is a relatively simple process. More important to this is that nano-Fe_3O_4 could self-assemble on the fungus template accompanied with forming mycelia pellet in a mild case, whereas the chemical synthesis to obtain similar product requires extreme pH and temperature environment. Moreover, *Penicillium* sp. formed mycelia pellets under submerged shaking cultivation conditions which enrich functional groups on the surface (Mishra 2013).

Fungus-Fe_3O_4 presented a composite structure by nano-Fe_3O_4 particles uniformly adhered on the surface of *Penicillium* sp. which improve the dispersion and stability of nano-Fe_3O_4 particles, avoiding the pollution resulting from the nano-Fe_3O_4 particles (Ding et al. 2015).

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

5.2.4 Other Metal Nanoparticles

Although the silver, gold, and magnetic NPs are the most studied NPs synthesized by fungi, there are other NPs with interesting properties. In fact, the first NP synthesis by fungus was reported as CdS NPs in addition to the formation of PbS, ZnS, and MoS_2 NPs (Iram et al. 2016).

Zirconia nanoparticles have been produced by *F. oxysporum* with aqueous ZrF_6^{-2} anions. Extracellular protein-mediated hydrolysis of the anionic complexes at room temperature results in the synthesis of nanocrystalline zirconia (Bansal et al. 2004). A strain of *F. oxysporum f.* sp. *lycopersici* was screened and successfully produced intra- and extracellular platinum NPs. Riddin et al. (2006) reported the synthesis of zinc, magnesium, and titanium NPs by using six *Aspergillus* by employing several salt precursors of nitrates, sulfates, chlorides, and oxides.

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

Iram et al. (2016) report for the first time the synthesis of terbium oxide NPs using *Fusarium oxysporum*. The biocompatible terbium oxide NPs (Tb_2O_3 NPs) were synthesized by incubating Tb_4O_7 with the biomass of the fungus. Physical characteriza-

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

5.3 Mechanisms of Nanoparticle Biosynthesis

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

5.3.1 Intracellular Synthesis

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

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

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

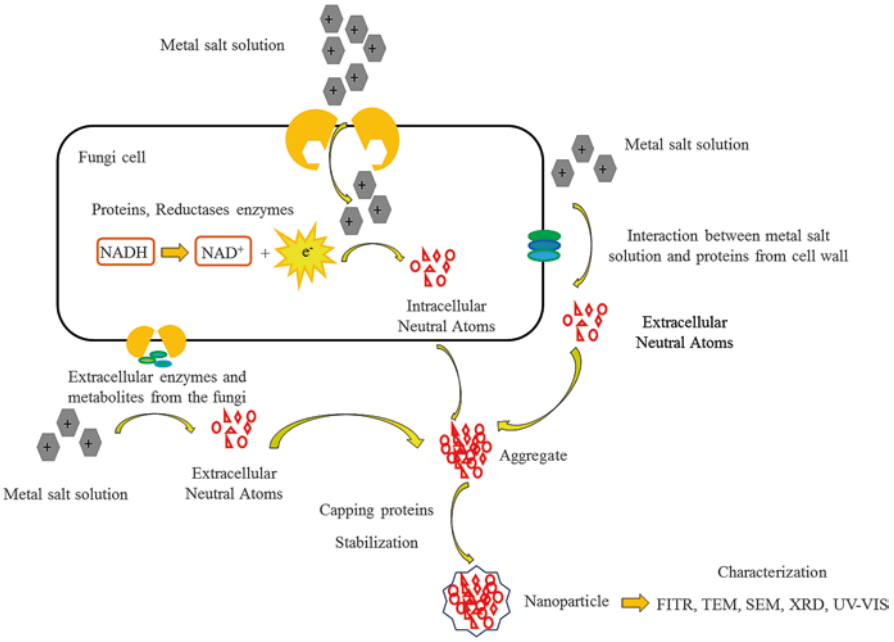

Fig. 5.1 Schematic synthesis of nanoparticles (NPs) by fungi

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

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

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

5.3.2 Extracellular Synthesis

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

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

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

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

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

5.3.3 Biomolecules Responsible for Nanoparticle Synthesis

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

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

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

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

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

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

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

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

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

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

5.4 Nanoparticle Applications

5.4.1 Antimicrobial Activity

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

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

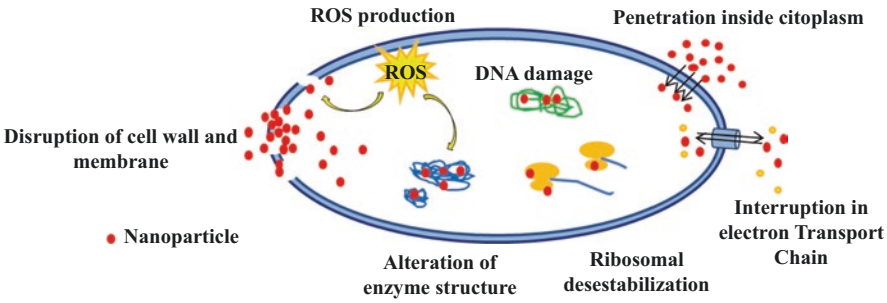

Fig. 5.2 Schematic mechanism of antibacterial activity of nanoparticles (NPs) produced by fungi

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

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

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

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

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

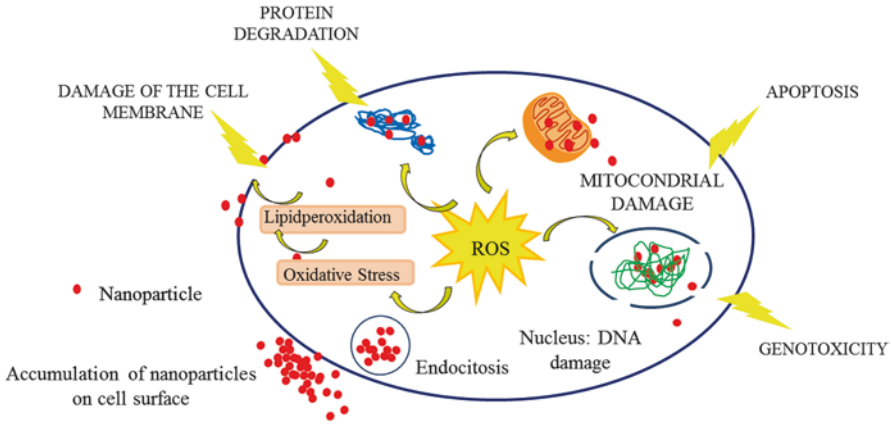

Fig. 5.3 Schematic mechanism of cytotoxic activity of nanoparticles (NPs) produced by fungi

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

5.4.2 Cytotoxicity

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

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

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

Netala et al. (2016) biosynthesized silver NPs from fungus *Pestalotiopsis microspora* VJ1/VS1 and probe its effects on the following cancer cells: B16F10 (mouse melanoma), SKOV3 (human ovarian carcinoma), A549 (human lung adenocarcinoma), PC3 (human prostate carcinoma), and NPs biocompatible toward normal cells (Chinese hamster ovary cell line). Cytological observations of SKOV3 cells (which were the most susceptible) showed apoptotic changes including pyknotic nuclei, cell membrane blebbing, cell shrinkage, and karyorrhexis followed by destructive fragmentation of nuclei. The mentioned results were very hopeful and provide the bases for the development of versatile biomedical applications of biosynthesized silver NPs for cancer therapy.

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

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

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

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

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

5.4.3 Fine Chemical and Pharmacology

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

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

Magnetic particles have been increasingly used as carriers for enzymes, binding proteins, antibodies, and drugs. Thus, the new biological material can be used directly as affinity ligands to capture or modify target molecules or cells or for a bioassay (Bickerstaff 1997). Immobilization of proteins and enzymes on magnetic NPs is an important area of interest. Several magnetic NPs – magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) – have been evaluated with promising applications.

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

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

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

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

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

5.4.4 Bioremediation

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

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

5.4.5 Food Safety

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

5.4.6 Plant Disease Management

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

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

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

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

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Chapter 6
Advances in Biomedical Application of Chitosan and Its Functionalized Nano-derivatives

Jobina Rajkumari and Siddhardha Busi

Abstract The recent advances in nanotechnology open new avenues for the development of functionalized nanomaterials with wide potential application. Chitosan has become one of the most promising biopolymers with wide application in diagnostics and therapeutics. It is a linear copolymer of β-(1–4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glycopyranose, with a varying content of N-acetyl groups. It is obtained by deacetylation of parent polymer, chitin, and also occurs naturally in fungal species such as *Absidia glauca*, *Absidia coerulea*, *Aspergillus niger*, *Mucor rouxii*, *Gongronella butleri*, *Phycomyces blakesleeanus*, *Absidia blakesleeanus*, *Rhizopus oryzae*, *Trichoderma reesei*, and *Lentinus edodes*. Chitosan can also be directly extracted from fungi by alkaline/acid treatment and by use of microorganisms/proteolytic enzymes. Unlike chitin, chitosan is readily soluble in dilute acetic acid and widely used in preparation of gels, films, and fibers. The production of the biopolymer is generally influenced by parameters such as the nutritional factors, mode of cultivation, temperature, pH, and mineral salts. In therapeutics, chitosan and chitosan-based materials are used as antimicrobial, antitumor, antiulcer, antidiabetic, and a cholesterol-lowering agent. Being a naturally occurring polysaccharide, chitosan and its functionalized derivatives exhibit unique properties, such as biocompatibility, biodegradability, biological activity, and low toxicity. The conformational flexibility of chitosan is attributed to the presence of the free primary amino groups which makes chitosan an ideal candidate for biofabrication. Various methods, such as ionic gelation, desolvation, spray-drying, and covalent cross-linking, have been employed for functionalization of chitosan. Nanoparticles and its biofabrication impart desirable functional characteristics to chitosan. The molecular weight and the concentration of chitosan used along with an amount of cross-linking govern the physical properties of chitosan nanoparticles formed. Chitosan nanocomposites have shown to improve the dissolution rate of poorly soluble drugs and, thus, are exploited for enhancement of drug

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bioavailability and delivery. Various therapeutic agents, such as anticancer, anti-inflammatory, antibiotics, antithrombotic, steroids, proteins, amino acids, antidiabetic, and diuretics, have been incorporated in chitosan nanocomposites. The controlled release of therapeutic agents opened new windows in drug delivery and bio-imaging techniques using chitosan. Hence, chitosan and its nano-derivatives serve as one of the sustainable and ecofriendly alternative to synthetic polymers in biomedical applications.

Keywords Chitosan · Drug delivery · Peptide · Antimicrobial · Emulsions · Antibiotics

6.1 Introduction

Henri Braconnot (1811) discovered the first natural polysaccharide, fungine, from the cell wall of fungi almost three decades before the isolation of cellulose, and in 1823, Odier renamed fungine as chitin (Akila 2014). The discovery of chitin was based on several studies carried out on fungal material isolated from mushroom, *Agaricus volvaceus*, *A. acris*, *A. cantharellus*, *A. piperatus*, *Hydnum repandum*, *H. hybridum*, and *Boletus viscidus* (Muzzarelli et al. 2012). Chitin, poly-(β-(1-4)-N-acetyl-d-glucosamine), is a natural polysaccharide synthesized by an enormous number of living organisms and is the second most abundant biopolymer after cellulose. In a wide number of invertebrates, chitin serves as a structural component and provides tensile strength. The well-ordered crystalline microfibrils of chitin occur in nature as exoskeleton of arthropods and crustaceans and as a cell wall component of fungi and yeast (Jayakumar et al. 2010). However, the structural complexity, tedious extraction procedure, and insolubility in aqueous and organic solvents greatly limit its practical applications (Dash et al. 2011; Cheung et al. 2015).

In 1859, Rouget, while studying the effect of deacetylation on the thermal and chemical changes of the chitin, discovered chitosan (Akila 2014). Chitosan (CS) is a high molecular weight linear polycationic heteropolysaccharide comprising copolymers of β-1,4-linked D-glucosamine and *N*-acetyl-D-glucosamine. Chitosan can be obtained either by (partial) deacetylation of chitin under alkaline conditions (concentrated NaOH) or by enzymatic hydrolysis by the aid of chitin deacetylase as shown in Fig. 6.1 (Duttagupta et al. 2015). However, for commercial production, chemical deacetylation is more preferred commonly due to economic issues and feasibility for scale up (Cheung et al. 2015).

The natural polysaccharide, chitosan, is biocompatible, biodegradable, and non-toxic. It also exhibits antimicrobial activity against a wide range of pathogens including bacteria, fungi, and yeast (Kong et al. 2010). All these characteristics make CS a promising agent for fabrication of antimicrobial compounds and development of more efficient nano-therapeutics. The chemical structure and molecular weight of chitosan govern its physiochemical properties. Hence, the low solubility

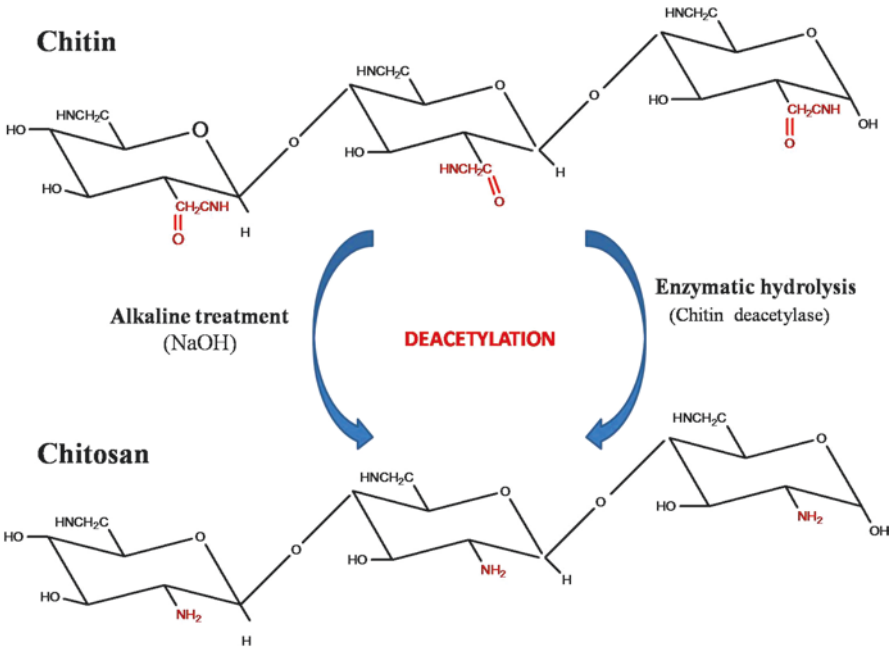

Fig. 6.1 Conversion of chitin to chitosan

of chitosan in acid-free aqueous media resulting due to its high molecular weight and highly viscous nature hinders its practical application. In recent years, advances have been made on modification and functionalization of chitosan for better applicability (Zhang et al. 2010). An increase in interest and developments are made in recent years in pharmaceutical as well as biomedical applications of chitosan and its nano-derivatives. Apart from its biocompatibility, the easily controllable size, surface charge density, loading efficiency, controlled release, etc. make chitosan nanoparticles a promising tool for biomedical applications (Mazancova et al. 2017).

Chitosan possess antitumor, immunoenhancing, antimicrobial, and hypocholesterolemic properties, and numerous research works have been attempted on chitosan and its nano-derivatives for their application in tissue engineering, drug delivery, wound healing, antitumor, and antimicrobial therapy (Zhang et al. 2010; Piras et al. 2015; Cheung et al. 2015).

6.2 Fungal Sources of Chitosan

In fungi, chitin is an important structural component which significantly contributes to the tensile strength and integrity of the fungal cell wall. It is the major component found in the septa between mother and daughter cells of *Saccharomyces cerevisiae*. It is also one of the main components of the hyaline outer wall of spores of

Table 6.1 Fungal sources of chitosan and their physiological properties

Fungal species	Degree of deacetylation (%)	Molecular weight (kDa)	References
Absidia coerulea	–	4.5×10^4	Niederhofer and Muller (2004)
Absidia spp	86	7×10^3	Kuhlmann et al. (2000)
Agaricus bisporus (SMR 13)	91	–	Mario et al. (2008)
Armillaria mellea (SMR 439)	92.7	–	Mario et al. (2008)
Aspergillus niger	90	1.4×10^5	Pochanavanich and Suntornsuk (2002)
Aspergillus niger ATCC 9642	89.6	2.5×10^4	Tayel et al. (2011)
Auricularia auricula-judae (SMR 54)	95.1	–	Mario et al. (2008)
Candida albicans	83.8	1.1×10^5	Pochanavanich and Suntornsuk (2002)
Lentinus edodes	86.5	1.9×10^5	Pochanavanich and Suntornsuk (2002)
Lentinus edodes (SMR 90)	92	–	Mario et al. (2008)
Mucor racemosus	84.4	–	Tajdini et al. (2010)
Mucor rouxii	89.8	4.5×10^4	Chatterjee et al. (2005)
Mucor rouxii DSM-1191	95	2.1×10^4	Tayel et al. (2010)
Pleurotus eryngii (SMR 755)	94.2	–	Mario et al. (2008)
Pleurotus ostreatus (SMR 684)	98.7	–	Mario et al. (2008)
Pleurotus sajor-caju	83.8	1.1×10^5	Pochanavanich and Suntornsuk (2002)
Rhizomucor miehei	80.6	–	Tajdini et al. (2010)
Rhizopus oryzae	86–90	$80–128 \times 10^3$	Kleekayai and Suntornsuk (2011)
Rhizopus oryzae	87.9	6.9×10^4	Pochanavanich and Suntornsuk (2002)
Trametes versicolor (SMR 117)	97	–	Mario et al. (2008)

arbuscular mycorrhizal glomus species. Chitosan occurs naturally in the mucorales such as *Mucor*, *Absidia*, and *Rhizopus* species (Table 6.1). In four edible mushrooms, *Lentinus edodes*, *Lycophyllum shimeji*, *Caju*, and *Volvariella volvacea*, chitin is present as a minor component in mycelia, the caps and stalks of fruiting bodies. Mario et al. (2008) reported the isolation of chitosan from the mycelium of seven species of *Basidiomycetes*, viz., *Mucor rouxii*, *Absidia glauca*, *Aspergillus niger*, *Gongronella butleri*, *P. sajor-caju*, *Rhizopus oryzae*, *L. edodes*, and *Trichoderma reesei*. The chitin yield was estimated between 8.5% and 19.6% dry

weight with <90% degree of deacetylation. However, chitin is not necessarily present in all fungi. Slime molds (*Myxomycetes*) and bacteria (*Schizomycetes*) are devoid of chitin (Akila 2014). However, chitosan is the most abundant component of both filamentous and yeast-like forms of *Mucor rouxii* (Tayel et al. 2010; Muzzarelli et al. 2012). In single-celled fungi-like yeast, the cell wall compose of 1–2% of chitin by dry weight, while in the case of filamentous fungi-like *Neurospora* and *Aspergillus*, chitin constitutes about 10–20% of the fungal dry weight (Bowman and Free 2008; Tayel et al. 2011). Pochanavanich and Suntornsuk (2002) investigated the ability to produce chitosan by four species of filamentous fungi, *Aspergillus niger*, *Rhizopus oryzae*, *Lentinus edodes*, and *Pleurotus sajor-caju*, and two yeast strains, *Zygosaccharomyces rouxii* TISTR5058 and *Candida albicans* TISTR5239. Fungal chitosan was produced at 10–140 mg/g cell dry weight, with the degree of deacetylation at 84–90% and a molecular weight of 2.7×10^4–1.9×10^5 Da with a viscosity of 3.1–6.2 centipoises (cP).

The physical and chemical condition of fermentation process can be manipulated to provide chitosan of more consistent physicochemical properties as compared to the ones derived chemically from chitin. Four fungal strains, *Aspergillus niger* TISTR3245, *Rhizopus oryzae* TISTR3189, *Zygosaccharomyces rouxii* TISTR5058, and *Candida albicans* TISTR5239, grown on soybean and mung bean residues resulted in the chitosan yields of 0.4–4.3 g/kg in soybean residue and 0.5–1.6 g/kg for mung bean residue. The highest amount of chitosan (4.3 g/kg) was obtained when *R. oryzae* was cultivated on soybean residue (Suntornsuk et al. 2002). Chitosan has been isolated from *Mucor rouxii* cultured in three different media, viz., molasses salt medium (MSM), potato dextrose broth (PDB), and yeast extract peptone glucose (YPG) medium under submerged condition, and their yield has been found to be almost the same, being 0.61 g/l for MSM, 0.51 g/l for PDB, and 0.56 g/l for YPG, respectively (Chatterjee et al. 2005). Kuhlmann et al. (2000) reported *Absidia* spp producing low molecular weight chitosan with a MW of 700 kDa and degree of deacetylation of 0.86. Niederhofer and Muller (2004) extracted chitosan with average MW 45 kDa from *Absidia coerulea*. Kleekayai and Suntornsuk (2011) documented the production of chitosan using *Rhizopus oryzae* with 86–90% degree of deacetylation, molecular weight of 80–128 kDa, and viscosity of 3.1–6.1 mPa.

6.3 Structure and Composition

Chitin serves as a fibrous structural component responsible for tensile strength and rigidity of fungal cell wall. The fungal chitin possesses predominantly the same structure to the ones present in exoskeleton of arthropods. However, a major difference is that the fungal chitin exists in association with other polysaccharides such as glucans and mannans which are absent in crustacean (Akila 2014). As shown in Fig. 6.1, Chitosan is a copolymer composed of N-acetylglucosamine and glucosamine units which are linked by β(1→4) linkage (Chattopadhyay and Inamdar 2013). In general, the individual chains of β-(1→4)-2-acetamido-D-glucose and

β-(1→4)-2-amino-D-glucose assume an essentially linear structure, which undergoes one full twist every 10.1–10.5°A along the axis of the chain (Dash et al. 2011). Chitosan exist in two allomorph forms, α and β, which could be elucidated by X-ray model and NMR studies. The α-chitin isomorphs are by far the most abundant and occur in the cell walls of fungi and yeast, in krill, tendons of crab, shrimp shells, insect cuticle, etc. On the other hand, β-chitin is found in association with proteins in squid pens and in the tubes synthesized by pogonophoran and vestimentiferan worms (Younes and Rinaudo 2015). Chitosan compose of three major reactive functional groups, an amino/acetamido group along with primary and secondary hydroxyl groups at the C-2, C-3, and C-6 positions, respectively. The amino groups contribute to the structural differences and physicochemical properties of the chitosan (Dash et al. 2011). The exact molecular mass of chitin has not yet been documented; however, it was estimated that *S. cerevisiae* synthesizes uniform chains of chitin constituting of 120–170 GlcNAc monomer units (~24,000–34,500 Da). In *S. cerevisiae* terminal reducing ends of chitin chains are attached though β (1, 4) or β (1, 2) linkages to the nonreducing end of β (1, 6) glucan. Attachment of chitin to glucan is catalyzed by chitin synthase. In addition, a mannoprotein is attached to β (1, 6) glucan through a glycosylphosphatidylinositol anchors containing five α-linked mannosyl residues (Akila 2014).

6.4 Physiochemical Properties of Chitosan

Chitin constitutes the cell walls and septa of fungi class, *Ascomycetes*, *Zygomycetes*, *Basidiomycetes*, and *Deuteromycetes*. It is a structural component and helps in maintaining the shape and integrity of fungi. Although many reports are available for the preparation of chitosan-based particles, a spectrum of chemical factors like molecular weight, degree of deacetylation, pH, ionic strength, temperature, rate of stirring, etc. greatly affects the overall physicochemical and biological properties of the resultant particles. The preparation process employed ultimately leads to the formation of inherently different particles in terms of size, cross-linking, density, loading capacity, surface charge density, colloidal stability, and release kinetics. The biological properties, such as analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobial, and antioxidant properties, also govern the physical properties of chitosan (Mazancova et al. 2017).

Modification of chitosan at the molecular level increases its solubility and stability (Cheung et al. 2015). The parent chitin is insoluble in most organic solvents; on the contrary due to the quaternization of the amine groups that have a pKa value of 6.3, chitosan is readily soluble in dilute acidic solutions below pH 6.0. At low pH, these amines present in chitosan get protonated making chitosan a water-soluble cationic polyelectrolyte. When the pH increases above 6, amines of chitosan gets deprotonated and the polymer loses its positive charge and becomes insoluble. The soluble-insoluble transition which occurs at its pKa value between pH 6 and 6.5 aids

in the functionalization of the chitosan. As the pKa value is highly dependent on the degree of deacetylation, the solubility of chitosan is in turn dependent on the degree of deacetylation (Dash et al. 2011). The degree of deacetylation and the molecular weight of chitosan were found to influence the physiochemical properties of chitosan (Cheung et al. 2015). The molecular weight and degree of deacetylation (DD) is determined by the conditions used for deacetylation of the polymer. The treatment of chitin with an aqueous solution of 40–45% (w/v) NaOH at 90–120 °C for 4–5 h results in N-deacetylation of chitin (Dash et al. 2011).

The characterization of a chitosan requires the determination of its average degree of deacetylation (DD) and molecular weight. The DD can be determined by different techniques, such as infrared spectroscopy, potentiometric titration, and NMR (Cheung et al. 2015). The DD also influences the solubility of the polymer, the interchain interactions due to H-bonds, and the hydrophobic character of the acetyl group (Zhang et al. 2010). Commercially available low molecular weight chitosan (LMWC) grade is characterized by molecular weight comprised between 20 and 190 kDa with DD <75% and that of high molecular weight chitosan (HMWC) is generally characterized by molecular weight comprised between 190 and 375 kDa with DD >75% (Dash et al. 2011). The mycelia of *Rhizomucor miehei* and *Mucor racemosus* were employed for the isolation of chitosan in which the degree of deacetylation were determined to be 98.6% and 97.1%, respectively (Tajdini et al. 2010). Production of LMWC and chitooligosaccharides (COS) from chitosan can be brought about either by chemical or enzymatic methods. The enzymatic hydrolysis of chitosan is the method of choice as it offers many advantages as compared to the energy intensive chemical method (Zhang et al. 2010). Unlike most polysaccharides, LMWC and COS possess positive charges, which allows them to bind strongly to negatively charged surfaces; this property is in turn responsible for many of the biological activities of chitosan (Cheung et al. 2015).

Crystallinity is maximum for chitin and fully deacetylated chitosan. In acidic environments, linear and high molecular chitosan acts as an excellent viscosity enhancer and a pseudoplastic material. The viscosity of chitosan solution increases with an increase in the concentration of chitosan used, decrease in temperature, and increasing DD (Dash et al. 2011). Chitosan shows different biological activities depending on its structures. Bioactive chitosan has been developed by various chemical modification and enzymatic hydrolysis (Zhang et al. 2010). The modified chitosan can be subsequently used for its potential biomedical applications.

6.5 Synthesis of Fungal Chitosan

Synthesis of chitin and chitosan from fungal mycelium has recently received increased attention due to its wide range of applications. Chitin and chitosan of crustacean may vary in the physicochemical properties, while fungal chitin and chitosan have relatively consistent properties because of the controlled fermentation conditions (Akila 2014).

The synthesis of chitin is mediated by an integral membrane enzyme; chitin synthase catalyzes the transfer of N-acetylglucosamine from uridine diphosphate (UDP)-N-acetylglucosamine to a growing chitin chain. In the solid state, chitin chains congregated by the H-bonds network which governs the solubility, swelling, and reactivity of the polymer. The hydrogen bonding between the newly formed polymers of chitin results in microfibril formation and subsequent crystallization of chitin in the extracellular space immediately adjacent to the plasma membrane (Younes and Rinaudo 2015). This process of crystalline chitin synthesis primarily occurs at sites of active growth and cell wall remodeling. For yeasts, this includes areas such as the bud tip during polarized growth and the bud neck during cytokinesis. In filamentous fungi, the synthesis occurs at the hyphal apex. So far three chitin synthases are identified in *S. cerevisiae*, namely, Chs1p, Chs2p, and Chs3p. Chs1p aids in cell wall repair and replenish chitin polymers lost during cytokinesis. Chs2p is involved in the formation of the primary septum within the dividing yeast cell. The Chs3p chitin synthase is majorly responsible for producing ~80 to 90% of the total cellular chitin. *A. fumigatus* has seven chitin synthase encoding genes, designated as CHSA through CHSF. Similarly, in *N. crassa* four specific chitin synthases have been reported (Bowman and Free 2008).

6.6 Methods for Preparation of Chitosan-Based Nanocomposites

Different methods have been employed for the preparation of chitosan-based nanocomposites and conjugated to different bioactive compounds (hydrophilic molecules, hydrophobic molecules, and macromolecules) either by covalent or reversible bonds. These compounds can be simply embedded through physical and irreversible interactions (hydrogen bonds, van der Waals forces, hydrophobic effects, electrostatic interactions) or can be also be loaded onto the surface of nanoparticles (Bugnicourt and Ladaviere 2016). However, the method to be employed depends on factors such as particle size, stability of the active agents and final product, loading efficiency, release kinetics, residual toxicity, the nature of the active molecule, and the delivery agent (Agnihotri et al. 2004). The lack of target specificity of bioactive species leading systemic distribution results in wastage of large doses of drugs and harmful side effects. Physiological environment such as the gastric environment at pH ~2 can also degrade bioactive agent before reaching the targeted site.

The advances in the area of nanoparticles research bring promising solutions to overcome the abovementioned problems. The nanoparticles possess the ability to encapsulate drug molecule, thereby shielding it from the harsh physiological environment. Moreover, the controlled release and targeted delivery of the loaded drug is possible through the surface modification of nanoparticles (Bugnicourt and Ladaviere 2016). Chitosan nanoparticles (CS-NPs) have been employed to encapsulate bioactive compounds, through the following approaches (Table 6.2).

Table 6.2 A summary of various methods employed for encapsulation of bioactive components to chitosan nanoparticles with few examples

Methods	Examples
Ionotropic gelation	Chitosan-silver nanocomposites (Potara et al. 2011)
	Copper-loaded chitosan nanocomposites (Qi et al. 2005)
	Zn^{2+}-loaded chitosan nanoparticles (Du et al. 2009)
	Superparamagnetic iron oxide nanoparticles (Sanjai et al. 2014)
	Gadolinium-loaded chitosan nanoparticles (Jahanbin et al. 2015)
	Tea polyphenol (TP)-Zn complex-loaded β-CS NPs (Zhang and Zhao 2015)
Coprecipitation	Chitosan-DNA nanoparticles (Mao et al. 2001)
	Magnetic Fe_3O_4-chitosan nanoparticles (Liu et al. 2011)
Emulsion cross-linking	Chitosan microspheres of pentazocine (Sankar et al. 2001)
	Fe_3O_4-chitosan nanoparticles (Qu et al. 2010)
	Chitosan-based mucoadhesive microspheres of clarithromycin (Majithiya and Murthy 2005)
Droplet coalescence method	Gadolinium-loaded chitosan nanoparticles (Ichikawa et al. 2014; Shikata et al. 2001)
Reverse micellar method	Doxorubicin-coupled dextran encapsulated in chitosan nanoparticles (Mitra et al. 2001)
Spray-drying	Betamethasone disodium phosphate chitosan microspheres (Huang et al. 2002)
	Chitosan-iron oxide nanoparticles (Huang et al. 2010)
Sieving method	Clozapine-loaded chitosan (Agnihotri and Aminabhavi 2004)

6.6.1 Ionotropic Gelation

The ionic gelation technique is by far the most simple and widely used methods used to prepare chitosan nanoparticles (CS-NPs). The polymeric nanoparticles are formed by electrostatic cross-links between the positively charged amino groups in CS molecules and the negatively charged sodium TPP (Zhang et al. 2016). For ionic gelation, chitosan is dissolved in aqueous acidic solution which quaternizes the chitosan amino groups making it soluble; this solution is then added dropwise under constant stirring to polyanionic TPP solution. The complexation between oppositely charged species causes the chitosan to undergo ionic gelation and precipitate as spherical particles. Various formulations of chitosan nanoparticles produced by the ionic gelation of TPP and chitosan were studied by Xu and Du (2003). As the CS reacts with TPP by simple electrostatic interaction, there is no permanent chemical cross-linking. In addition, the use of toxic chemicals is also avoided throughout the preparation and loading procedure (Jamil et al. 2016). The chitosan/TPP nanoparticles have also been used to incorporate metal ions, such as silver, copper, and zinc, to enhance their antimicrobial activity (Qi et al. 2005; Du et al. 2009; Potara et al. 2011) or iron oxide (Sanjai et al. 2014) and gadolinium (Jahanbin et al. 2015). Zhang and Zhao (2015) prepared TP-loaded β-chitosan (CS) nanoparticles (NPs) based on the principle of ionic gelation between CS and sodium tripolyphosphate

(TPP). The tea polyphenol (TP)-Zn complex-loaded β-CS NPs had an encapsulation efficacy of 97.33% and average particle size of 84.55 nm. Further, TP-Zn complex-loaded β-CS NPs exhibited higher antioxidant activity than that of TP-loaded β-CS NPs (Zhang and Zhao 2015).

6.6.2 Coprecipitation

This method is based on the principle that chitosan is insoluble in alkaline pH and hence precipitates/coacervates when it in contact with alkaline solution. In this method, chitosan solution is added into an alkali solution, for example, sodium hydroxide, NaOH-methanol, or ethanediamine, by means of a compressed air nozzle to form nanoparticles (Mao et al. 2001). Under the action of emulsified solvent, the water phase containing chitosan is dispersed in the organic phase encapsulating the drug, where turbulence appears between the interfaces of the two phases and chitosan is precipitated, resulting in the generation of nanoparticles (Wang et al. 2011). The size can be controlled by regulating the compressed air pressure or spray-nozzle diameter. The release of drug is controlled by using appropriate cross-linking agent (Madureira et al. 2015). This technique has been used to prepare chitosan-DNA nanoparticles by Mao et al. to encapsulate and protect the plasmid DNA from nuclease degradation. The particle size was successfully optimized to 100–250 nm with a narrow distribution by keeping the amino to phosphate group ratio between 3 and 8 and chitosan concentration of 100 μg/ml (Mao et al. 2001). Liu et al. (2011) prepared magnetic Fe_3O_4-chitosan nanoparticles by coprecipitation using glutaraldehyde as a cross-linking agent. The synthesized nanoparticles were used subsequently used to immobilize lipase (Liu et al. 2011).

6.6.3 Emulsion Cross-Linking

This method exploits the reactive functional amine group of chitosan to interact with the available reactive groups of the cross-linking agent. In this method water-in-oil (w/o) emulsion is obtained by emulsifying the chitosan aqueous solution in the oil phase. A suitable surfactant is used to stabilize the aqueous droplets and obtain the final particles. Consequently, a stable emulsion is cross-linked using a suitable cross-linking agent to solidify the particles. This method is useful to control the dimension of the NPs, because the dimension of the final product is dependent on the amount of cross-linking agent used. The emulsion cross-linking method involves a few drawbacks, such as the use of organic solvents which affects the proteins and cell viability. Moreover, complete removal of the unreacted cross-linking agent may be a challenge (Shi et al. 2011; Wang et al. 2011; Madureira et al. 2015). Sankar et al. (2001) used this method to prepare chitosan-based pentazocine

microspheres for intranasal delivery. The formulation parameters such as drug loading, polymer concentration, stirring speed during cross-linking, and oil phase were controlled to develop the desired microspheres. The in vivo and in vitro studies indicated a significant enhancement in bioavailability of pentazocine and diffusion-controlled release kinetics, respectively (Sankar et al. 2001). Majithiya and Murthy (2005) developed chitosan-based mucoadhesive microspheres of clarithromycin using glutaraldehyde as a cross-linking agent. The prepared microspheres exhibited an entrapment up to 74% along with sustained release of the drug. In another study, oleic acid-coated Fe_3O_4 nanoparticles are absorbed by chitosan and cross-linked with glutaraldehyde, resulting in Fe_3O_4-chitosan nanoparticles of average size of 10.5 nm with a narrow size distribution. These nanoparticles have highly saturated magnetization effect, superparamagnetic properties, and a sufficiently high temperature to induce hyperthermia (Qu et al. 2010).

6.6.4 Droplet Coalescence Method

Developed by Tokumitsu et al. (1999), in this method precipitation is caused by enabling coalescence of chitosan particles with NaOH, rather than cross-linking stable particles. Two separate emulsions are prepared, one containing aqueous solution of chitosan along with drug and another containing chitosan aqueous solution on NaOH in liquid paraffin oil. When the emulsions are blended under high-speed stirring, the particles of each emulsion collide at random and coalesce to generate small-sized particles that precipitate (Tokumitsu et al. 1999). This method was employed to prepare gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs) with different particle sizes by using Gd-DTPA and chitosan with varying molecular weight. The nanoparticles formed exhibited significant tumor growth suppression (Shikata et al. 2001; Ichikawa et al. 2014).

6.6.5 Reverse Micellar Method

Reverse micelles are thermodynamically stable liquid mixtures of water, oil, and surfactant. The size, polydispersity, and thermodynamic stability of these droplets are maintained by the rapid dynamic equilibrium. Ultrafine polymeric nanoparticles with narrow size distribution could be achieved by using this method (Leong and Candau 1982). To prepare reverse micelles, the surfactant is dissolved in an organic solvent followed by the addition of chitosan and drug with constant agitation. To the transparent solution obtained, a cross-linking agent is added and incubated overnight with constant stirring. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug until the clear microemulsion turns translucent (Agnihotri et al. 2004; Mitra and Dey 2011). Anticancer drug, doxorubicin (DXR),

coupled with dextran (DEX) encapsulated in chitosan nanoparticles was prepared by this method. The antitumor effect of these DEX-DXR nanoparticles (100 ± 10 nm diameter) when evaluated in J774A.1 macrophage tumor cells implanted in Balb/c mice resulted in the enhanced permeability and retention effect (EPR) in solid tumors and further reduction in undesirable side effects such as cardiotoxicity (Mitra et al. 2001).

6.6.6 Spray-Drying

This method is employed to create powders, granules, or agglomerates by the combination of drug and carrier solutions. The process is based on drying of atomized particles in a flow of hot air. For a short time, chitosan is diluted in aqueous acetic acid solution, and then drug is diluted or dispersed in the solution, followed by the addition of an appropriate cross-linking agent. This solution or dispersion is atomized in a flow of hot air that results in the formation of small particles, from which the solvent evaporates and immediately leads to the formation of nanoparticles. Various process parameters, e.g., the size of nozzle, spray flow rate, atomization pressure, inlet air temperature, and extent of cross-linking, are controlled to get the desired dimension of particles (He et al. 1999). Huang et al. (2002) prepared betamethasone disodium phosphate chitosan microspheres by this method using type-A gelatin and ethylene oxide-propylene oxide block copolymer poloxamer as modifiers. A good drug stability (less 1% hydrolysis product), high entrapment efficiency (95%), and positive surface charge (37.5 mV) was achieved. The gelatin/chitosan ratio of 0.4–0.6 (w/w) showed a fairly prolonged drug release up to 12 h (Huang et al. 2002). Similarly, Huang et al. (2010) prepared chitosan-iron oxide nanoparticles with various chitosan: iron oxide ratios by spray-drying. These nanoparticles were stable in water with strong superparamagnetic.

6.6.7 Sieving Method

It is a simple method developed by Agnihotri and Aminabhavi (2004) to produce chitosan microparticles containing the drug clozapine. In this method, the microparticles are prepared by cross-linking 4% acetic acid chitosan solution to form glassy hydrogels that are passed through a sieve. Clozapine ($C_{18}H_{19}C_lN_4$) was incorporated into chitosan gel before cross-linking with 99% efficiency. Irregular shaped microparticles (540–700 nm) were formed on sieving, and in vivo studies indicated a slow release of clozapine (Agnihotri and Aminabhavi 2004).

6.7 Chemical Modification and Functionalization of Chitosan

Chitosan usually reacts with other bioactive molecules or polymers and changes into derivatives or composites. The presence of free hydroxyl and amino groups on the chitosan chains allows chemical modifications and provides sites for a variety of side group attachment under mild conditions (Cheung et al. 2015; Zhang et al. 2010). In addition, the characteristic features of chitosan, such as being cationic, hemostatic, and insoluble at high pH, can be modified by sulfating the amine group which makes the nano-derivative anionic and water-soluble (Dash et al. 2011). The mucoadhesive property of chitosan aids in transport of molecules across mucosal membrane and subsequent delivery of vaccines (Sawaengsak et al. 2014; Del Guidice and Baudner 2015). Chitosan nanoparticles are used as drug excipients as they are (i) biocompatible and biodegradable, (ii) soluble in water, (iii) available in a wide range of molecular weights, (iv) easy in derivatization, (v) good loading efficiency, and (vi) controlled drug release (Liu et al. 2014; Lu et al. 2014; Ragelle et al. 2014).

Generally, the process of drug loading in chitosan systems are achieved either (i) by incorporating the drug simultaneously during the preparation of the particles or (ii) by loading the drug to the preformed particles by incubating with them. The first method is employed to incorporate water-soluble drugs with chitosan, while in case of water-insoluble drugs, the loading of drug involves incubation of preformed particles in a saturated solution of drug (Kumbar et al. 2002). Chitosan microspheres were loaded with tetracycline using two different methods, i.e., cross-linking and precipitation, by Hejazi and Amiji (2002). The loading efficacy was found to be 69% (w/w), and the release of about 30% of tetracycline in solution at pH 1.2 in 12 h was achieved (Hejazi and Amji 2002).

6.8 Biomedical Applications of Chitosan-Based Systems

6.8.1 Chitosan-Based Systems for Antibiotics

Drug delivery systems are designed to reduce drug side effects and to allow a specific drug to be delivered to the targeted tissue in a controlled manner. Chitosan nanoparticles (CS-NPs) have been widely used as drug carriers in diagnosis and therapeutics. Because of its nanosize, these nanoparticles can easily penetrate the targeted tissues (Jamil et al. 2016). Aranaz et al. (2016) synthesized ciprofloxacin hydrochloride (CFX)-loaded chitosan films to achieve controlled drug delivery of CFX. It was found that the amount of drug release was influenced by the thickness of the film and the degree of cross-linking. The antimicrobial effect of CFX and AgNPs-CFX-loaded chitosan films against *P. aeruginosa* was tested, and it was observed that the antimicrobial loaded films exhibited higher antimicrobial efficacy

than the chitosan films alone (Aranaz et al. 2016). Jamil et al. (2016) reported the synthesis of cefazolin-fabricated CS-NPs by ionic gelation method. It was demonstrated that cefazolin-loaded CS-NPs possess excellent antimicrobial potential against multidrug-resistant *K. pneumoniae* and *P. aeruginosa* and extended spectrum beta-lactamase (ESBL)-positive *E. coli*.

Limited cellular penetration reduces the effectiveness of many antimicrobial treatments and also results in various side effects. Ibrahim et al. (2015) explored the possible improvement in cellular penetration and antimicrobial activity of the antibiotics (ciprofloxacin, chlortetracycline hydrochloride, and gentamicin sulfate) when incorporated into chitosan-based nanoparticles. Chitosan nanoparticles were prepared via the ionic gelation of chitosan with tri-polyphosphate anions. The nanoparticles exhibited higher antibacterial activity against gram-positive (*S. aureus*) bacteria than gram-negative bacteria (*E. coli*) (Ibrahim et al. 2015).

6.8.2 Chitosan-Based Systems for Metals

Chitosan hybrid metal and metal oxide nanoparticles have been developed with excellent properties and synergistic effects. Currently, the research on the combination of CS and metal oxide has focused on titanium dioxide (TiO_2), as it possesses excellent photocatalytic performance and is stable in acidic and alkaline solvents. In addition to TiO_2, CS has tremendous ability to form metal complexes with Zn metal (Dhillon et al. 2014). The ZnO-CS NPs were synthesized by nano-spray-drying and precipitation significantly inhibited biofilm formation and growth in both *M. luteus* and *S. aureus* at a concentration ranging from 0.625 to 0.156 mg/ml. Hebeish et al. (2014) prepared chitosan-grafted-poly acrylonitrile silver nanocomposites (Cs-g-PAN/Ag) via in situ chemical reduction of Ag ions in graft copolymerization of acrylonitrile onto chitosan. The nanoparticles with an average of 15–20 nm showed excellent antimicrobial performance toward both *E. coli* and *S. aureus* (Hebeish et al. 2014). Synergistic antibacterial activity of chitosan-silver nanocomposites on *S. aureus* results in the changes in morphology of *S. aureus* cells due to disruption of bacterial cell wall integrity (Potara et al. 2011). Chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions and copper ion sorption. It was observed that chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles exerts dose-dependent cytotoxic effects on the proliferation of tumor cell lines, BEL7402, BGC823, and Colo320 tumor cells, while having little effect on the growth of L-02 human normal liver cells (Qi et al. 2005). Metal ions, Ag^{+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}, loaded onto chitosan nanoparticles were prepared based on ionic gelation between chitosan and sodium tripolyphosphate. The metal ion-loaded nanoparticles showed enhanced antibacterial activity against both gram-positive and gram-negative bacteria (*E. coli* 25922, *S. choleraesuis* ATCC 50020, and *S. aureus* 25923) (Du et al. 2009).

6.8.3 Chitosan-Based Systems for Protein and Peptides

Antimicrobial protein and peptides have received growing interest due to their broad spectrum of activities. It is well known that lysozyme can inhibit some gram-positive bacteria due to its unique ability to damage bacterial cell wall by hydrolyzing 1,4-β-linkage between N-acetyl-muramic acid and N-acetyl-D-glucosamine of cell wall peptidoglycan (Yuan et al. 2013). CS-NPs and chitosan-lysozyme nanoparticles (CS-Lys-NPs) were prepared according to the ionic gelation technique with tripolyphosphate anions. The integration of lysozyme into CS-NPs enhanced the antibacterial activity against negative bacteria, *E. coli*, and gram-positive bacteria, *B. subtilis*. The antibacterial action may be attributed to the ability of CS-NPs/CS-Lys-NPs to penetrate the cell membrane, which results in the leakage of the cytoplasm components and eventual cell death. CS-NPs/CS-Lys-NPs also influence metabolism enzyme activities and interfere with bacterial metabolism (Wu et al. 2017). In another report, Piras et al. (2014) explored the use of CS-NPs as delivery systems for lysozyme (LZ). The chitosan nanoparticles loaded with lysozyme (LZ-NPs) were successfully prepared by means of a mild ionic gelation technique. LZ loading in the NPs was up to 8% and the release up to 20% over 3 weeks, in a controlled and sustained manner. The nanoparticles showed in vitro cytocompatibility and a good antimicrobial activity on *S. epidermidis* (Piras et al. 2015). The encapsulation of the frogskin-derived antimicrobial peptide, AMP temporin B (TB), into chitosan nanoparticles(CS-NPs) resulted in enhanced activity of the antimicrobial peptide, while reducing its toxic potential. TB-loaded CS-NPs were prepared, based on the ionotropic gelation between CS and sodium tripolyphosphate. The encapsulation efficiency of TB in the formulation was up to 75%. The encapsulation of TB in CS-NPs significantly reduces the peptide's cytotoxicity against mammalian cells. Moreover, the nanocarrier evidenced a sustained antibacterial action against various strains of *S. epidermidis* (Piras et al. 2015).

6.8.4 Chitosan-Based Systems for Dye

Immobilization of photosensitizers on polymeric supports prevents the toxic side effects of residual photosensitizers and also provides an added advantage of stability in the physiological environment. Nanoparticles either encapsulated or surface modified with photosensitizer have been studied for enhanced antimicrobial photodynamic therapy (PDT). Shrestha et al. (2014) reported the synthesis of photoactivated rose Bengal (RB) dye-functionalized CS nanoparticles (CSRBnp) by conjugating CS-NPs with RB via chemical cross-linking. CSRBnp exerted antibacterial mechanism by adhering to bacterial cell surface, permeabilizing the membrane and lysing the cells subsequent to photodynamic treatment. CSRBnp combined with PDT showed complete disruption of the biofilm structure and reduced viability of *E. faecalis* biofilms (Shrestha et al. 2014).

6.9 Conclusion

Chitosan nanoparticles have attracted increasing attention because of their biocompatible, biodegradable, and nontoxic nature. In the area of therapeutics, biocidal and biostatic natural polymers are incorporated into fibers, membrane, or hydrogel and used for various biomedical applications, including wound dressing, tissue engineering, drug delivery, cancer diagnosis, etc. Functionalized nanoparticles decorated with various bioactive molecules and peptides have opened up new avenues in therapeutics and diagnosis. These modified nanomaterials offer unique physicochemical properties, such as ultrasmall sizes, large surface area/mass ratio, and increased chemical reactivity. Developing targeted chitosan carriers is an area of future development for sustained/controlled release drugs and targeted delivery. The absorption and bioavailability of drug encapsulated into chitosan nanoparticles renders improved delivery of bioactive molecules/drugs and also protect them from enzymatic degradation. Great progress has been achieved in the application of chitosan nanoparticles as drug carriers. However, further investigation is required on the biocompatibility of modified chitosan and its derivatives for wider applications.

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Chapter 7
Biosynthesis of Metal Nanoparticles via Fungal Dead Biomass in Industrial Bioremediation Process

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Abstract The nanoparticle biosynthesis in bioremediation process is a promising technique to deal with the continuous demands of waste removal, especially concerning the heavy metal industry activity. It is environmentally sustainable, green, safe, and cost-effective. Once synthesized, the nanoparticles can be considered as future construction blocks of the next generation of photoelectric materials, electronics, biomedical devices, chemical and biochemical sensors, and others. The designing of nanomaterials using fungal biomass is a relatively recent research field, and the utilization of fungal dead biomass is very promising in this area due to their operational versatility, such as absence of toxicity limitations, the possibility of storage for a prolonged period of time, and the fact that it does not require growth media and nutrients for its maintenance. It has been reported in literature the use of fungal dead biomass in the synthesis of metallic nanoparticles, metallic oxides nanoparticles, metal/oxide (core-shell) nanoparticles, magnetic nanoparticles, and thin films that can be synthesized extra- and/or intracellularly. This approach opens new perspectives for the biosynthesis of nanomaterials by fungal dead biomass, and at the same time it has the advantage to be a low cost-effective bioremediation process.

Keywords Fungi · Yeast · Nanoparticles · Mycoremediation · Biosynthesis · Metal oxides · Magnetic · Nanofilm

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Abbreviations

AFM	Atomic force microscopy
EDS	Energy dispersive X-ray spectroscopy
EDXRF	Energy dispersive X-ray fluorescence spectroscopy
FT-IR	Fourier transform infrared spectroscopy
HRTEM	High-resolution transmission electron microscopy
NPs	Nanoparticles
SEM	Scanning electron microscopy
SPR	Surface plasmon resonance
SQUID	Superconducting quantum interference device
TEM	Transmission electron microscopy
VSM	Vibrating sample magnetometry
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

7.1 Introduction

The nanotechnology arises from the chemical, physical, and biological science, wherein novel approaches are being developed to explore and manipulate single atoms and molecules, involving the production of nanoparticles (NPs) with new properties, which differ significantly from their corresponding bulk solid-state material. Synthetic methods of NPs production include complex chemical and physical process that require the use of large amounts of energy, high pressures and temperatures, and toxic waste production, posing environmental and human health risk (Varshney et al. 2012). Currently the metal NPs have reached importance in many applications as chemical sensing and imaging, information storage, catalysis, drug delivery, biological labeling, electronics, photonics, and environmental remediation (Daniel and Astruc 2004; Guo et al. 2005; Huang et al. 2007; Prasad et al. 2016).

The use of biological materials for the synthesis of metal NPs has emerged as a more efficient and greener approach (Bhattacharya and Gupta 2005). The fungus-mediated green chemistry approach toward the production of NPs has many advantages, economic viability, easy processing and scaling up, simple biomass handling, and recovery of large surface areas with optimum growth of mycelia (Varshney et al. 2012; Prasad et al. 2016; Prasad 2016, 2017a).

In literature review, many studies report the biosynthesis of metal NPs using live fungal biomass (Narayanan and Sakthivel 2010). The use of live biomass for the binding of metal ions depends on nutrient availability, environmental conditions, and cell age (Kapoor and Viraraghavan 1995). In addition, live biomass may be subject to the toxic effect of metals at high concentrations. Therefore, dead biomass is preferred to overcome these drawbacks (Butter et al. 1998). Dead biomass has several advantages in the synthesis of NPs such as few risk of handling, the possibil-

ity of storage for a prolonged period of time, it not requiring growth media and nutrients for its maintenance, and the adsorbed metal ions being easily desorbed for the dead biomass reutilization (Salvadori et al. 2014).

There are few investigations regarding the production of NPs by fungal dead biomass, for example, the fungus *Hypocrea lixii* can produce extracellularly metallic copper NPs (Salvadori et al. 2013) and intra- and extracellularly nickel oxide NPs (Salvadori et al. 2015); the fungus *Trichoderma koningiopsis* was able to synthesize extracellularly metallic copper NPs (Salvadori et al. 2014a); the *Aspergillus aculeatus* have the ability to produces extracellularly nickel oxide NPs in film form (Salvadori et al. 2014); and the yeast *Rhodotorula mucilaginosa* was able to produce, intracellularly, metallic copper NPs (Salvadori et al. 2014b) and magnetic Ni/NiO core-shell NPs nanostructured in film form (Salvadori et al. 2016).

The metal ions are not biodegradable; they are generally removed from contaminated water and soil by chemical or physical treatment. The available treatment processes are reverse osmosis, precipitation, electrolysis, ion exchange, and membrane separation. However, such processes are often not feasible because of the high costs, the production of toxic sludge, and the need for continuous input of chemicals (Han et al. 2006). In this context, the bioremediation of metal pollutants has emerged as an environmental-friendly technology. Some microorganisms are potent bioremediators that remove metals ions through biosorption mechanisms. This novel approach is competitive, effective, and cheap (Volesky 2001). In this respect, fungi have been used in bioremediation processes since they are a versatile group that can adapt to and grow under various extreme conditions of pH, temperature, and nutrient availability, as well as at high metal concentration (Anand et al. 2006). The microbial fungal systems also have an important role in NP production due to their natural mechanisms for detoxification of metallic ions through bioreduction. In fact, the bioreduction is one of the primary processes of biosynthesis and can be a feasible approach in both the biosynthesis of NPs and in industrial bioremediation (Malik 2004; Srivastava et al. 2013).

In this chapter it is discussed a versatile approach for the biosynthesis of metal and oxide NPs via fungal dead biomass in industrial cleaning process of metals. The main objective is deeper understanding of the utilization of the fungi in a sustainable environmentally system of production NPs associated with their role as nanoadsorbent in bioremediation.

7.2 Filamentous Fungi in NP Biosynthesis

The fungi are eukaryotic organisms, are heterotrophs, do not synthesize chlorophyll, are spore-producing, are ubiquitous, and generally contain filamentous branches with multicellular structures consisting of somatic and reproductive cells.

Currently in the nanobiotechnology area, the exploration of the implication of fungi is considered very important, since these organisms can uptake metals from the environment (Veglio and Bolchini 1997) and bioaccumulate metals in a

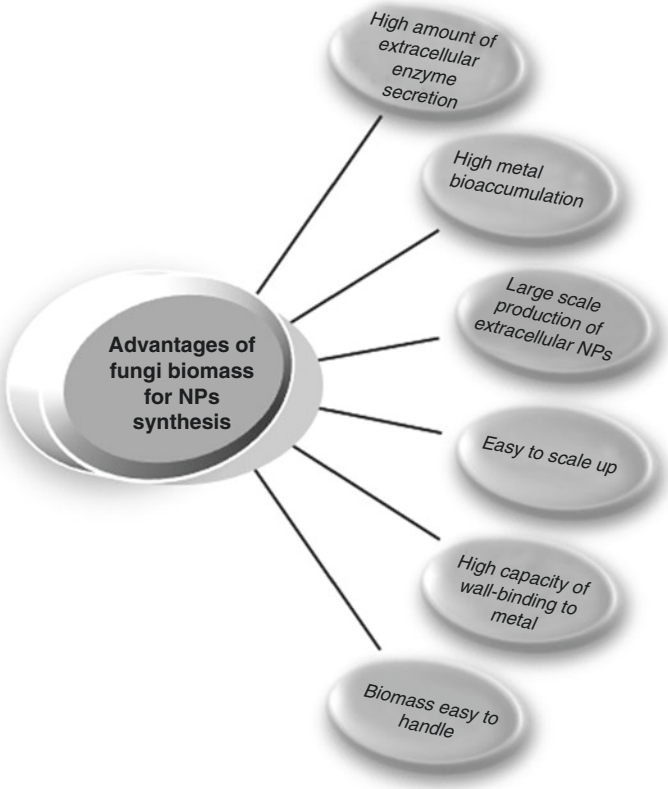

Fig. 7.1 Some advantages of fungal biomass in synthesis of metal NPs

metabolism-dependent process (Sastry et al. 2003). It occurs generally by the extracellular binding via metabolites and polymers present in the cell wall composed of structural polysaccharides, proteins, and lipids that contain metal-binding functional groups.

The fungi are considered promising for large-scale NP production due to the peculiarity of these organisms as effective secretors of reductive enzymes involved in metal NP synthesis, an advantage comparing to other microorganisms (Kitching et al. 2015; Prasad 2016, 2017a). The easy and economic fungal cultivation both in the laboratory and in the industrial scale become the employment of the fungal biomass, another merit for the utilization of green approach using fungi to synthesize metallic NPs (Moghaddam et al. 2015) (Fig. 7.1). The fungi are able to synthesize metal meso/nanoparticles and nanostructured via reducing enzyme intracellularly or extracellularly and through the procedure of the biomimetic mineralization (Ahmad et al. 2003). The utilization of fungal dead biomass is very promising in the designing of nanomaterials area due to their operational versatility and cost-effectiveness (Salvadori et al. 2013).

Table 7.1 Metal and metal oxide NPs biosynthesized by filamentous fungal biomass

Filamentous fungi species	Metal NPs	Location	Size (nm)	Shape	References
Verticillium sp.	Ag	Intracellular	25	Spherical	Mukherjee et al. (2001)
Aspergillus niger	Ag	Extracellular	3–30	Spherical	Jaidev and Narasimha (2010)
Phoma glomerata	Ag	Extracellular	60–80	Spherical	Birla et al. (2009)
Aspergillus fumigatus	Ag	–	15–45	Mostly spherical	Alani et al. (2012)
Rhizopus oryzae	Au	Cell surface	10	Nanocrystalline	Das et al. (2009)
Aspergillus niger	Au	Extracellular	12.79 ± 5.61	Spherical	Bhambure et al. (2009)
Verticillium luteoalbum	Au	Intracellular	˂10	Spheres and rods	Gericke and Pinches (2006a)
Aureobasidium pullulans	Au	Intracellular	29 ± 6	Spherical	Zhang et al. (2011b)
Colletotrichum sp.	Au	Extracellular	8–40	Spherical	Shankar et al. (2003)
Aspergillus tubingensis	$Ca_3P_2O_8$	Extracellular	28.2	Spherical	Tarafdar et al. (2012)
Fusarium oxysporum	Cd	Extracellular	9–15	Spherical	Kumar et al. (2007b)
Hypocrea lixii	Cu	Extracellular	24.5	Spherical	Salvadori et al. (2013)
Trichoderma koningiopsis	Cu	Extracellular	87.5	Spherical	Salvadori et al. (2014a)
Fusarium oxysporum	Fe_3O_4	Extracellular	20–50	Quasi-spherical	Bharde et al. (2006)
Aspergillus aculeatus	NiO	Extracellular	5.89	Spherical	Salvadori et al. (2014)
Hypocrea lixii	NiO	Extra and intracellular	3.8 1.25	Spherical	Salvadori et al. (2013)
Aspergillus flavus	TiO_2	–	62.74	Spherical	Rajakumar et al. (2012)
Aspergillus fumigatus	ZnO	Extracellular	12–68	Spherical and hexagonal	Raliya and Tarafdar (2013)

There are many species of fungal biomass, employed in the biosynthesis of metal NPs (Table 7.1). The biosynthesis of silver NPs mediated by the filamentous fungus *Verticillium* sp. (Mukherjee et al. 2001) illustrates one of the first reports of synthesis of NPs by fungi. The *Rhizopus oryzae* produced another noble metal NPs as nano gold-bio conjugated in the surface cell, with a size of 10 nm and nanocrystalline shape, showing antimicrobial activity against bacteria as *Staphylococcus*

aureus, *Escherichia coli*, *Salmonella* sp., and others (Das et al. 2009). Fe_3O_4 NPs are produced extracellularly by *Fusarium oxysporum* with an average size of 20–50 nm (Bharde et al. 2006). Recently, it was reported that the *Aspergillus flavus* synthesizes extracellularly TiO_2 NPs with a probable antibacterial property (Rajakumar et al. 2012).

Currently there is the promising employment of the fungal dead biomass in the NP biosynthesis. Gold NPs are being synthesized by *Penicillium brevicompactum*, as potential anticancer agents (Mishra et al. 2011b). Fungi strains isolated from wastewater of a copper mine in Amazon Brazilian region as *Hypocrea lixii* and *Trichoderma koningiopsis* (Salvadori et al. 2013, 2014a) synthesized metallic copper NPs extracellularly. Nickel oxide NPs in film form were produced by *Aspergillus aculeatus* in average size of 5.89 nm (Salvadori et al. 2014), and intra- and extracellular nickel oxide NPs were also synthesized by *Hypocrea lixii* with spherical shape (Salvadori et al. 2015). All these fungi showed high capacity as nano-adsorbent of metals in bioremediation process.

7.3 Yeast in NP Biosynthesis

Yeasts are non-filamentous, unicellular fungi that are typically spherical or oval and can be of facultative anaerobic growth. Yeasts can use oxygen or an organic compound as the final electron acceptor. This is a valuable attribute because it allows these fungi to survive in various environments.

The high production of NPs as well as the easiness of controlling yeasts in laboratory conditions, the rapid growth, and the production of various enzymes present more benefits in comparison with the bacteria (Kumar et al. 2011). Various studies have been performed to obtain the synthesis of metal NPs utilizing yeasts (Table 7.2).

Yeasts such as *Schizosaccharomyces pombe* and *Candida glabrata* produced CdS NPs intracellularly, being one of the first processes using biological materials for this objective (Dameron et al. 1989). It is worth to emphasize that the NPs synthesized intracellularly by *Schizosaccharomyces pombe* sulfide were used for fabrication of a cadmium diode, whose properties can form the artificial structure of a perfect diode (Kowshik et al. 2002). The synthesis of noble metal NPs such as gold NPs has been produced by the tropical marine yeast *Yarrowia lipolytica* NCIM 3589 (Agnihotri et al. 2009), silver NPs by yeast strain MKY3 (Kowshik et al. 2003), and the synthesis of gold and silver NPs by the *Candida guilliermondii* (Mishra et al. 2011a) and *Saccharomyces cerevisiae* (Lim et al. 2011).

An innovative sustainable protocol of nanobiotechnology was developed employing the dead biomass of the yeast *Rhodotorula mucilaginosa* isolated from a copper mine in Amazon Brazilian region. The yeast was able to synthesize metallic copper NPs intracellularly with size of 10.5 nm and spherical shape and concomitant nano-adsorbent of this metal ion in wastewater in bioremediation process (Salvadori et al. 2014b). Moreover using the same dead organic matrix of the yeast *Rhodotorula mucilaginosa*, it was biosynthesized magnetic NPs of Ni/NiO core-shell nanostruc-

Table 7.2 Biosynthesis of metal and metal oxide NPs mediated by yeast

Yeasts species	Metal NPs	Location	Size (nm)	Shape	References
Pichia jadinii (*Candida utilis*)	Au	Intracellular	–	Various	Gericke and Pinches (2006b)
Candida albicans	Au	Cell-free extract	5	Spherical	Ahmad et al. (2013)
Yarrowia lipolytica NCIM3589	Au	Cell surface	Varying	Particles and plates	Agnihotri et al. (2009)
Saccharomyces cerevisiae	Au	Cell wall cytoplasm	15	Spherical	Sen et al. (2011)
Yeast strain MKY3	Ag	Extracellular	2–5	Twinned or multitwinned, some hexagonal	Kowshik et al. (2003)
Candida glabrata	CdS	Intra and extracellular	20 Å, 29 Å	Hexamer	Dameron et al. (1989)
Schizosaccharomyces pombe	CdS	Intra and extracellular	18 Å, 29 Å	–	Dameron et al. (1989)
Schizosaccharomyces pombe	CdS	Intracellular	1–1.5	Hexagonal	Kowshik et al. (2002)
Rhodotorula mucilaginosa	Cu	Intracellular	10.5	Spherical	Salvadori et al. (2014b)
Rhodotorula mucilaginosa	Ni/ NiO	Extracellular	5.5	Spherical	Salvadori et al. (2016)
Yeast	Zr	–	–	Irregular mesoporous	Tian et al. (2010)

tured in film form, demonstrating its applications as a sustainable system in nanobioremediation of metals from wastewater (Salvadori et al. 2016).

7.4 Potential Mechanisms of NP Biosynthesis

Although several microorganisms as fungi and yeasts are able of synthesizing metal NPs, the mechanisms of NPs synthesis have not been well elucidated. The metabolic complexity of the organisms hinders the study and identification of active species in the nucleation and growth of NPs. Many questions still need to be addressed in nanomycotechnology, before these bioprotocols can dispute with the conventional procedures, mainly with respect to the comprehension of the metabolic pathways to create an ecological-rational approach to NPs synthesis.

As example of mechanisms of NP synthesis by fungi, we can cite Kumar et al. (2007a) that reported the enzymatic synthesis of silver NPs through α-NADPH-dependent nitrate reductase purified from *Fusarium oxysporum*. Another mechanism described for the synthesis of silver NPs was demonstrated by

Vigneshwaran et al. (2007) using the fungus *Aspergillus flavus* that produced extracellularly NPs stabilized by proteins. These researchers also found four high molecular weight fungal proteins associated with NPs. The fungi *Trichoderma asperellum* (Mukherjee et al. 2008) and *Coriolus versicolor* (Sanghi and Verma 2009) had their capacity of silver NP production attributed to fungal protein. Das et al. (2009) reported, using *Rhizopus oryzae*, the biosynthesis of gold NPs concluding that their production is associated with cellular surface-bound protein molecules.

However, the mechanisms of NPs synthesis by dead biomass of fungi have been not elucidated yet, needing future studies to the understanding of this promising green approach in nanotechnology.

7.5 Mycoremediation: Current Situation

Industrial wastes are often found as contaminants in soils, water sources, rivers, and seas (Fig. 7.2) due to the human activity such as metal smelting, sludge dumping, fuel production, intensive agriculture, energy conversion, metal-rich mine tailings, and electroplating (Nedelkoska and Doran 2000). As well known, some heavy metals are toxic and poisonous, especially to humans, while various other metals can be used in high-tech nanotechnology applications or in the production of high-value

Fig. 7.2 Local impacted with metal copper

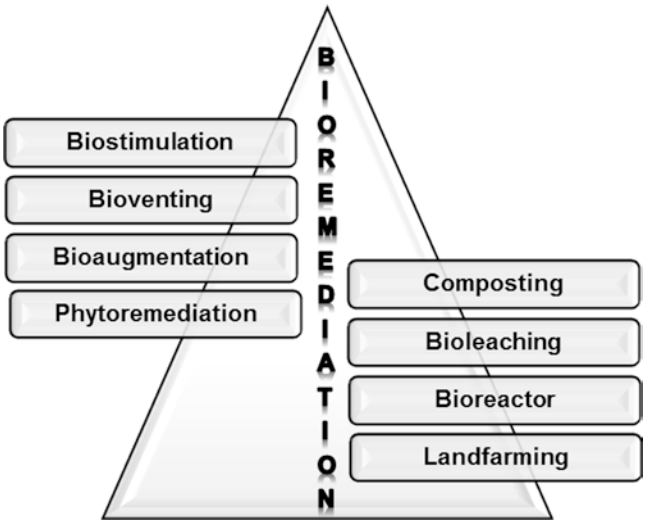

Fig. 7.3 Procedures of bioremediation

materials (Fu and Wang 2011; Hashim et al. 2011). At present, chemical and physical processes are commonly used to remove heavy metals from wastes; however these technologies have several disadvantages such as incomplete metal removal, high reagent concentration, high energy requirement, generation of toxic sludge, and hazardous to environment (Ahalya et al. 2003). The microbial bioremediation has emerged as an alternative technique to such traditional chemical and physical treatments (Brierley 1990; White and Gadd 1986).

The definition of bioremediation encompasses all the processes and actions required to biotransform a polluted environment back into its original pristine condition (Karman et al. 2015). Figure 7.3 outlines some processes of bioremediation. Inside the bioremediation process, the mycoremediation has emerged as an efficient process, where the fungi are utilized to degrade or remove contaminants from the environment, to repair or restore the weakened immune system of the environment (Stamets 2005; Prasad 2017b, 2018).

In the last 20 years, researchers have concentrated their attention in the key components of the biosorption processes (the most effective process in the mycoremediation) in function of the fungal live and dead biomasses (Fig. 7.4). The biosorption process using dead biomass is more rapid in comparison to live biomass, as their binding of cellular surface with metals, displaying also a high affinity for metal removal from aqueous solution. The utilization of the dead biomass becomes the biosorption process easy and nondestructive for recovery of metal ions, which allows regeneration and reuse of biosorbents (Junlian et al. 2010).

Fungi have been used as cation biosorbents for the uptake of metals from industrial wastewater or for the recovery of these materials (Babel and Kurniawan 2003). Ramasamy et al. (2011) investigated the *Aspergillus fumigatus* for uptake Pb

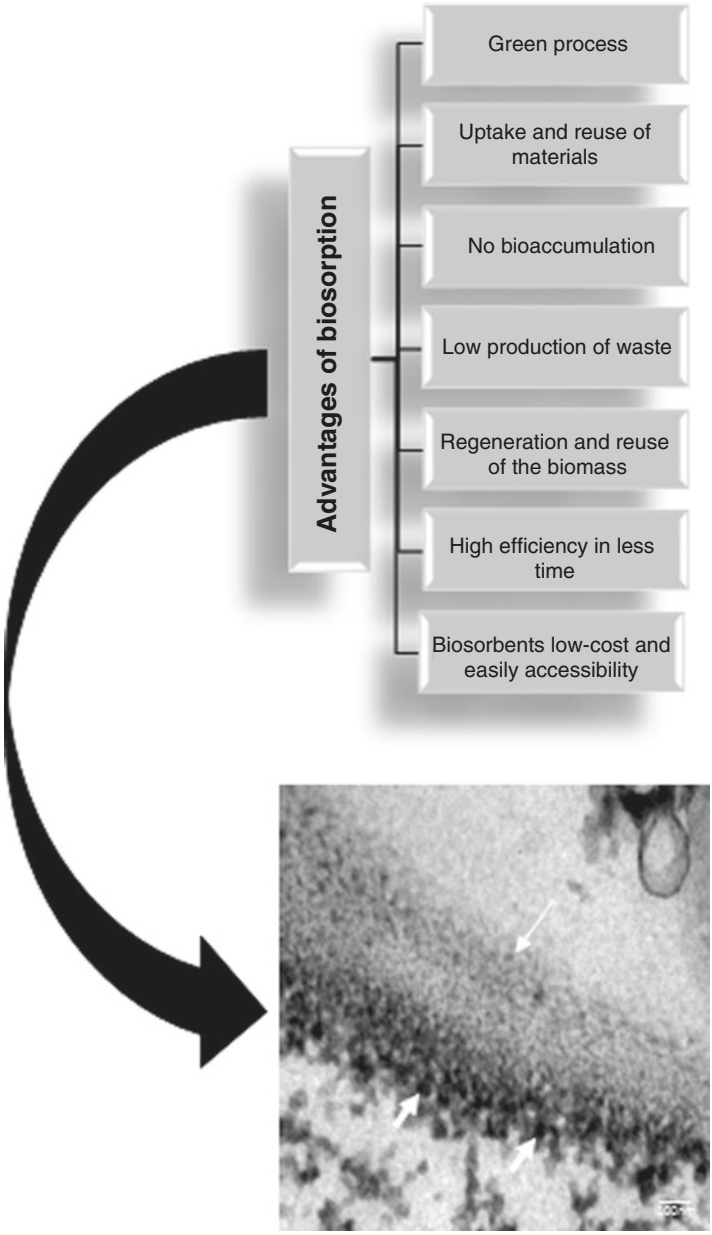

Fig. 7.4 Schematization (above) of the advantages of biosorption process (Photomicrograph (below) by transmission electron microscopy of a section of dead biomass of the filamentous fungus Hypocrea lixii containing metal ions copper (darkest white arrows) in the fungal cell wall (lighter white arrow) after the biosorption process of the metal)

Table 7.3 Fungi and yeasts used in mycoremediation

Organisms	Elements	References
Fusarium sp.	Cu	Majumder (2014)
Yeast: *Rhodotorula mucilaginosa*	Cu, Ni	Salvadori et al. (2014b, 2016)
Yeast: *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*	Cd	Li et al. (2014))
Aspergillus fumigatus	Pb	Ramasamy et al. (2011)
Ganoderma lucidum, *Penicillium* sp.	Ar	Loukidou et al. (2003)
Aspergillus aculeatus	Ni	Salvadori et al. (2014)
Penicillium canescens	Cr	Say et al. (2003)
Aspergillus versicolor	Cr, Ni, Cu	Tastan et al. (2010)
Hypocrea lixii	Cu, Ni	Salvadori et al. (2013, 2015)
Cladonia rangiformis (lichen)	Pb	Ekmekyapar et al. (2012)
Aspergillus sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp.	Cd, Cu, Fe	Fulekar et al. (2012)
Trichoderma koningiopsis	Cu	Salvadori et al. (2014a)

(II) from electronic waste and observed an adsorption of 85.41% of the metal. Zeftawy and Mulligan (2011) reported in a micellar-enhanced ultrafiltration (MEUF) of phosphorous wastewater that could treat metals with rejection ratios of 99%. Li et al. (2014) observed that the yeasts *Zygosaccharomyces rouxii* and *Saccharomyces cerevisiae* were efficient biosorbents of cadmium in a complex food environment. The fungus *Aspergillus versicolor* was investigated in relation to its adsorption capacity of the ions Cu (II), Ni (II), and Cr (VI), and it was observed that this filamentous fungus is a good biosorbent of chromium in wastewater effluents (Taştan et al. 2010). It were also reported that fungi such as *Cladosporium resinae*, *Penicillium* sp., *Aspergillus niger*, *Funalia trogii*, *Ganoderma lucidum*, *Aureobasidium pullulans*, *Trametes versicolor*, and *Rhizopus arrhizus* (Loukidou et al. 2003; Say et al. 2003) are able to biosorb heavy metals from polluted sites (Table 7.3). Salvadori et al. (2013) observed that the dead biomass of fungus *Hypocrea lixii* is able to uptake efficiently Cu (II) ions.

7.6 Biosynthesis of NPs: An Approach Using Fungal Dead Biomass in Bioremediation Process

The relationship between the bioremediation process and the synthesis of metal NPs has increasingly being explored nowadays (Gadd 2010). The fundamental process common for both NPs biosynthesis and bioremediation by fungal dead biomass comprises the surface binding of metal ions to the cell wall known as biosorption (Bishnoi and Garima 2005; Dhankhar and Guriyan 2011). The fungal biomass walls are formed of chitin, chitosan, glucan, lipid, and phospholipids, which contain

carboxyl groups, amino group phosphates, lipids, melanin, sulfates, and hydroxides (Caesartonthat et al. 1995; Kapoor et al. 1999; Siddhanta et al. 2017); these functional groups are the sorption sites of the metals (Mullen et al. 1992; Zhou 1999).

The employment of dead biomass instead of live biomass has been a better alternative to the removal of metals. The high acceptability of dead biomass is due to their higher capacity to bind metals than live cells, the mathematic modulation in the metal removal reactors is simpler, it does not require growth media and nutrients in the feed solution, and the regenerated biomass can be reused and absence of toxicity (Kapoor and Viraraghavan 1996; Kogej and Pavko 2001; Merrin et al. 1998; Salvadori et al. 2013). The preparation of dead biomass can be made through chemical treatment methods using chemicals as acids, alkalies, detergents (Akthar et al. 1996; Fernandes and Nazareth 1999; Tan and Cheng 2003), and others or physical treatment methods as mechanical disruption (Yakubu and Dudeney 1986), heat treatment (Siegel et al. 1986), and autoclaving (Salvadori et al. 2013, 2016).

Among conventional analysis methods used in detection and characterization of metal NPs, we can cite transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM), atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDS), energy dispersive X-ray fluorescence spectroscopy (EDXRF), UV-vis spectroscopy, superconducting quantum interference device (SQUID), vibrating sample magnetometry (VSM), and surface plasmon resonance (SPR) (Wang et al. 2009; Binupriya et al. 2010; Narayanan and Sakthivel 2010; Salvadori et al. 2013, 2014, 2016).

7.6.1 Biosynthesis of Metallic NPs Mediated by Dead Biomass of Fungi and Yeast

The synthesis of metallic NPs mediated by fungi is viewed as a novel basis building pillar of nanotechnology. In order to enlarge the scope of biological systems for the biosynthesis of metallic nanomaterials and bioremediation of wastewater, Salvadori et al. (2013) explored for the first time the potential of the dead biomass of the fungi *Hypocrea lixii* and *Trichoderma koningiopsis* (Salvadori et al. 2014a) and also the yeast *Rhodotorula mucilaginosa* (Salvadori et al. 2014b) isolated from the metal mine in the Brazilian Amazon region to the uptake and reduction of copper ions to copper NPs.

It has previously been demonstrated that fungi are potential biosorbents of heavy metals, being that fungi possess a wide range of chemical groups present in the biomass that can sequester metals (Veglio and Beolchini 1997; Volesky and Holan 1995). The effect of various copper concentrations was analyzed in agar medium, and the results showed that the fungi *Hypocrea lixii* and *Trichoderma koningiopsis* exhibited high tolerance to copper of up to 528 mg L^{-1} and 1057 mg L^{-1}, respectively (Salvadori et al. 2013, 2014a), and the yeast *Rhodotorula mucilaginosa* up to 2000 mg L^{-1} (Salvadori et al. 2014b).

It is important to highlight the influence of physicochemical factors on biosorption in order to develop an efficient NP biosynthesis process; such factors are biomass concentration, pH, temperature, rate of agitation, contact time, and metal ion concentration. The amount of biosorbents is an important factor to determine the sorbent-sorbate equilibrium of the system (Hanif et al. 2007; Sari et al. 2007). The efficiency of ionization of functional groups present on the surface of the biomass cell wall depends on the pH of the solution (Ozer and Ozer 2003). According to the theory of adsorption, adsorption decreases with increasing temperature, as molecules adsorbed earlier on a surface tend to desorb from the surface at higher temperatures (Iftikhar et al. 2009). Under high agitation speeds, vortex phenomena occur, and the suspension is no longer homogeneous, a fact impairing metal removal (Liu et al. 2006). The initial metal concentration provides an important driving force to overcome all mass transfer resistance of the metal between the aqueous and solid phases (Xuejiang et al. 2006).

The optimal adsorption conditions of copper obtained for the fungi *Hypocrea lixii* and *Trichoderma koningiopsis* were the following: use of dead biomass, pH 5.0, temperature of 40 °C, agitation rate of 150 rpm, and time of contact of 60 min. The efficient physicochemical conditions for the synthesis of metallic copper NPs by yeast *Rhodotorula mucilaginosa* were the following: use of dead biomass, pH 5.0, temperature of 30 °C, agitation rate of 150 rpm, and time of contact of 60 min. In these conditions the maximum adsorptions of copper by dead biomass of *Hypocrea lixii*, *Trichoderma koningiopsis*, and *Rhodotorula mucilaginosa* following the Langmuir isotherm model were 19.0 mg g^{-1}, 21.1 mg g^{-1}, and 26.2 mg g^{-1}, respectively. Both fungi and yeast followed the pseudo-second-order kinetic model in copper adsorption (Salvadori et al. 2013, 2014a, b). This adsorption kinetics is typical of the adsorption of divalent metals onto biosorbents (Reddad et al. 2002).

Salvadori et al. (2013) showed by the analysis of TEM images of copper NPs synthesized by dead biomass of the fungus *Hypocrea lixii* the NPs size of 24.5 nm and spherical form and found that they were produced extracellularly. By XRD analysis it was observed the metallic nature of the copper NPs, and, by the SEM-EDS and FT-IR analyses, it was possible to verify the presence of protein (interaction of copper NPs and amide groups) capping the NPs that could possibly act as a stabilization agent (Fig. 7.5).

The fraction of dead biomass of the fungus *Trichoderma koningiopsis* impregnated with copper examined by TEM showed extracellular NPs in the cell wall with an average diameter of 87.5 nm, with predominantly spherical shape and few aggregates of NPs with an average diameter of 328.27 nm. The EDS and XPS spectra revealed the presence of peaks corresponding to the presence of proteins as capping material on the surface of the copper NPs. The XPS spectra also demonstrated the presence of metallic copper NPs (Salvadori et al. 2014a) (Fig. 7.6).

Salvadori et al. (2014b) observed by XPS analysis that the metallic copper NPs synthesized by the dead biomass of yeast *Rhodotorula mucilaginosa* are capped by proteins. In addition, by the analysis of TEM images, it was observed the NPs are produced intracellularly and are uniformly distributed (monodispersed) with spherical shape and an average size of 10.5 nm (Fig. 7.7).

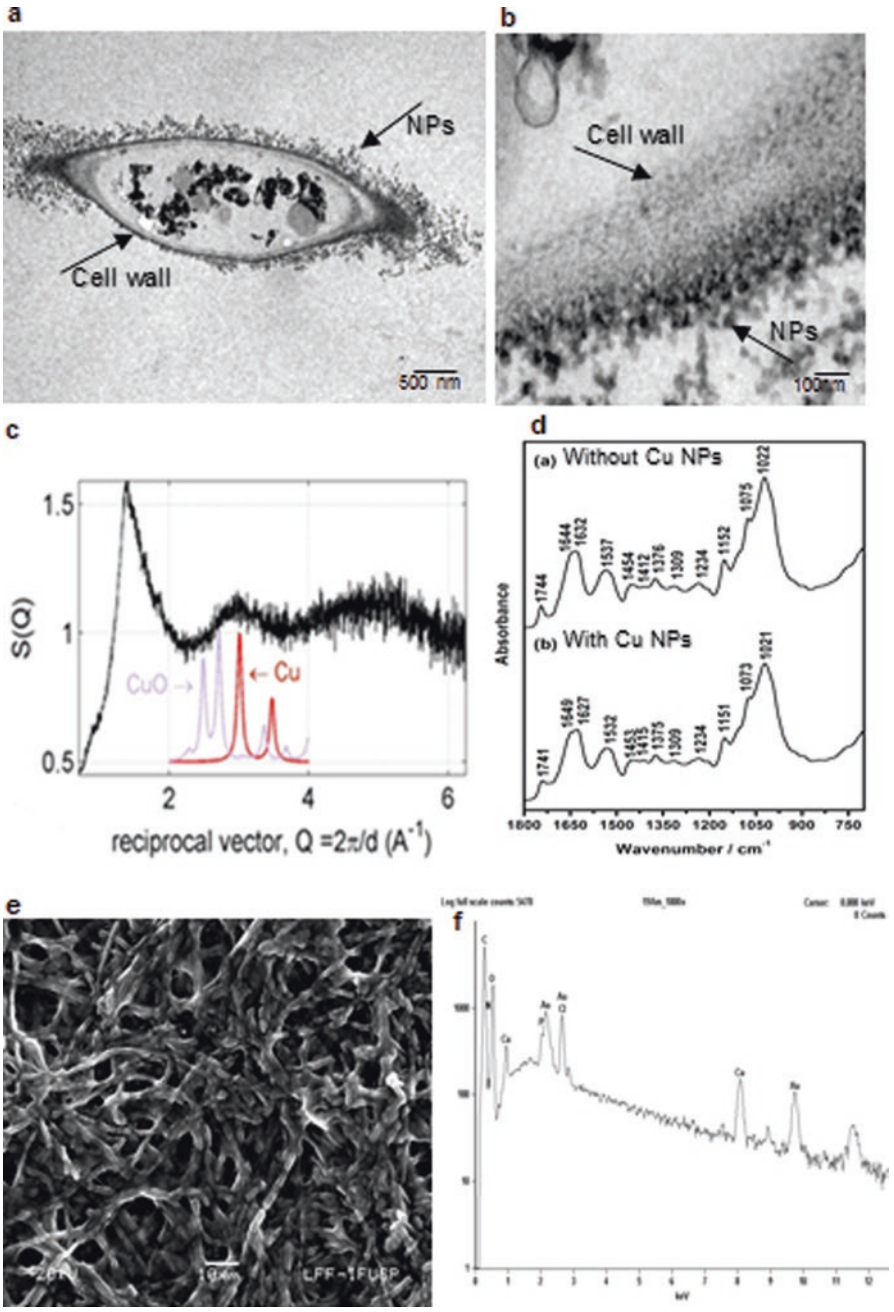

Fig. 7.5 Green synthesis of copper metallic NPs by dead biomass of fungus *Hypocrea lixii*. (**a**, **b**) TEM micrographs of *Hypocrea lixii* sections showing extracellular localization of copper NPs; (**c**) XRD analysis showed the metallic nature of copper NPs; (**d**) FT-IR spectra of dead biomass of *Hypocrea lixii* without and with copper NPs; (**e**) SEM images of dead biomass of *Hypocrea lixii* impregnated with copper NPs; and **f** EDS spectrum recorded of dead biomass of *Hypocrea lixii* after exposure to copper NPs. (From Salvadori et al. 2013)

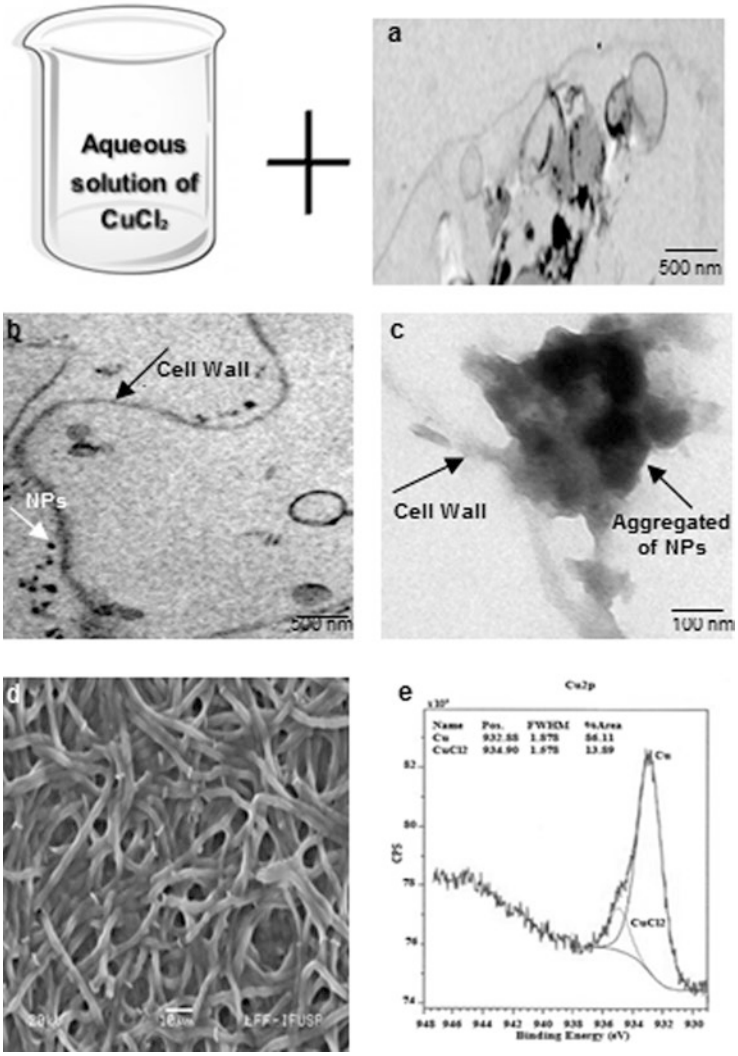

Fig. 7.6 Biosynthesis of copper metallic NPs by dead biomass of fungus *Trichoderma koningiopsis*. TEM images (**a**) section of dead biomass without copper NPs; (**b**) section of the fungus showing the extracellular localization of copper NPs and (**c**) aggregated of NPs; (**d**) SEM micrograph of biomass exposed to copper; and (**e**) XPS spectrum of Cu 2p core-level binding energies after the biosynthesis of copper NPs. (From Salvadori et al. 2014a)

7.6.2 Biosynthesis of Metal Oxide NPs Mediated by Dead Biomass of Fungi and Yeast

Metal oxide NPs, nano-powders, and nanotubes, especially from transition metal oxides, are currently used in various industrial segments as medicine, household

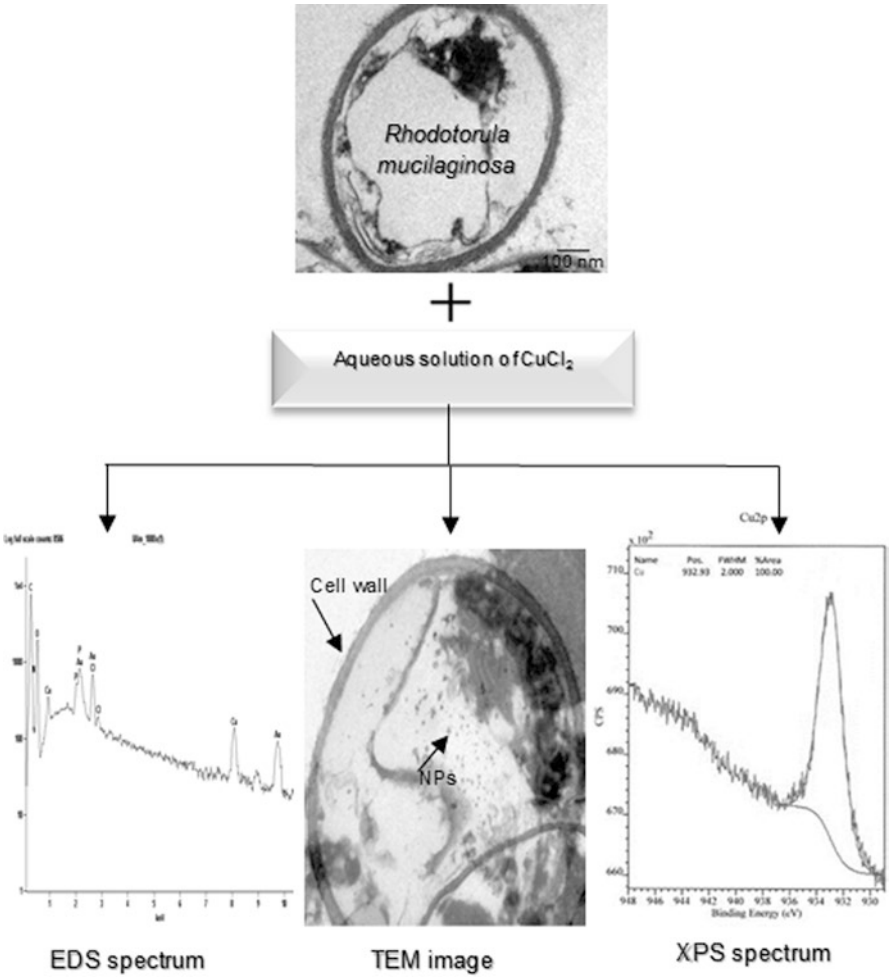

Fig. 7.7 Schematization of biosynthesis of metallic copper NPs by dead biomass of yeast *Rhodotorula mucilaginosa.* (From Salvadori et al. 2014b)

applications, and environmental remediation (Sharma et al. 2015). Synthetic methods such as microwave, hydrothermal, vapor deposition, spray pyrolysis, wet-chemical, solvothermal, and others are used for the production of metal oxide NPs (Gotic´ and Music 2008; Hayashi and Hakuta 2010; Kim et al. 2003; Leonelli and Lojkowski 2007). There are few descriptions in literature about the synthesis of metal oxide NPs. The research in this field is nowadays focusing more in the biosynthesis of NPs using microorganisms than conventional synthetic methods (Sharma et al. 2015). There are only few studies whose approach in the synthesis of metallic oxide NPs uses dead biomass of fungi and yeast.

Salvadori et al. (2014) reported that the fungus *Aspergillus aculeatus* isolated from copper mine in the Amazon region exhibited a high tolerance to nickel (up to

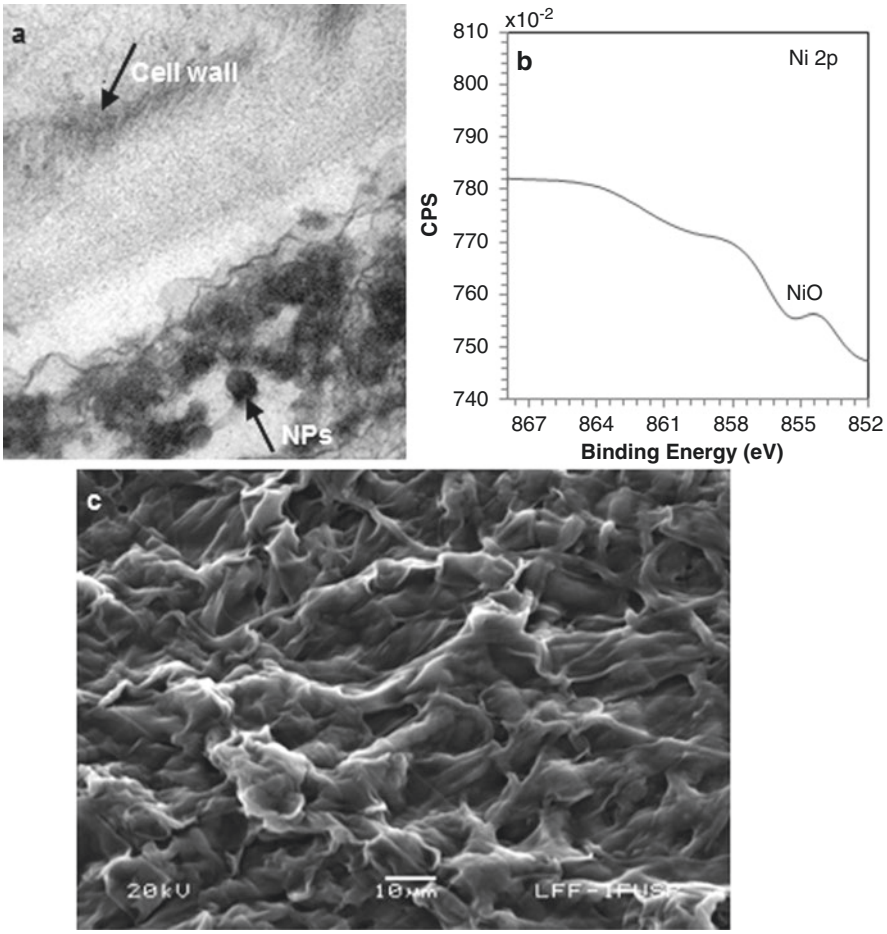

Fig. 7.8 Synthesis of nickel oxide NPs by the dead biomass of the filamentous fungus *Aspergillus aculeatus*. (**a**) TEM image showing the extracellular synthesis of nickel oxide NPs; (**b**) XPS spectrum of the NiO NPs; and (**c**) SEM image of the surface of dead biomass impregnated with NiO NPs. (From Salvadori et al. 2014)

1473 mg L^{-1}), and their use in dead biomass form under physicochemical conditions, as pH 4.0, temperature of 30 °C, contact time of 90 min, agitation speed of 150 rpm, initial Ni (II) concentration of 100 mg L^{-1}, and biosorbent dose of 1 g, showed a maximum uptake capacity of Ni (II) estimated by Langmuir isotherm model of 19.6 mg g^{-1}. The dead biomass of the fungus synthesized NiO NPs extracellularly, with an average size of 5.89 nm and predominantly spherical shape. The elements C, N, and O were determined by EDS analysis indicating the presence of proteins as a capping material on the surface of the NPs. The peak at 854.1 eV corresponding to the Ni $2p_{3/2}$ level observed in XPS analysis confirmed the nature of metallic oxide of the NPs (Fig. 7.8).

The use of dead biomass of the fungus *Hypocrea lixii* was also studied concerning their capacity to produce NiO NPs (Salvadori et al. 2015). It was observed by TEM and HRTEM analysis that the dead biomass of this fungus was able to synthesize NiO NPs extra- and intracellularly with average size of 3.8 nm and 1.25 nm, respectively, and are mainly spherical, in optimal physicochemical conditions to uptake the metal as temperature of 30 °C, pH 4.0, agitation speed of 150 rpm, and with a contact time of 90 min. A maximum retention capacity of 20.1 mg nickel g^{-1} was obtained by Langmuir isotherm model. FT-IR analysis of nickel oxide NPs showed a band at 1535 cm^{-1} that was shifted to 1542 cm^{-1}, assigned to the N-H deformation of amide II linkages of polypeptides or proteins (Zhang et al. 2011a), corroborating the results of EDS analysis that showed C, N, and O signals, confirming the presence of protein as capping agent involving the NPs. The XPS analysis confirmed the presence of NiO NPs. Salvadori et al. (2015) proposed a two-step process that involves the reduction of Ni^{2+} ions and their subsequent extra- and intracellular oxidation to NiO by dead biomass of *Hypocrea lixii*. The first step involves the interaction between nickel ions and amide groups found in the fungal cell wall and the subsequent ions bioreduction to metallic Ni, probably by enzymes present in the cell wall. The dead biomass probably used enzymes released from the cell during the autoclaving process and bound to the cell surface (Salvadori et al. 2013). The second step involves the oxidation of metallic NPs by water and oxygen present in the medium, due to the negative reduction potential of nickel. The formation of an oxide passivation layer would be expected, but the XPS results showed that the NPs are formed solely by NiO, which may be a consequence of the very small size (high superficial area) of the NPs formed (1.25 nm and 3.8 nm for intracellular and extracellular NPs, respectively). The intracellular synthesis of NPs is very similar to their extracellular synthesis: the Ni^{2+} ions interact with the fungal cell wall as a result of eletrostatic interaction with enzymatic groups present in the mycelial cell wall, and then they are probably reduced by enzyme inside the cell wall, leading to the aggregation of nickel ions and formation of NPs. The difference in this case is that the formed NPs are smaller than extracellularly. In this case it was also observed the complete oxidation leading to the formation of nickel oxide NPs (Fig. 7.9).

Salvadori et al. (2016) reported that the yeast *Rhodotorula mucilaginosa*, isolated from a wastewater of a mine in Amazon region, showed a high tolerance to nickel (up to 2971 mg L^{-1}). The dead biomass of yeast revealed an uptake capacity of nickel estimated by Langmuir isotherm model of 29.4 mg g^{-1} in these following conditions: pH 4.0, temperature of 30 °C, agitation speed of 150 rpm, contact time of 60 min, initial Ni (II) concentration of 100 mg L^{-1}, and biosorbent dose of 0.75 g. The TEM and HRTEM analysis indicated the extracellular synthesis of Ni/NiO NPs on the cell wall surface of the yeast and the NP size of 5.5 nm and spherical shape. The FT-IR spectra provided results that indicate the presence of proteins by the analysis of the band at 1529 cm^{-1}, which was shifted to 1534 cm^{-1} suggesting the capping of the NPs by the proteins. The high-resolution XPS spectrum of the Ni2p core-level photoemission signal after the synthesis of Ni/NiO NPs shows two components, a peak at 852.6 eV, which would be characteristic of the Ni $2p_{3/2}$ level of Ni (0) (Furstenau et al. 1985), and a peak at 854.2 eV that corresponds to the Ni $2p_{3/2}$

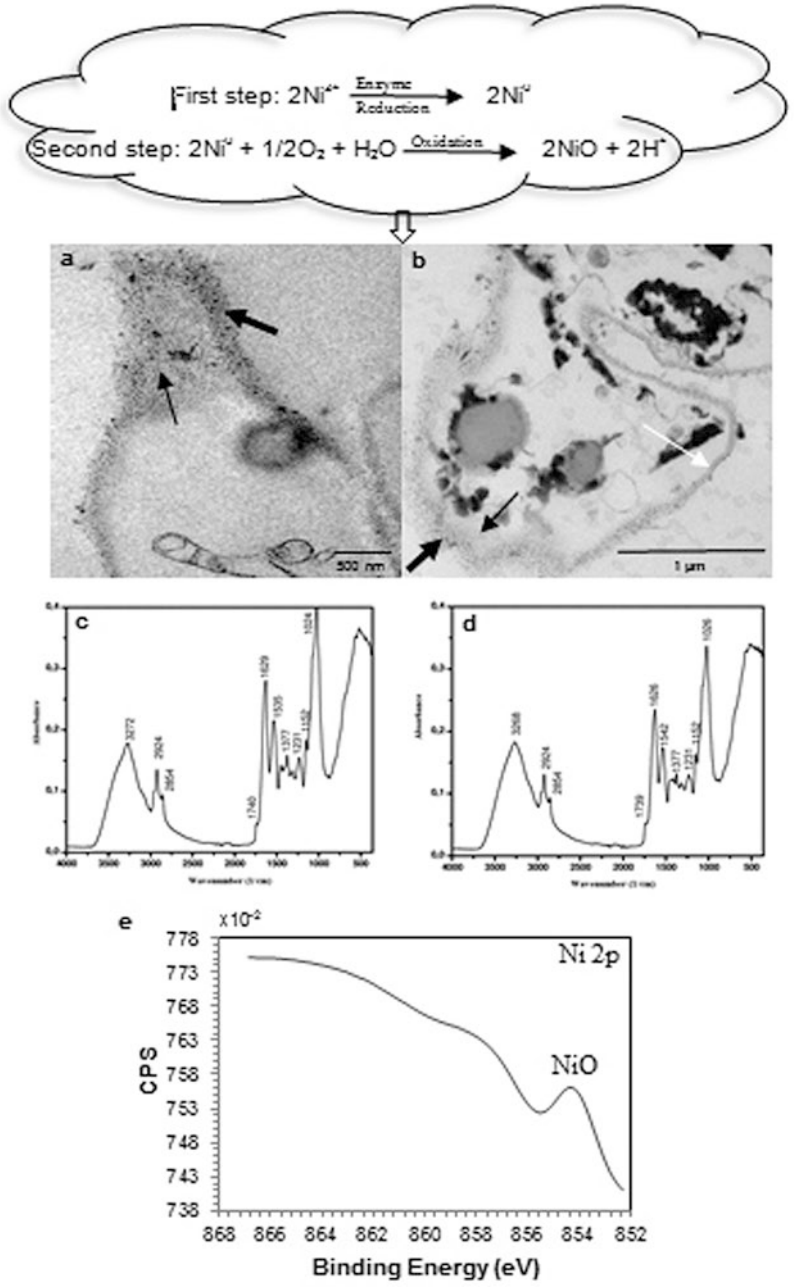

Fig. 7.9 Extra- and intracellular biosynthesis of nickel oxide NPs by dead biomass of *Hypocrea lixii*. (**a**) TEM image of a section of dead *Hypocrea lixii* biomass showing extracellular (darkest arrow) and intracellular (lighter arrow) nickel oxide NPs; (**b**) HRTEM photomicrograph of a section of dead *Hypocrea lixii* biomass showing the cell wall (white arrow), extracellular (darkest black arrow) and intracellular (lighter black arrow) nickel oxide NPs; FT-IR spectra of dead biomass of *Hypocrea lixii* (**c**) before and (**d**) after saturation with nickel; and (**e**) XPS spectrum Ni 2p core-level binding energy after synthesis of nickel oxide NPs by dead biomass of *Hypocrea lixii.* (From Salvadori et al. 2015)

level and is characteristic of NiO (Song et al. 2008) confirming the formation of the core-shell NPs of Ni/NiO (Fig. 7.10). Salvadori et al. (2016) propose a process that comprise two stages: in the first occurs the interaction between Ni^{+2} and amide groups located in the cell wall of the organic matrix and its subsequent bioreduction to Ni^0, possibly due to the presence of extracellular enzymes. These proteins present in dead biomass of the yeast probably are thermostable enzymes, able to resist to autoclaving temperature. In the second stage, the oxidation of Ni^0 NPs occurs by water and oxygen present in the medium, due to the negative reduction potential of nickel. There is a formation of an oxide passivation layer forming the Ni/NiO NPs as observed in XPS analysis.

7.6.3 *Biosynthesis of Metal and Metal Oxide Nanofilms Mediated by Dead Biomass of Fungi and Yeast*

Transparent conducting oxide thin films are attracting much attention due to their wide application such as solar cells, liquid crystal displays, detectors, and light-emitting diodes (Hosono et al. 2002; Wager 2003). NiO thin films have received considerable importance due to their technological and scientific applications in special electrical, optical, and magnetic properties (Ismail et al. 2013). There are scarce reports in the literature concerning the production of metal and metal oxide nanofilms using fungi and yeast, especially with regard to the use of dead biomass.

The synthesis of nanofilm using dead biomass of fungi was described by Salvadori et al. (2014), using the fungus *Aspergillus aculeatus* isolated from a mine of Amazon region. Morphological analysis of cross-sectional of the sample *Aspergillus aculeatus* surface by AFM provided an image of the NiO film formed by NiO NPs (Fig. 7.11).

Salvadori et al. (2016) described the organization of the Ni/NiO NPs synthesized by the dead biomass of yeast *Rhodotorula mucilaginosa* by the morphological characterization of the dead biomass surface after the synthesis of the NPs in the SEM and AFM images, which revealed the formation of a nanofilm on the surface of the dead biomass of the yeast structured by the Ni/NiO NPs. There are still no reports in the literature about the production of NPs of Ni/NiO by the dead biomass of the yeast *Rhodotorula mucilaginosa*, as well as their natural structuration in nanofilm (Fig. 7.12).

7.6.4 *Biosynthesis of Metal and Metal Oxide Magnetic NPs Mediated by Dead Biomass of Yeast*

There is high interest in developing nanoparticulate magnetic materials due to their potential application in areas such as ferrofluids (Raj et al. 1995), enhancement in magnetic resonance imaging (Schüler and Frankel 1999), magnetic recording

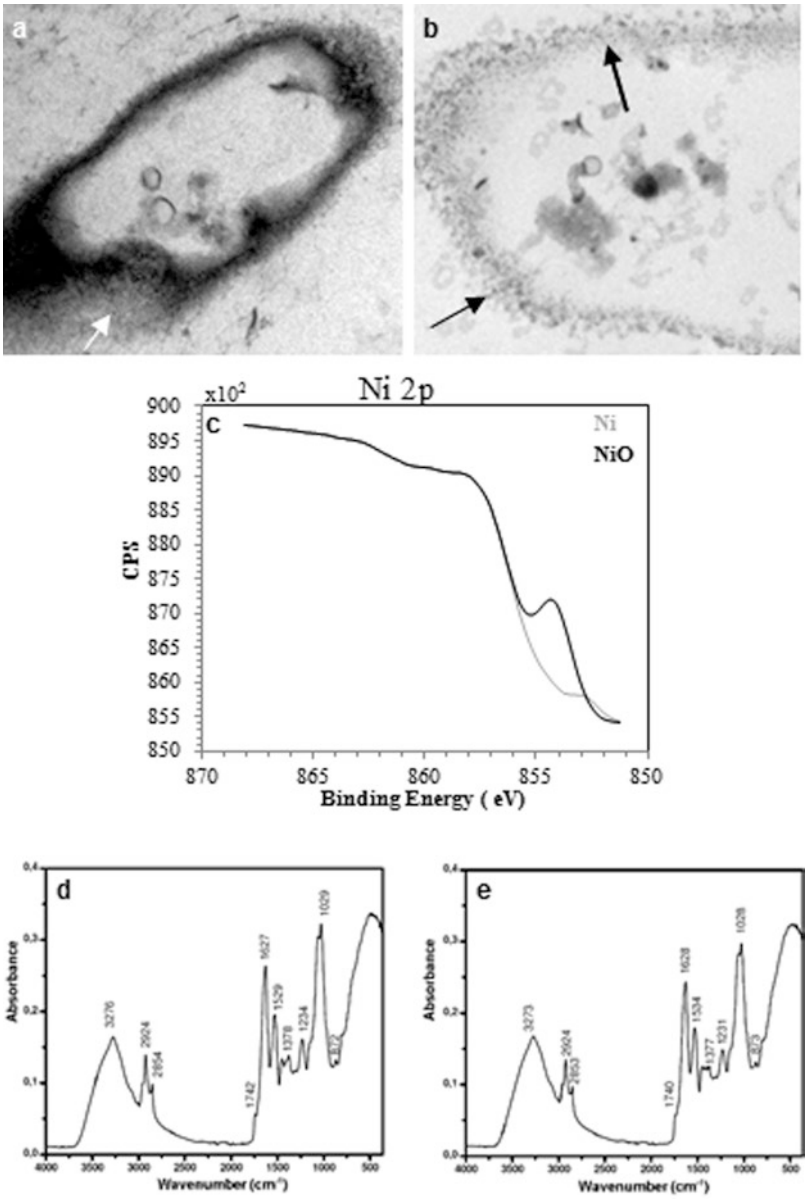

Fig. 7.10 Production of metallic copper NPs by dead biomass of the yeast *Rhodotorula mucilaginosa*. (**a**) TEM image of thin section of stained dead biomass of the yeast revealing the presence of Ni/NiO NPs extracellularly (*white arrow*); (**b**) HRTEM micrograph showing the cell wall (*darkest black arrow*), with deposition of NPs extracellular on the cell wall (*lighter black arrow*); (**c**) high-resolution XPS spectrum of the Ni/NiO NPs of the Ni2p core-level binding energy; FT-IR spectra of the dead biomass of the yeast *Rhodotorula mucilaginosa* (**d**) before and (**e**) after with nickel. (From Salvadori et al. 2016)

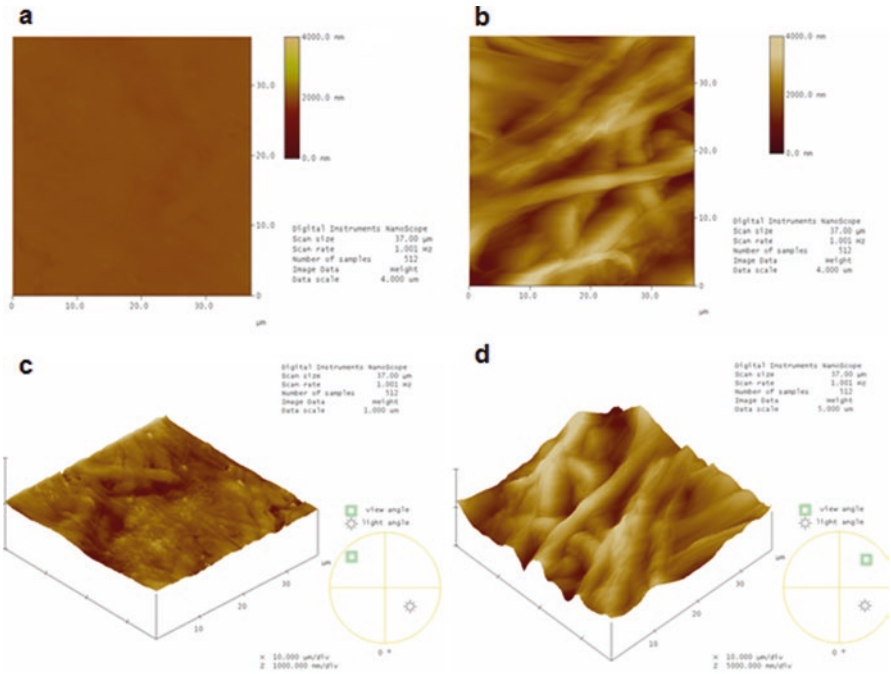

Fig. 7.11 AFM images of the surface of dead *Aspergillus aculeatus* biomass showing the NiO nanofilm formation. (**a**) AFM image (2D) of the dead fungal biomass without NiO NPs; (**b**) AFM image (2D) of the dead fungal biomass impregnated with NiO NPs, showing the formation of a film coating the surface of the dead fungal biomass; (**c**) AFM image (3D) showing the dead fungal biomass without NiO NPs; and (**d**) AFM image (3D) showing the dead fungal biomass after impregnation with NiO NPs which form a film on the surface of the dead biomass. (From Salvadori et al. 2014)

(enhanced blocking temperature) (Skumryev et al. 2003), shielding (superior microwave absorption) (Xi et al. 2011), and others. Salvadori et al. (2016) developed a natural technique that provides a process of industrial interest due to the concomitant uptake and production of magnetic metallic NPs from toxic metal of liquid waste that can result in detoxification and safe environmental discharge, by the synthesis of magnetic NPs of Ni/NiO by dead biomass of yeast *Rhodotorula mucilaginosa* (Fig. 7.13).

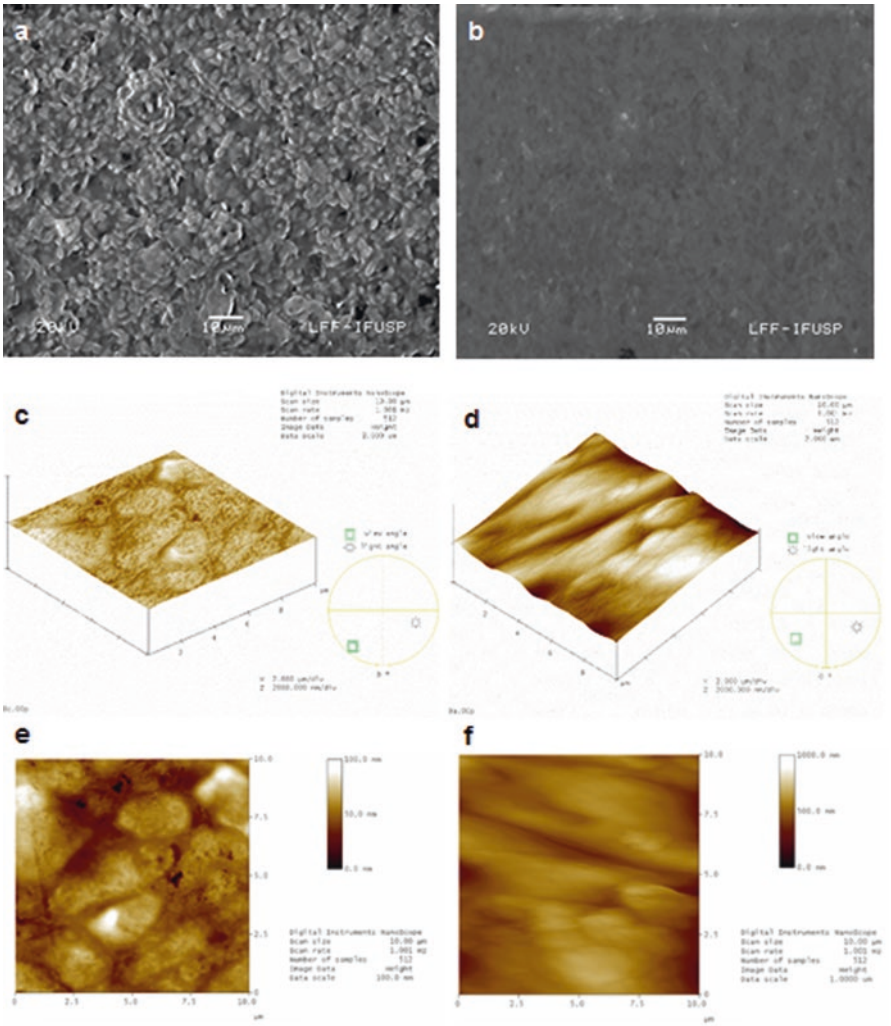

Fig. 7.12 Synthesis of nanofilm formed by Ni/NiO NPs produced by dead biomass of the yeast *Rhodotorula mucilaginosa*. (**a**) SEM micrograph of the surface of dead biomass of the yeast without Ni/NiO NPs; (**b**) SEM micrograph showing the formation of a film coating the surface of the dead yeast biomass after binding to the Ni/NiO NPs; (**c**) AFM image (3D) showing the dead biomass without Ni/NiO NPs; (**d**) AFM image (3D) showing the dead biomass after exposition of the Ni/NiO NPs, which form a film on the surface of the dead biomass; (**e**) AFM topographic image (height) showing the dead biomass without Ni/NiO NPs; and (**f**) AFM topographic image (height) showing the dead biomass after exposition of the Ni/NiO NPs, which form a film on the surface of the dead biomass. (From Salvadori et al. 2016)

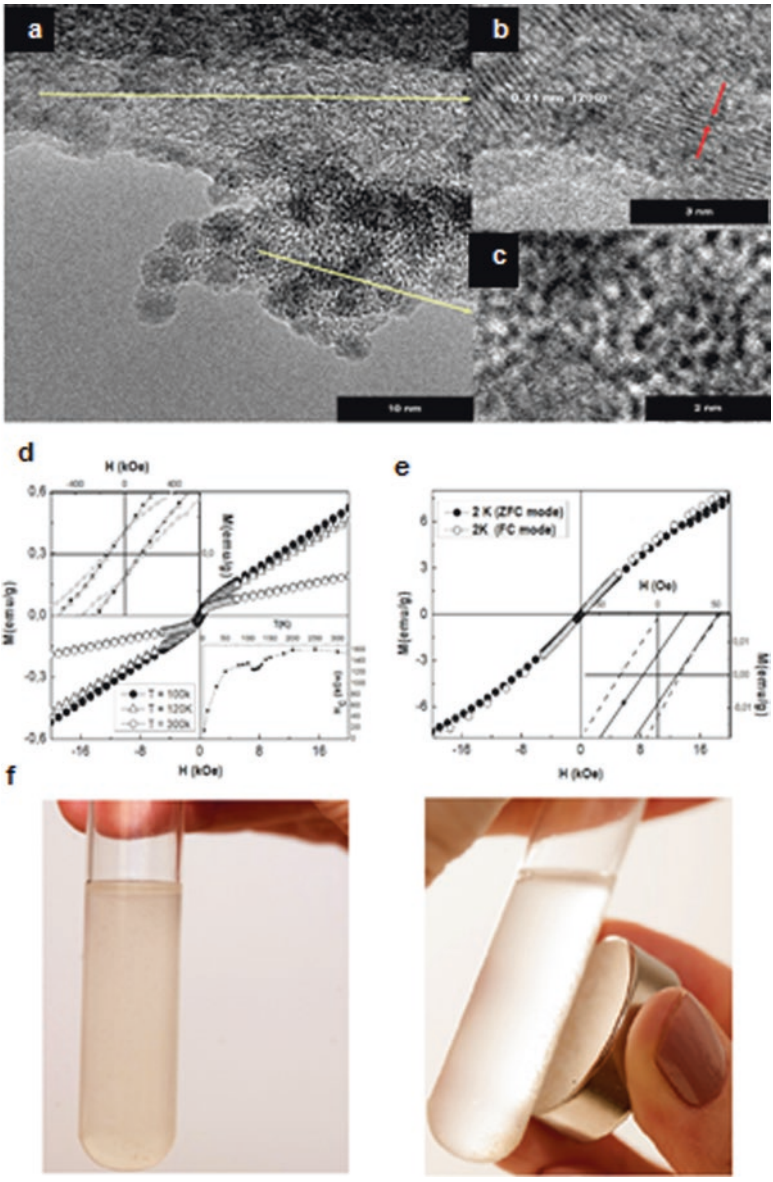

Fig. 7.13 Biosynthesis of magnetic Ni/NiO NPs by dead biomass of the yeast *Rhodotorula mucilaginosa*. HRTEM micrographs of the magnetic Ni/NiO NPs organized in film form on surface of the dead biomass (**a**); NPs details of the select regions, showing crystallized NPs with well-defined Bragg planes (**b**); amorphous NPs (**c**); (**d**) magnetization loops between 2 T and −2 T at 100 K, 120 K, and 300 K. Inset top left: detail of low field of the three measures. Inset down high: coercive field (*Hc*) obtained from magnetization loops between 2 K and 300 K; (**e**) magnetization loops at 2 K on ZFC and FC modes; and (**f**) image showing magnetic attraction of the Ni/NiO NPs. (From Salvadori et al. 2016)

7.7 Nanobiotechnology from Industrial Bioremediation Processes: Perspectives of Future Applications

The nanobiotechnology is emerging as a novel science, attracting the attention of researchers around the world, due to their wide applications such as cosmetics, fuel additives, textile engineering, lubricants, food packaging, paints, photonics, electronics, catalysis, sensing, drug delivery, imaging, biolabeling, agrochemicals, and environmental cleanup (Gajbhiye et al. 2009; Jelveh and Chithrani 2011; Khan et al. 2013; Khwaja and Husein 2016; Li et al. 2011; Nair et al. 2010; Prabhu and Poulose 2012; Rico et al. 2011; Salata 2004; Sperling et al. 2008; Prasad and Swamy 2013; Swamy and Prasad 2012; Prasad et al. 2014, 2016, 2017; Prasad 2014) (Fig. 7.14). The live and dead fungal biomass have a high potential for the biosynthesis of nanoparticles/nanodevices producing through reaction mixture nanomaterials with controlled shape and size.

7.7.1 Sensors

Due to their submicron size, nanosensors and other nanosystems are innovating the areas of biological and chemical analysis, making possible fast analysis of various substances in vivo (Jiarong et al. 2004). The Au-Ag alloy NPs biosynthesized by yeast cells were reported to be applied in the production of a sensitive electrochemical vanillin sensor (Zheng et al. 2010).

7.7.2 Catalysis

Industrial catalysts frequently depend on processes that occur on the surface of metals. Therefore metallic NPs with large surface-area-to-volume ratios are efficient catalysts. Bigall et al. (2008) utilizing common fungi as *Penicillium* sp. as template in media containing gold, silver, platinum, or palladium NPs observed in microscopy the formation of a crust of NPs on fungi surface, being these (fungi) metal-coated, used as catalysts.

7.7.3 Drug Delivery

The target of the creation and developing novel drug delivery systems is its specific and safe delivery to the targeted sites. The objectives reach a controlled release and obtain a high therapeutic effect. The nano-conveyors to reach target cells need to surpass the blood tissue obstacles (Häfeli et al. 2009), besides to have a contact with

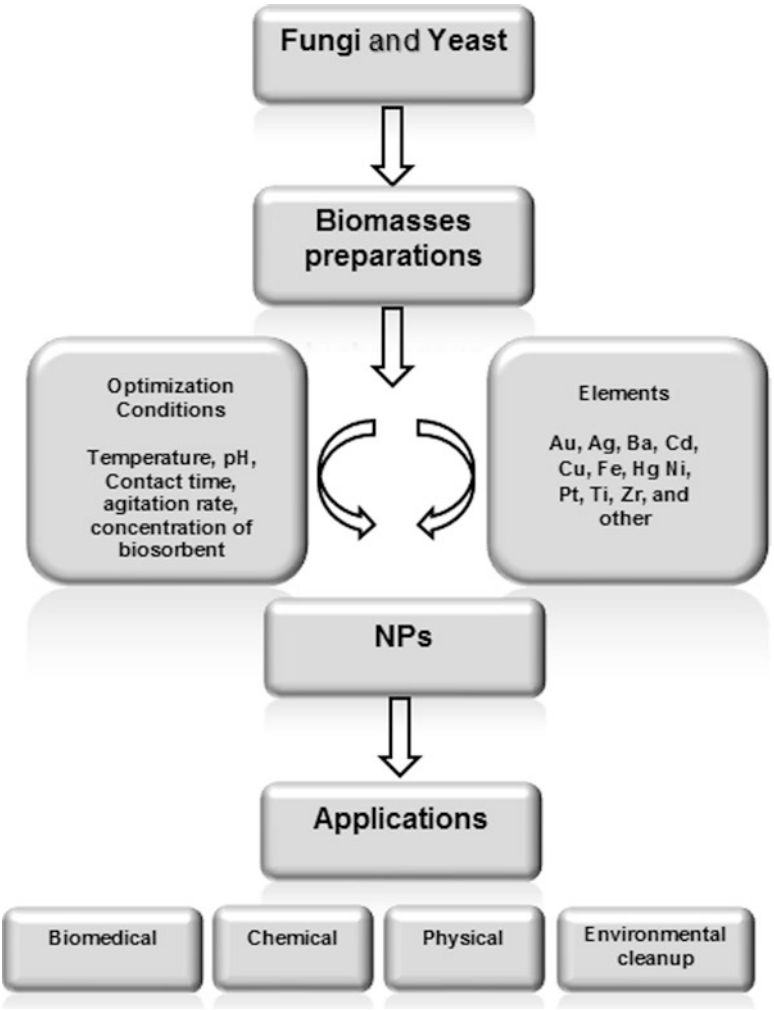

Fig. 7.14 Applications of metal NPs biosynthesized by fungi and yeast

cytoplasmic targets through special transcytotic and endocytotic transfer mechanisms crossing cellular obstacles up until target cells (Fadeel and Garcia-Bennett 2010). It is known that magnetic NPs as magnetite (Fe_3O_4) and maghemite (Fe_2O_3) are biocompatible; therefore they have applications in the gene treatment, examination of DNA, manipulation of stem cell, drug delivery, and target cancer therapy. These NPs are the aim of many studies (Xiang et al. 2007).

7.7.4 Antimicrobial NPs

The researchers have high interest of metal NPs because of their inherent antimicrobial activity, being used as antimicrobial agents in a wide range of commercially medical products (Pollini et al. 2011; Aziz et al. 2014, 2015, 2016). Au NPs synthesized by the fungus *Rhizopus oryzae* showed a growth inhibition of G- and G+ bacterial strains and also of the yeasts *Candida albicans* and *Saccharomyces cerevisiae* (Das et al. 2009). NPs produced by the fungi *Fusarium semitectum*, *Candida albicans*, *Phoma glomerata*, *Trichoderma* sp., and *Penicillium herbarum* in combination with fluconazole showed potential antifungal properties (Gajbhiye et al. 2009). Using the fungus *Amylomyces rouxii*, Musarrat et al. (2010) synthesized Ag NPs as efficient antimicrobial against the fungi *Fusarium oxysporum* and *Candida albicans* as well as the bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* type I, *Bacillus subtilis*, and *Citrobacter* sp. Fayaz et al. (2010) described the synthesis of Ag NPs by the filamentous fungus *Trichoderma viride* investigating the augmented antimicrobial activities of the antibiotics enriched of NPs, against Gram-positive and Gram-negative bacteria, where antibacterial activities of chloramphenicol, erythromycin, ampicillin, and kanamycin were augmented against test strains.

7.7.5 Medical Imaging Devices

The Ag NPs produced by the filtrate of the fungus *Trichoderma viride*, when submitted the laser excitation, showed in photoluminescence measurements emissions in the range of 320–520 nm, allowing the use of Ag NPs for labeling and imaging (Sarkar et al. 2010). Another example of medical imaging devices is the case of cadmium telluride quantum dots (CdTe QDs), covered with protein and highly solvable in water, produced by the yeast *Saccharomyces cerevisiae* (Bao et al. 2010a) and by UV-visible spectroscopy and spectrofluorimetry with photoluminescence emission from 488 to 551 nm, presented optical features, and when associated with folic acid could be used to investigated in vitro imaging of cancer cells (Bao et al. 2010b).

7.7.6 Environmental Cleanup

The pollution of water bodies by chemicals caused by industrial and anthropogenic activities became one of the principal stimuli to development of nanomaterials, employing fungal biomass. One of the utilizations of nanomaterials is in the treatment of water contaminated with pesticides and metals. There are reports of the removal of heavy metals through adsorption processes from nanomaterials by alloy

formation. Bootharaju and Pradeep (2010) studied the interaction of Ag NPs and Hg^{+2} ions and observed the incorporation of mercury into the Ag NPs. The ability of Ag NPs to reduce various metals can be seen as a technique to prepare alloys of NPs. For example, the electroplating process illustrates the preparation of alloy NPs, through the preparation of Pd-Ag and Pt-Ag NPs. Some heavy metals as mercury, arsenic, lead, and others have been detected by colorimetric techniques using nanomaterials. As an example we can cite the study of Ono and Togashi (2004) that uses the combination of metal ions with nucleotides, where the Hg^{+2} enabled the formation of thymine-thymine base pairs. In similar studies, functionalized nanomaterials are being used for the detection of metal ions; we can cite as examples the ligands cysteine (Hg^{+2}, Cu^{+2}), gallic acid (Pb^{+2}), etc.

The employment of nanomaterials to remove pesticides is a novel promising technology to solve this serious environmental problem, mainly toxic residues in potable water, due to the wide use of not controlled pesticides in agricultural practices as organophosphorus groups. In recent decades, for minimizing the environmental impact of pesticides, various processes were developed employing the nanotechnology. Das et al. (2009) observed the adsorption of organophosphorus pesticides by Au NPs produced by the fungus *Rhizopus oryzae*.

Recently, techniques for mining metals using fungi – mycomining – nowadays associated with nanotechnology (Salvadori et al. 2014a). Myconanomining has demonstrated new eco-friendly and cost-effective approaches to extract metals from low-grade ores, mill tailing, mineralized soil, or overburdens that would not be viable cost to mining industry to employ conventional processes. For example, we have the use of dead biomass of fungus *Trichoderma koningiopsis* to uptake ion copper and the concomitant synthesis of copper metallic NPs (Salvadori et al. 2014a).

7.8 Conclusions

In this chapter we explored the great potential of the employment of fungal dead biomass via mycoremediation process in synthesis of NPs, in polluted areas by toxic metals wasted by industry (soil and water bodies).

The application of dead biomass (biosorption-based bioremediation) by their easy handling and low operational cost contributes to a sustainable solution that can be used both in minor and large scale in bioremediation processes (mining, foundry, galvanoplasty industries, and others). This is an attractive industrial waste cleaning process (fungal dead biomass biosorption), which is able to synthesize metal NPs, in a cheap and efficient manner, opening new venues in novel natural technologies in nanoscience.

In function of the present reports using fungal dead biomass in bioremediation in conjunction with the nanotechnology, promising great advances were demonstrated in the successful implementation of the nanomycoremediation.

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Chapter 8
Nanofabrication of Myconanoparticles: A Future Prospect

Rajender Boddula, Priyanka Dubey, Saurabh Gautam, Ramyakrishna Pothu, and Aditya Saran

Abstract Nanofabrications of nanomaterials are widely used in electronic industries related to integrated circuits and visual display. There in electronics the more popular word is nanolithography. The synthesis methods of myconanoparticles (MNPs) are eco-friendly, easy and less costly than chemically synthesized nanomaterials. Till now a full control over the fungal growth is possible, but the same is not possible for the synthesis of MNPs either through intra- or extracellular environment. Myconanoparticles are generally large in size with high standard deviation and less uniformity. The lack of uniformity and some specific structural requirement for optical properties is a big challenge for the applicability of MNPs. Fabrication is a method which can be applied for reshaping the MNPs. Nanofabrication is the future of MNPs processing for its wide-scale practical and industrial applications.

Keywords Nanofabrication · Myconanoparticles · Reshaping of nanoparticles · Etching · Surface functionalization

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8.1 Introduction

Fungi are eukaryotic organism which occurs as a single cellular entity to multicellular form. As being more evolutionary developed from Kingdom Monera, fungi have more complex cytology and genetics. Fungi secrete many intracellular and extracellular enzymes other than various other excretions. These different excretions play as a reducing agent for the synthesis of nanoparticles. Myconanoparticles have low production cost and are easy to synthesize, and synthesis procedures are devoid of any harmful chemicals (Prasad 2016, 2017). Many different nanoparticles can be synthesized from fungi such as gold, silver, zinc, cadmium, silica, platinum, titanium and ferrous nanoparticles (Ravindra and Rajasab 2014). Nanoparticles have large applications in different sectors such as pharmaceuticals, diagnostics, catalysis, electronics, etc (Prasad et al. 2016). The potential of application of metal nanoparticles (MNPs) is too high, but in reality it was not being utilized commercially. Still on the commercial and industrial scale, chemically synthesized nanoparticles are used. There has been a lot of work done on the green synthesis of nanoparticles via fungi. Here this chapter will address the scientific reasons behind the hurdles in the application of MNPs and suggest some ways out to overcome it.

8.2 Size, Shape and Morphology of Myconanoparticles

As we know the synthesis process of nanoparticles through fungi is not fully controlled. Only the growth of fungi can be controlled. Mainly the nanoparticle follows the bottom-up synthesis process via reduction. Due to presence of different enzymes and molecules, the size, shape and morphology are not fully controlled. The size and the shape vary greatly. The variations in the size of myconanoparticle are shown by few examples listed below in Table 8.1.

There are many ingredients present in the extra- and intracellular environment of fungi whose functions in the nanoparticle synthesis are unknown. Additionally for a precise synthesis, the concentration of the ingredients and their molar ratio must be maintained which is not possible in the case of fungi or its crude extract. This is the main reason behind the wide size variations of MNPs.

8.2.1 Obstacles in the Application of Myconanoparticles

Mostly the standard deviation for the size is so high for the MNPs that it cannot be accepted for practical application. For a targeted drug delivery through nanoparticles as carrier inside the nucleus, the size of nanoparticle must be $\leq$5 nm. Nearly same types of limitations are there in every application. Even if the size and shape are in control, morphology needs to be addressed. For an example, if a nanoparticle is to be used as catalyst in ELISA, then its surface must be modified so that it can be

Table 8.1 Myconanoparticles synthesized through different fungi

Nanoparticle type	Fungal species	Size (in nm)	References
Gold	*Aspergillus oryzae*	10–60	Ni et al. (2008)
	Trichothecium sp.	5–200	Ahmad et al. (2005)
	Colletotrichum sp.	20–40	Tsung et al. (2006)
	Verticillium luteoalbum	<10–100	Wen et al. (2013)
	Verticillium sp.	2–20	Tsung et al. (2006) and Saa et al. (2014)
Silver	*Rhizopus nigricans*	35–48	Kim and McIntyre (2006)
	Trichoderma sp.	5–40	Foroughi-Abari and Cadien (2012)
	Mucor hiemalis	5–15	Aziz et al. (2016)
	Penicillium fellutanum	5–25	Leskelä and Ritala (2002)
	Cladosporium cladosporioides	10–100	Balaji et al. (2009)
	Fusarium semitectum	10–60	Stepanova and Dew (2011)
Zirconia	*Fusarium oxysporum*	3–11	Cui (2011a)
Zinc oxide	*Aspergillus terreus*	54.8–82.6	Binupriya et al. (2010)
Cadmium	*Fusarium oxysporum*	5–20	Ahmad et al. (2002)
Silica	*Fusarium oxysporum*	5–15	Gericke and Pinches (2006)
Platinum	*F. oxysporum f.* sp. *lycopersici*	10–100	Mukherjee et al. (2001a)
Titanium	*Fusarium oxysporum*	6–13	Gericke and Pinches (2006)
Magnetite	*Fusarium oxysporum*	20–50	Mukherjee et al. (2001b)

A high size variation of myconanoparticles synthesized through a particular species in the same set of growth condition

attached to detection antibody. Nanoparticle bio interfaces must be addressed properly. For the practical application of MNPs, there is a strong need of fabrication in order to get desired shape, size and surface modification.

To overcome these obstacles, two approaches can be made: (1) development of methods to synthesize MNPs precisely and (2) reshape the synthesized MNPs to desired shape, size and morphology. At present there are some approaches already in use for reshaping the nanoparticles. Nanolithography is a technique being used in electronic industry and R&D to get desired pattern, size and functional surface.

8.3 Nanofabrication

It is method of fabricating nanoparticles to a desired shape, size and surface modification. For the nanofabrication, two types of approach are possible – 'bottom up' and 'top down'. In the bottom-up approach, nanomaterial is synthesized precisely by adding atom by atom, molecule by molecule or cluster by cluster. Here in 'bottom-up' approach, synthesis process is well focused and addressed. Majority of the

chemical synthesis follows bottom-up approach. So the ratio, concentration of different ingredients, chemical kinetics and other physical factors must be precise in order to get uniform desired nanoparticles.

Top-down approach is to cut down or etch the bulk or nanomaterial to desired shape and size. Top-down approach has originated from the conventional lithographic technique, which is used as a standard in semiconductor industries. For the proper application, a definite shape, size, morphology and surface modified nanoparticles are required. Top-down approach plays most important role in redefining the nanoparticle and makes it suitable as per requirement for its application. At many places both bottom-up and top-down approaches are utilized to get the desired shape and size of nanoparticles.

The synthesis of nanoparticles by the fungus in extracellular or intracellular environment takes place via bottom-up approach. Biological nanoparticles including MNPs are usually not uniform in size and shape. Because of uncontrolled/semi-controlled synthesis process, myconanoparticles tend to grow large in size if synthesis process is not stopped. The easiest way to stop the synthesis process of myconanoparticles is the extraction of MNPs from fungal culture or extract. There are many other ingredients other than needed for MNPs present in the fungal cell/extract. It is not necessary that ingredients which are responsible for the synthesis of MNPs will also present with MNPs after extraction. So it is difficult to know the exact ingredients responsible for the synthesis of particular MNPs. Hence it is difficult to get the uniformly shaped and sized MNPs which are highly required for their practical application at large scale. Top-down approach of reshaping the nanoparticles is a problem-solving option. There are several options from harsh to mild etch process. The optimized one can get the full control over the etching process to get the desired shape and size of nanoparticles.

8.3.1 Deposition at the Nanoscale

Conventionally monolayer or multilayer deposition of non-reactive or functional material is done through evaporation, sputter deposition, chemical vapour deposition or electrochemical deposition. Deposition becomes necessary when selective etching is required. Sometimes one end of a nanoparticle is required to etch, and other times the end needs to be protected from etching. Hence as per requirement, deposition at nanoscale is done to achieve the desired shape and size of nanoparticles.

8.3.2 Surface Functionalization

For the purpose of nanofabrication, many surface modification procedures are available. Nanofabrication procedures are dependent upon the strength of the interaction between the modifier and the substrate surface, which is in turn controlled by the

surface bonding chemistry. Along with other criteria, hydrophilic or hydrophobic nature of surface or its modifiers are important aspect to consider. As per need some depositions are made to protect the surface from corrosion or etching.

Spin-coating and vapour deposition are commonly used for surface films substantially thicker than a monolayer. Multilayer surface modification can be done through diazonium reduction, electropolymerization, layer by layer deposition of molecular and atomic multilayers, etc. (Stepanova and Dew 2011).

Atomic layer deposition (ALD) is a thin film chemical vapour deposition technique. Its concept was first proposed by Professor Aleskovskii in his PhD thesis published in 1952. This technique is able to deliver extremely conformal, pin hole-free, nanometre-thick films. This technique is applied in various applications like display technology, integrated circuit fabrication, solar cells, catalysis, etc. For the deposition of metals and the low temperature deposition of oxides and nitrides, plasma ALD is used. ALD forms ultra-thin films efficiently and where the geometry is also complex (Foroughi-Abari and Cadien 2012; Kim and McIntyre 2006). The self-limiting growth also enables large area uniformity, reproducibility and excellent adhesion. AVD technique has process temperature less than 400 °C, and there is no gas phase nucleation, and hence the defects are much lower (Leskelä and Ritala 2002).

8.4 Etching

Etching is a process by which a material is removed from its or another material's surface. There are three important formats of etching which are electrolytic etching (EE) format, dry chemical etching (DCE) format and wet chemical etching (WCE) format. For an etching process, some information and physical factors are important. These are etchants, types of etching process, compositions of ingredients, time, temperature and rate of reaction. In case of electrolytic etching (EE), additional information of anode, cathode and power is required, and for dry etching process, addition information of gas flow and pressure is required.

There are more than 200 different methods for etching. Here in this chapter we will only focus on those etching methods which can be applied for the nanofabrication of MNPs. Some of the selected etching methods and their applicability are listed in Table 8.2.

On the basis of process, there are main two types: dry etching and wet etching. Dry etching process involves plasma etching, chemical reactions by using reactive gases, physical removal by momentum transfer or the combination of chemical reactions and physical removal. On the other hand, wet etching process involves the liquid chemicals or etchants.

Table 8.2 Some selected etching methods which can be applied for reshaping of myconanoparticles (Walker and Tarn 1990)

Etching methods	Description/application
Acid etch	Strong acids H_2SO_4, HNO_3 to weak acids
Adhesion etch	To etch thin films
Aged etch	Slow etching process
Agitation etch	To prevent bubbles from adhering to surfaces being etched
Air etch	Actual etching is done by oxygen
Isotropic etch	To etch equally at all facets
Anisotropic etch	Here etchants attach crystallographic planes different rates
Contamination removal etch	For cleaning the surface of nanoparticles from undesired ions and molecules
Oxide etch	To clean oxide surface
Ozone etch	Ozone is extremely strong oxidizing agent
Plasma etch	Uses ionized gas particles
Redox etch	Both oxidation and reductions take place. One acts as reducer, while other acts as oxidizer. Example: HF:HNO_3
Removal etch	To reduce the thickness of metal
Seeded etch	Small materials are added to an etching solution which initially attacks rapidly for first few seconds, and then etching goes on by a controllable linear rate
Selective etch	Wet chemical etch and dry chemical etch methods are common
Sequence etch	Many etchants are used one by one as per requirement
Ultrasonic etch	Useful for breaking large nanoparticles into smaller units

8.4.1 Dry Etching

Dry etching techniques are mostly used in electronic industry and its R&D for integrated circuits and semiconductors (Table 8.3). Dry etching process may have following steps:

1. Generation and transport of reactive species (atoms, molecules, ions) within the plasma-sheath surface system
2. Physisorption or chemisorptions of reactive species on the surface
3. Dissociation of reactants, formation of chemical bonds to the surface, diffusion, and formation of desorbing species
4. Desorption and transport of product species from surface to plasma
5. Possible redeposition of etching products

Pulsed lasers have more potential to get utilized for the fabrication of metallic MNPs. By using a pulsed laser, on an average a power density of 10 MW/cm^2 is needed to melt a metal up to 200 nm thickness, and on average 100 MW/cm^2 power density is required for substantial vaporization (Berggren et al. 1995).

Table 8.3 Some of the important dry etching techniques listed

Dry etching methods	Description	References
Electron beam lithography (EBL)	Fabrication of semiconductor devices and nanometre-sized pattern for direct writing	Cobley et al. (2009)
Focused ion beam (FIB) lithography	Enhanced capabilities over EBL and can work with a resolution of 10 nm and can do direct material modification by ion-induced mixing	Cui (2011b)
Atom lithography	Fabrication of arrays of micro- and nanostructures	Doherty (1979)
Pulsed laser	Ultrafast fabrication of metal nanostructures	Berggren et al. (1995)
Soft UV nanoimprint lithography	Suitable for nanostructuration at the 20 nm scale	Wanzenboeck and Waid (2011)
Interferometric lithography	Applied when periodical patterns are required	Cattoni et al. (2011)
Ultrashort pulsed lasers	It involves the removal of material through the ablation process	Soppera et al. (2011)

8.4.2 Wet Etching

Wet etching processes are simple, need less equipment, have high etching rate and have high selectivity. Wet etching process is popular in the mass production of electronic devices especially in integrated circuit manufacturing and devices. Wet etching process is not suitable to fine-line patterning with critical dimension (CD) control, because of the isotropic nature of chemical reaction processes. The wet etching process is advantageous over dry etching process because it doesn't damage the under layers induced by high energetic ionic plasma species or laser irradiation (Table 8.4).

To keep an etching process selective, it is necessary that rate of etching of unwanted material must be higher than the desired material. This selectivity can be achieved through the vastly different chemical reactions between the unwanted and the desired material with the selected wet etchant. Etching selectivity (S) between the first and second material is defined through an equation $S = (r1/r2)$. Here $r1$ is the etching rate of unwanted material, and $r2$ is the etching rate of desired material. Hence, the higher the value of 'S', the more the selectivity. Selectivity is important where there is more than one layer of different materials. It is to be noted that selectivity is not considered in the case of only reducing the size. Over etching is required when there is a need of complete removal of unwanted material and it is suitable to reduce the size (Liu et al. 1997).

A typical wet etching process consists of three steps. First, the reactive ions are transported to the surface to be etched by the bulk solution through diffusion. Then the ions react with the surface, and then finally the reaction products dissolve and diffuse out from the structure. The etching process is controlled by concentration of etchants and its ratio, time, temperature and agitation.

Table 8.4 A comparison between dry etching and wet etching (Chen et al. 2012)

	Dry etching	Wet etching
Etching rate	Low	High
Uniformity	Good for small plane and fine-pitch patterning	Poor for small plane and fine-pitch patterning
Reproducibility	Good	Poor
CD loss	Small	Large
Selectivity to under layer	Poor	Good
Profile control	Good	Very poor
Multilayer etch	Possible	Difficult
Advantage	Anisotropic	High selectivity
	Fine-pattern definition	Free of damage
	Fewer waste problems	High throughput
	Better process control	
Disadvantages	Damage issue	Isotropic
	Selectivity issue	Fine-pattern limitation
	Low throughput	Incomplete etching
		Bubble formation
		Scum remainder
		Adhesion problem

Iodine and potassium iodide solution are used as common etchants for gold, while aqua regia can be used for gold as well as silver. H_3PO_4-HNO_3-H_2O (80:4:16) is a good etchant for aluminium.

8.4.3 Reshaping of Nanoparticles

Even chemically synthesized nanoparticles are reshaped as per required/desired characteristics such as LSPR and optical properties. Gold nanoparticles have good biocompatibility and can be conjugated with bacteria, antibodies, biomolecules, etc. The transverse and longitudinal surface plasmon resonances correspond to electron oscillations perpendicular and parallel to the rod length direction, respectively. Their longitudinal surface plasmon wavelengths (LSPWs) can be tuned from the visible to infrared regions. Their absorption cross sections are at least five orders larger than those of conventional dyes, and the light scattering by Au nanorods is several orders larger than the light emission from strongly fluorescent dyes (Saglam et al. 2016; Lee and El-Sayed 2005; Jain et al. 2006). The tenability in the LSPW, together with strongly enhanced scattering and absorption at the LSPW, makes Au nanorods useful for the formation of many functional composite materials, for example, with hydrogel (Chen et al. 1997; Gorelikov et al. 2004), polymers (Karg et al. 2007; Pérez-Juste et al. 2005), silica (Murphy and Orendorff 2005) and bacteria (Chon et al. 2007).

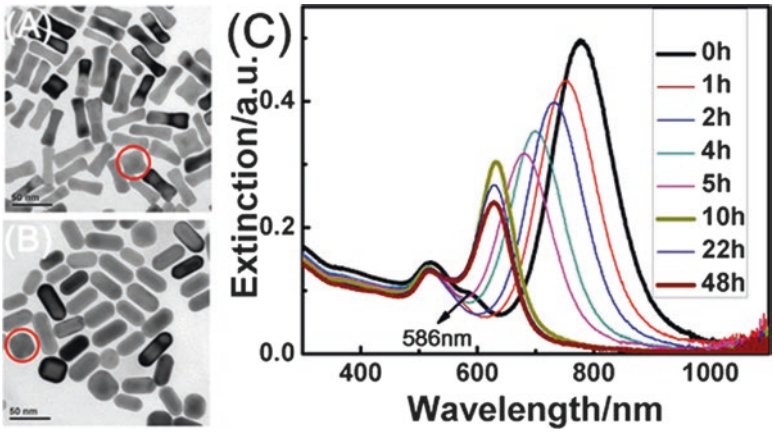

Fig. 8.1 Typical TEM images of original dog-bone GNRs (**a**) and after 10 h shape conversion (**b**) and evolution of UV-vis-NIR spectra of the GNRs during shape conversion (**C**). Reaction conditions: [GNRs] = 0.5 nM, [CTAB] = 0.1 M, [H2SO4] = 10 mM, [Cu^{2+}] = 100 μM. Notice that the dog-bone cuboids (red circle in this figure) also become rounded at the corners (Wen et al. 2013)

The reshaping of gold nanoparticles by making use of dissolved oxygen was first reported by Tsung et al. (2006). The dissolved oxygen etches by mild oxidation. Mild oxidation process gives better control over the reaction, and the oxidation rate can further be controlled by temperature and acid concentration. Surface of Au atoms with high surface energy is stabilized by forming Au-O complexes. In this complex the oxygen is called static adsorbed oxygen. At low concentration of copper ions, the Au-O complexes are removed by oxidative etching leaving a clean surface. This clean surface gets stabilized via surface atom diffusion. At high concentration of copper ions, etching is initiated by dissolved oxygen. Here the dissolved oxygen is called dynamic adsorbed oxygen. In the case of gold nanorods, at high concentration of copper ions, the etching is anisotropic that results in short length gold nanorods. Copper ions produce synergistic effect in presence of other etch agents such as H_2O_2 and Fe^{3+} via scavenging adsorbed oxygen species and providing more Au atoms for etchants. Due to high affinity of oxygen to silver and palladium, such synergistic effect is expected to be more prone in silver and palladium. Tao et al. demonstrated the reshaping and etching of gold nanorods through the assistance of copper ions. Purified dog-bone-like GNRs were dispersed in a CTAB aqueous solution. Different concentration combinations of CTAB, Cu^{2+} ions, and H_2SO_4 were used for reshaping at different temperatures. Post 10 h incubation, the morphology at ends of dog-bone gold nanorods changed to hemisphere. Due to this, the localized surface plasmon resonance (LSPR) features affects greatly (Wen et al. 2013) (Fig. 8.1).

Thermodynamically the stability is higher for spherical nanoparticles. So the mild etching process will make less spherical nanoparticles to more spherical nanoparticles (Wen et al. 2013). Hence it is easy to reshape the roughly spherical nanoparticles to spherical. The spherical nanoparticles would show narrow and high peak at specific wavelength.

The strategy of etching of gold nanorods through the mediation of iodine to generate a plasmonic effect can detect up to 100 pg/ml of IgG (Bansal et al. 2005). In another work for the detection of glucose, gold nanorods were etched by the gradual oxidation in presence of trace concentration of H_2O_2 through the activity of HRP assisted by halide ions by Saa et al. (Baskar et al. 2013). Silver nanoprism etching-based plasmonic ELISA can detect 100 pg/ml with limit of detection 4.1 fg/ml (Kumar et al. 2007). In the chemical etching, halide ions act as a ligand to reduce the electron potential of the gold species, which enables ferric ions to oxidize the gold nanorods and results in the etching of the gold nanorods. The redox etching leads to a significant decrease of the gold nanorods in length but little change in diameter, which could be attributed to less surface passivation or higher chemical reactivity of the tips of the gold nanorods (Riddin et al. 2006).

Ni et al. (2008) demonstrated the shortening the length of gold nanorods while keeping the diameter constant by etching through H_2O_2. By this the longitudinal surface plasmon resonance peak gradually blue-shifts, and decrease in intensity was observed during oxidation. For thickening the diameter of gold nanorods by keeping the length constant, an optimized concentration of cysteine is added which attaches with the end of gold nanorods. The cysteine end capped nanorods can be put in growth solution. Cysteine will inhibit the growth of gold nanorods at the end region, and hence growth at transverse region will occur only which will lower the aspect ratio (Fayaz et al. 2010).

Zou et al. (2009) reported chemical etching of gold nanorods by ferric chloride at room temperature. Here, halide ions act as a ligand to reduce the electron potential of the gold species, which enables ferric ions to oxidize the gold nanorods and results in the etching of the gold nanorods. The redox etching leads to a significant decrease of the gold nanorods in length but little change in diameter (Kathiresan et al. 2009).

A new method of wet etching was developed by Cobley et al. for the production of high-quality single crystalline Ag nanospheres. They used ferric nitrate-based etching solution to truncate the sharp corners of Ag nanocubes to make it sphere of same size. The method can be prolonged to get spherical Ag nanoparticles of smaller sizes. The change in size will affect the surface-enhanced Raman scattering (SERS) (Berry et al. 2005).

8.5 Conclusion

Reshaping and surface modification myconanoparticles can make it applicable for practical application at commercial scale. It is possible to top down the myconanoparticles to desired shape and size with suitable surface modification for practical applications. The reshaped and fabricated myconanoparticles can find their application in various fields such as killing and inhibiting of microorganisms, treatment of cancer and detection of biomolecules both in vitro and in vivo in the

near future. In a far future, it may be hoped that these green synthesized nanoparticles get to find their place also in electronics and integrated circuit sector.

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Chapter 9
In Vitro Secondary Metabolite Production Through Fungal Elicitation: An Approach for Sustainability

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Abstract Being sessile, plants produce an array of secondary metabolites in response to different biotic and abiotic stimuli to survive under adverse conditions. Many of these secondary metabolites are valued for their medicinal properties and are known for their usage to cure diseases since ancient time. Even after the discoveries of synthetic drugs, the interest for drugs derived from medicinal plants is gaining importance as they are safe and devoid of any side effects. A number of important medicinal plants are yet to be cultivated for commercial purposes and are collected from wild habitat, posing a threat to their existence. To protect the biodiversity and achieve the sustainability, development of alternative strategies for production of secondary metabolites is necessary. Plant cell, tissue and organ culture including hairy root cultures have shown great potential for production of secondary metabolites. However, sometimes, these cultures fail to synthesise adequate secondary metabolites compared to plants growing under natural condition. The production of secondary metabolites can be enhanced by using different elicitors, either biotic or abiotic, which act as stress agents and enhance the production of secondary metabolites in plant callus/cell suspension culture, root culture, shoot culture and hairy root culture. Fungal elicitor (including yeast extract) is one of the preferred elicitors used for this purpose. In this chapter, attempts have been made to provide a comprehensive account of the strategies used for increasing the production of secondary metabolites in different in vitro culture system using fungal elicitors. A brief account of elicitors, elicitations and general mechanism of elicitations has also been provided.

Keywords Cell suspension culture · Fungal elicitors · Hairy root culture · Medicinal plants · Plant tissue culture · Secondary metabolites

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9.1 Introduction

Plants have been a major source of drugs to human population. The pharmaceutical properties of the plants are due to the presence of secondary metabolites, also named as phytochemicals or natural products (Bourgaud et al. 2001; Kliebenstein 2013) or specialised metabolites (Pichersky and Gang 2000; Yonekura-Sakakibara and Saito 2009). Higher plants not only produce primary metabolites, which are indispensable for normal functioning of plants, but also secondary metabolites for adaptation to the environment and defence against pathogens (Wink 1988; Bednarek and Osbourn 2009; Murthy et al. 2014). About 100,000 (Verpoorte et al. 1999; Wink 2015) to 200,000 (Dixon and Strack 2003) secondary metabolites are estimated to be produced by plants. But these numbers may significantly increase as metabolic profiling of a number of plant species is yet to be done (Pichersky and Lewinsohn 2011). Many of these secondary metabolites may be directly used as therapeutic agents or as starting material for synthesis of new drugs and are thus of great value to human society (Yadav and Dixit 2008). Due to their pharmacological properties, they are of huge commercial importance. For example, plant-derived compounds such as vinblastine, vincristine and camptothecin derivatives have played a major role in the development of several clinically useful anticancer drugs (Da Rocha et al. 2001). In addition, artemisinin, a sesquiterpene lactone isolated from *Artemisia annua*, has antimalarial properties and has the potential to kill multidrug-resistant strain of *Plasmodium falciparum* (Woodraw et al. 2005; Ulhemann et al. 2007).

Even at present, over three quarters of world population relies mainly on plants and their extracts for health causes (Hannan et al. 2011). In general, herbal products symbolise safety being devoid of any kind of side effects in contrast to synthetic compounds. Population rise, inadequate supply of drugs, inflated costs of treatment and development of multidrug-resistant pathogens led to shift in emphasis from synthetic drugs to the use of plant materials as a source of medicine for the treatment of wide variety of human ailments. Unfortunately, the bioactive secondary metabolites are produced in very low quantities in plants (Dixon 2001; Rao and Ravishankar 2002). Moreover, biosynthesis of many of these metabolites is developmentally regulated and accumulated in plants in response to specific environmental stimuli (Verpoorte et al. 2002). Besides, some secondary metabolites are species specific or limited to only a few species maybe due to their plant lineage-specific evolution (Pichersky and Lewinsohn 2011). For example, artemisinin is naturally produced in *Artemisia annua* and a few other species of Artemisia (Van der Kooy et al. 2008; Liu et al. 2009; Ali et al. 2017) and the anticancer compounds vincristine and vinblastine are produced exclusively in *Catharanthus roseus* (El-Sayed and Verpoorte 2007). In addition, sometimes, plants growing in a particular habitat are difficult to grow outside their biotopes and are so not amiable to systematic cultivation (Bourgaud et al. 2001). A number of valuable medicinal plants are collected from wild due to lack of cultivation practices for commercial uses and trades. Unregulated collection of such plants from wild habitats may eventually lead to their extinction. For example, *Blepharispermum subsessile*, an endemic medicinal

plant of India, is found in the states of Odisha, Chhattisgarh, Madhya Pradesh, Karnataka and Maharashtra (Saxena and Brahman 1995; Naidu et al. 2014; Jadhav et al. 2016). The plant, known for its use in arthritis treatment, has already been overexploited and now enlisted as threatened species of Odisha, India (Ved et al. 2008). It is also difficult to get plant materials of uniform quality when collected from wild as the growth conditions of the plants might be different. Under such circumstances, the chemical synthesis of the compounds may be an alternative. However, chemical synthesis is usually difficult and expensive, as in the case of artemisinin (Woodraw et al. 2005; Weathers et al. 2011). It is thus difficult to depend upon the natural or cultivated populations of plants for obtaining secondary metabolites in large quantity. Plant tissue culture technology including hairy root culture has the potential to overcome these limitations. These techniques can be used as an alternative platform for sustainable production of secondary metabolites.

In this chapter, a comprehensive account of the strategies used for enhancing the production of secondary metabolites of pharmaceutical importance in plant cell and tissue cultures through the intervention of fungal elicitors has been discussed. A brief account of elicitors, elicitations and mechanism of fungal elicitations has also been provided.

9.2 In Vitro Culture Systems for Secondary Metabolite Production

Over the past few decades, a wealth of information has been accumulated regarding production of valuable secondary metabolites in vitro using plant cell, tissue and organ culture techniques (Rao and Ravishankar 2002; Karrupusamy 2009). Plant tissue culture techniques successfully used for production of secondary metabolites include callus/cell suspension culture, shoot culture, adventitious root culture, in vitro regenerated plantlets, hairy root culture and callus-mediated somatic embryogenesis. The advantage of plant tissue culture for the production of secondary metabolites is availability of uniform quality products season independently throughout the year, in a relatively shorter duration. In addition, these in vitro culture methods can comparatively yield more secondary metabolites than natural plants. Novel compounds can also be isolated which is normally not present in the natural populations of that particular species. Sustainability can be achieved by the use of these in vitro culture methods to produce these compounds as dependency on wild population is reduced (Rao and Ravishankar 2002; Bhatia and Bera 2015; Fig. 9.1).

In early 1970s, it was a common belief that, unlike differentiated organs, the undifferentiated mass of cells including callus or cell suspension was unable to produce secondary metabolites (Krikorian and Steward 1969; Bourgaud et al. 2001). However, subsequent works provided evidences that plant cells are capable of producing secondary metabolites present in the mother plant as they are biosynthetically

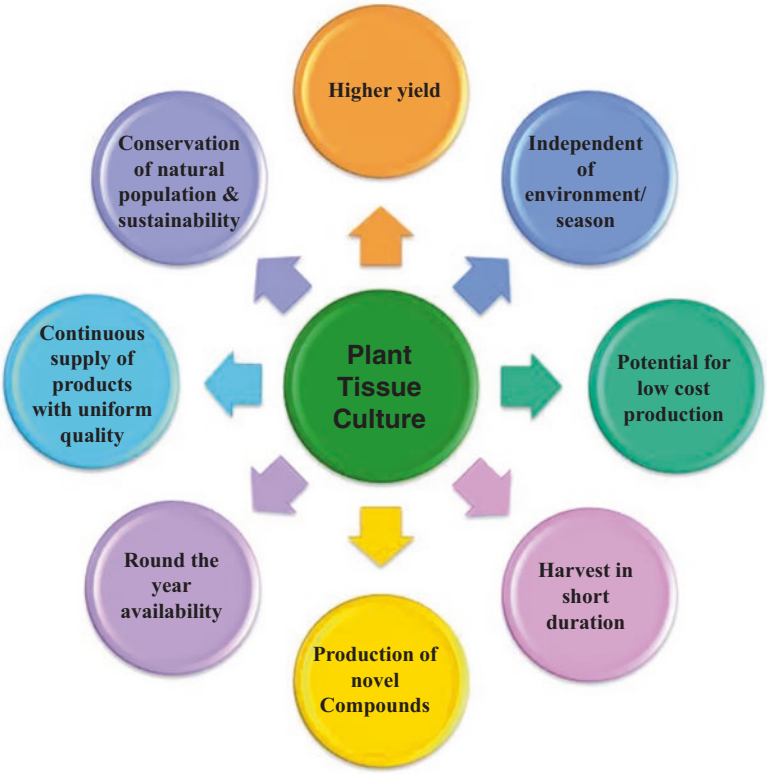

Fig. 9.1 Schematic representation of advantages of plant tissue culture for the production of secondary metabolites

totipotent due to retention of the genetic potential (Zenk 1991; Rao and Ravishankar 2002). The cell suspension culture has the potential for sustainable production of the industrially important secondary metabolites (Pattnaik et al. 2000). However, in a number of cases, the low yield and poor quality of secondary metabolites are the major bottleneck for their potential use in commercial scale (Yue et al. 2016). It is also assumed that regulations of a number of genes responsible for secondary metabolites production are cell, tissue and/or developmental stage specific (Facchini and St-Pierre 2005; Wink et al. 2005; Sharma et al. 2013) and accumulation of many of the precursors are restricted to specific cell types. It is also possible that many of these key regulatory and/or biosynthetic genes do not express in undifferentiated tissues and that may be the reason why callus and cell suspension systems failed to produce large amount of secondary metabolites than the normal plant system (Wink 1987; Wink et al. 2005). In spite of these facts, till date probably, cell suspension culture mediated in vitro secondary metabolite production is most documented method, if not the most efficient.

The past few decades have witnessed a significant progress in the development of tissue culture-mediated propagation of medicinal plants (Barik et al. 2007; Chaturvedi et al. 2007; Nayak et al. 2013; Mohanty et al. 2013; Moharana et al. 2018). The use of shoot and root cultures for the production of secondary metabolites are preferred by many as they are stable and produce comparatively higher amount of secondary metabolites. This may be attributed to their nature of being the differentiated tissues unlike undifferentiated callus or suspension culture (Bourgaud et al. 2001; Rao and Ravishankar 2002; Wink et al. 2005; Murthy et al. 2008). A number of studies have already been reported where shoot cultures and adventitious roots are being used for secondary metabolites production (Kovačević and Grubišić 2005; Vinterhalter et al. 2008; Mercy et al. 2012). The major limitation associated with shoot and root cultures is the availability of efficient in vitro propagation methods for rapid multiplication of plant materials for commercial scale isolation of secondary metabolites. The other drawback of in vitro root culture of higher plants is their slow growth rate (Sevon and Oksman-Caldentey 2002).

Hairy root cultures have the potential to overcome the drawbacks of shoot/root cultures as well as callus/cell suspension cultures. *Agrobacterium rhizogenes*-infected cells under suitable conditions differentiate into hairy roots in vitro. These hairy roots shows high growth rate in hormone-free media, unlimited branching and biochemical and genetic stability (Sharma et al. 2013). Most importantly, these hairy roots not only resemble normal roots but also have the potential to produce valuable secondary metabolites present in natural roots (Pistelli et al. 2010). As mentioned earlier, the synthesis of secondary metabolites is assumed to be associated with the degree of differentiation of tissues (Wink 1987; Sevon and Oksman-Caldentey 2002). Being an organised differentiated tissue, the hairy root culture has the potential to overcome the limitation of undifferentiated callus or cell suspension cultures as a source of secondary metabolites (Sevon and Oksman-Caldentey 2002; Sharma et al. 2013). The hairy root cultures for production of secondary metabolites have already been reported in a number of plant species with different degrees of success (Giri and Narasu 2000; Srivastava and Srivastava 2007; Mehrotra et al. 2015).

To overcome the limitations of plant tissue and cell cultures as discussed above and for enhanced production of secondary metabolites through plant tissue culture including hairy root culture, different strategies have been employed. The use of elicitors is one of extensively studied methods.

9.3 Elicitors and Elicitations

Elicitors are defined as ‘a substance which, when applied in small concentrations to a living system, initiates or improves the biosynthesis of specific compounds’ (Radman et al. 2003). The process of induction or enhancement of biosynthesis of secondary metabolites due to addition of trace amount of elicitors is known as ‘elicitation’ (Radman et al. 2003). Among the various available methods, elicitation is

the most practically acceptable strategy to enhance the production of secondary metabolites in plants (Poulev et al. 2003; Namdeo 2007). Elicitors are classified as biotic and abiotic, based on their nature of origin. Biotic elicitors are of biological in origin. They may include complex 'undefined' fungal mycelial preparations including yeast extract and fungal spores. Exact composition of these 'undefined' elicitors is not known, and they may comprise of several different molecular classes. However, mannan, cellulase, chitosan, alginate, pectin, etc. derived from biological sources with known 'defined' chemical structure are also act as biological elicitors (Radman et al. 2003; Vasconsuelo and Boland 2007). Abiotic elicitors are of non-biological origin. They may be physical (e.g. thermal stress, UV irradiation, etc.) or chemical (e.g. heavy metal salts, inorganic salts, osmotic stress, etc.) in nature (Vasconsuelo and Boland 2007).

9.4 Mechanism of Fungal Elicitor Action

The beginning of the elicitor action starts at the membranes. Plant has several receptors that are localised in the plasma membrane and organellar membranes of the cell and are key feature for the binding of large diverse group of elicitors. Such large variable number of elicitor molecules can stimulate several plant-based receptors indicating that the plants respond to different elicitors through these common receptors. Once the elicitor signals are perceived by the receptors, the process of elicitation begins. As the elicitors are recognised by their respective receptors, a series of signals are transduced in the form of secondary messengers into the cell. These downstream signals carry out a number of cellular activities necessary for the cell to survive and adapt to the changes in the environment.

Elicitors can act through different pathways and alter various biochemical and physiological processes in the cell. Although elicitor-mediated responses initiate at the membrane, it can have a series of responses to multiple cellular targets through multiple channels or pathways. Such an elaborative and extensive consequence of elicitor-mediated responses are not yet fully understood; however, the recent developments in this field of plant biotechnology and secondary metabolite production put forward new insights into the mechanism of elicitor action and its application. A hypothetical model depicting the sequence of events following recognition and binding of an elicitor to receptor in plant cells is shown in the Fig. 9.2.

One of the first biochemical and molecular responses that happens to the cell is change in the ionic fluxes across the plasma membrane. Binding of elicitor to the plasma membrane alters the flow of key ions such as H^+, K^+, Cl^- and Ca^{2+} by modulating the ion channels (i.e. influx of Ca^{2+}, efflux of K^+ and Cl^-) leading to acidification of the cytosol. Such change in ion fluxes affects the physiological responses within the cellular system (Mithöfer et al. 2005; Zhao et al. 2005; Siddiqui et al. 2013). Calcium influx during the elicitation is one of the early measurable signalling responses that play crucial role in the physiochemical state of the cell. A resting cell maintains a constant concentration of cytoplasmic calcium ions and this value

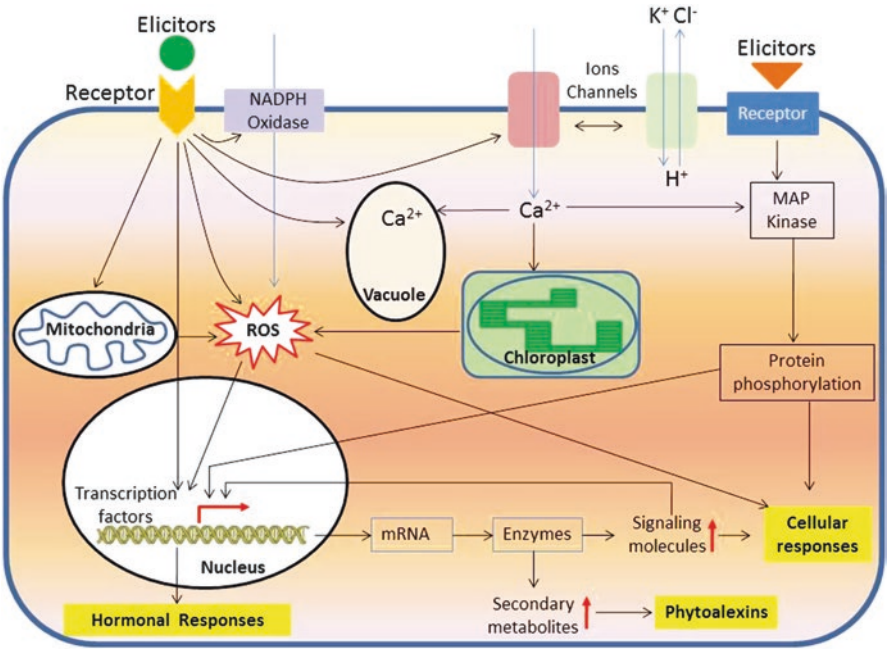

Fig. 9.2 A generalised schematic representation of elicitor-mediated responses in plant cell

is elevated under the effect of elicitation. Such concentration-based calcium signalling is specifically dependent on the magnitude and duration of the stimulus (White 2000; Garcia-Brugger et al. 2006; Lecourieux et al. 2006; Manzoor et al. 2012). Calcium pool in cytoplasm or in plant organelles like chloroplasts and vacuoles play a central role in the elicitor-mediated signalling cascades. Calcium ions acts as a secondary messenger in many elicitor-mediated responses including cAMP pathway (Hofer and Lefkimmiatis 2007); hormonal signalling like jasmonic acid, salicylic acid and ethylene pathway (Zhao et al. 2004; Arimura et al. 2008); inositol triphosphate mediate pathway (Berridge 2009); and calcium-dependant protein kinase pathway (Murillo et al. 2001; Cheng et al. 2002). In all these calcium ion-mediated pathways, both cytoplasmic and organellar concentrations induce the expression of defences and stress-related genes that promotes the formation of secondary metabolites and phytoalexins.

Another key response of elicitation is the activation of NADPH oxidase and the formation of reactive oxygen species (ROS) (Sagi and Fluhr 2006). Due to the decrease in the pH of the cytoplasm, NADPH oxidase gets activated that in turn increases the reactive oxygen and nitrogen radicals within the cellular systems. In addition, different oxidases and peroxidases present in mitochondria and chloroplasts can release large number of oxidised radicals leading to oxidative stress. Free radicals like hydrogen peroxide, hydroxide ion, superoxide ion, singlet oxygen (Jabs et al. 1997; Zhao et al. 2007; Sewelam et al. 2016; Mazars et al. 2017) and

nitric oxide (NO) (Palavan-Unsal and Arisan 2009) have been found to be associated with elicitation-based induction. The oxidative burst and formation of ROS in cell can activate, directly or indirectly, the expression of various stress-responsive transcription factor genes which in turn induce the downstream targets leading to the formation of secondary metabolites (Baxter et al. 2014; Rejeb et al. 2014).

In addition to above responses, elicitors can also induce mitogen-activated protein kinases (MAPKs). The MAPK cascade is known to participate in the phosphorylation of several transcription factors that can regulate the expression of genes responsible for the biosynthesis of signalling molecules like salicylic acid and jasmonic acid, as well as secondary metabolites and other secondary messengers that can protect the plant cell from foreign intruders (Pitzschke et al. 2009; Taj et al. 2010; Meng and Zhang 2013; Paul et al. 2017). A detailed review on the mechanism of elicitor-mediated signal transduction pathways that participate in the cellular and hormonal responses in plant defence and biosynthesis of secondary metabolites has recently been compiled by Zhai et al. (2017).

9.5 Fungal Elicitor-Mediated Enhanced In Vitro Secondary Metabolite Production

In vitro production of secondary metabolites using different biotic and abiotic elicitors has been reviewed previously by Namdeo (2007) and Jimenez-Garcia et al. (2013). In recent times, microbe-based biotic elicitors are gaining importance owing to their ability to mimic a disease response in plant cells which, in turn, switches on defence signal cascade and activate the biosynthesis of secondary metabolite in vitro (Jeong et al. 2005; Chandra and Chandra 2011; Biswas et al. 2016). Of the different biotic elicitors, fungal elicitors have been widely used for stimulation of secondary metabolite production in in vitro cultures (Baldi et al. 2009; Wang and Wu 2013). Production of secondary metabolites through fungal elicitation has been mostly studied in cell suspension culture or hairy root culture because it is relatively easy to scale up in bioreactor. Despite their limitations, use of shoot culture, root culture, in vitro seedlings and even somatic embryos derived from calli have also been reported for enhanced production of secondary metabolites through fungal elicitation (Table 9.1). Recently, Tonk et al. (2016) reported the use of fungal elicitation to increase the yield of vincristine and vinblastine through somatic embryogenesis in *Catharanthus roseus*. They found that fungal elicitor improved the growth of callus biomass as well as enhanced the yield of alkaloids from callus. Interestingly, the fungal elicitor significantly enhanced the number of somatic embryo formation compared to control. The germinating somatic embryos produced from elicitor-induced calli also showed high amount of alkaloids. Biosynthesis of a number of important secondary metabolites with diverse biological activities has been successfully produced in various medicinal plant species through fungal elicitation (Table 9.1). A few notable examples are the production of taxol (anticancer) in cell

Table 9.1 Secondary metabolite production in different in vitro culture systems using fungal elicitors

Plants (family)	Secondary metabolites enhanced	In vitro culture systems	Fungal species	Elicitor used for optimum result	Duration of elicitor treatment	Fold or % rise of secondary metabolites with compare to control	References
Dioscorea deltoidea (Dioscoreaceae)	Diosgenin	Cell suspension culture	*Rhizopus arrhizus*	Mycelia extract	3 d	72%	Rokem et al. (1984)
Thalictrum rugosum (Ranunculaceae)	Berberine	Cell suspension culture	–	Carbohydrate fraction of yeast extract	–	4	Funk et al. (1987)
Tagetes patula (Asteraceae)	Thiophene (bithienylbutine)	Hairy root culture	*Botrytis cinerea*	Cell wall filtrate	48 h	204%	Mukundan and Hjortso (1990)
	Thiophene (acetoxybutinylbithiophene)					259%	
Tagetes patula (Asteraceae)	Thiophene	Hairy root culture	*Aspergillus niger*	Mycelia extract	–	85%	Buitelaar et al. (1992)
Eschscholzia californica (Papaveraceae)	Benzophenanthridine alkaloids	Cell suspension culture	Commercial source	Yeast extract	–	3.5	Byun and Pedersen (1994)
Artemisia annua (Asteraceae)	Artemisinin	Hairy root culture	*Verticillium dahliae*	Mycelia extract	4 d	45%	Wang et al. (2000)
Salvia miltiorrhiza (Lamiaceae)	Cryptotanshinone	Cell suspension culture	Commercial source	Carbohydrate fraction from yeast extract	5 d	[a]	Chen and Chen (2000)
Artemisia annua (Asteraceae)	Artemisinin	Hairy root culture	*Colletotrichum* sp.	Mycelia extract	4 d	[a]	Wang et al. (2001)
Ocimum basilicum (Lamiaceae)	Rosmarinic acid	Hairy root culture	*Phytophthora cinnamomi*	Cell wall fragments	12 d	2.67	Bais et al. (2002)

(continued)

Table 9.1 (continued)

Plants (family)	Secondary metabolites enhanced	In vitro culture systems	Fungal species	Elicitor used for optimum result	Duration of elicitor treatment	Fold or % rise of secondary metabolites with compare to control	References
Catharanthus roseus (Apocynaceae)	Ajmalicine	Cell suspension culture	*Trichoderma viride*	Cell free filtrate	48 h	3	Namdeo et al. (2002)
Taxus chinensis (Taxaceae)	Paclitaxel	Cell suspension culture	An endophytic fungus of *Taxus*	Hydrolysate filtrate	22 d	>70	Su et al. (2002)
Taxus chinensis var. *mairei* (Taxaceae)	Taxol	Cell suspension culture	*Fusarium oxysporum*	Mycelia extract	96 h	3	Yuan et al. (2002)
Artemisia annua (Asteraceae)	Artemisinin	Hairy root culture	*Colletotrichum gloeosporioides*	Oligosaccharide B II extract	4 d	[a]	Wang et al. (2006)
Linum album (Linaceae)	Podophyllotoxins	Cell suspension culture	*Sebacina vermifera*	Coculture	24 h	3.9	Baldi et al. (2008a)
	6-Methoxy podophyllotoxin					7.6	
Linum album (Linaceae)	Podophyllotoxins	Cell suspension culture	*Sebacina vermifera*	Coculture	24 h	3.76	Baldi et al. (2010)
	6-Methoxy podophyllotoxin					8.74	
Euphorbia pekinensis (Euphorbiaceae)	Isoeuphpekinensin	Cell suspension culture	*Fusarium* sp.	Mycelia extract	6 d	5.81	Gao et al. (2011)
	Euphol					3.56	
Gymnema sylvestre (Apocynaceae)	Gymnemic acid (gymnemagenin)	Cell suspension culture	*Aspergillus niger*	Cell extract	–	9	Devi and Srinivasan (2011)
Drosera indica (Droseraceae)	Plumbagin	Whole plant culture	–	Yeast extract	6 d	5.4	Thaweesak et al. (2011)

Calophyllum inophyllum (Clusiaceae)	Inophyllum A	Cell suspension culture	*Phoma* sp.	Dried cell powder	45–50 d	751	Pawar et al. (2011)
	Inophyllum B		*Nigrospora sphaerica*	Dried cell powder		414	
	Inophyllum C			Culture filtrate		928	
	Inophyllum P					750	
Catharanthus roseus (Apocynaceae)	Alkaloid	Cell suspension culture	*Fusarium oxysporum*	Coculture	36 h	48%	Tang et al. (2011)
Hypericum perforatum (Hypericaceae)	Anthocyanins	Cell suspension culture	*Aspergillus flavus*	Mycelia extract	14 d	4	Gadzovska-Simic et al. (2012)
Atractylodes lancea (Asteraceae)	Volatile oil	In vitro plantlet	*Gilmaniella* sp.	Mycelia extract	10 d	–	Wang et al. (2012)
	β-Eudesmol					4.07	
Andrographis paniculata (Acanthaceae)	Andrographolides	Cell suspension culture	*Aspergillus niger*	Dried cell powder of mycelia	10 d	6.94	Vakil and Mendhulkar (2013)
Salvia miltiorrhiza (Lamiaceae)	Cryptotanshinone	Hairy root culture	*Trichoderma atroviride*	Mycelia extract	18 d	83	Ming et al. (2013)
	Dihydrotanshinone-I			Mycelia extract		35	
	Tanshinone I			Polysaccharide fraction		5	
	Tanshinone IIA			Polysaccharide fraction		11	
Artemisia annua (Asteraceae)	Artemisinin	Shoot culture	*Piriformospora indica*	Cocultivation	–	60%	Sharma and Agrawal (2013)
Centella asiatica (Apiaceae)	Asiaticoside	Shoot culture	*Trichoderma harzianum*	Culture filtrate	25 d	2.53	Prasad et al. (2013)
Linum album (Linaceae)	Podophyllotoxin	Hairy root culture	*Piriformospora indica*	Filter-sterilised culture filtrate	2 d	3.8	Kumar et al. (2013)
	6-Methoxy podophyllotoxin					4.4	

(continued)

Table 9.1 (continued)

Plants (family)	Secondary metabolites enhanced	In vitro culture systems	Fungal species	Elicitor used for optimum result	Duration of elicitor treatment	Fold or % rise of secondary metabolites with compare to control	References
Linum album (Linaceae)	Podophyllotoxin	Hairy root culture	*Fusarium graminearum*	Fungal extract	6 d	2	Bahabadi et al. (2014)
	Lariciresinol					3.1	
	6-Methoxy podophyllotoxin		*Trichoderma viride*			2.4	
Picrorhiza kurroa (Scrophulariaceae)	Picrotin	In vitro raised plants	Commercial source	Yeast extract	10 d	3.8	Rawat et al. (2014)
	Picrotoxinin					1.88	
Psoralea corylifolia (Fabaceae)	Psoralen	Cell suspension culture	*Aspergillus niger*	Mycelia with spores extract	72 h	9	Ahmed and Baig (2014)
Linum album Kotschy ex Boiss (Linaceae)	Podophyllotoxin	Cell suspension culture	*Fusarium graminearum*	Culture filtrate	5 d	3	Tahsili et al. (2014)
	Lariciresinol					2	
	Total phenolics				3 d	2	
Hypericum perforatum (Hypericaceae)	Hypericin	Cell suspension culture	*Fusarium oxysporum* f. sp. *Lini/Phoma exigua/Botrytis cinerea*	Mycelia extract	1–21 d	3–4	Gadzovska-Simic et al. (2015)
	Pseudohypericin				1-21 d	3–4	
	Total flavonols				21 d	8	
	Total anthocyanins				7 d	5	
	Total phenolics		*Fusarium oxysporum* f. sp. *Lini*		4 d	3.5	
	Total flavonoids		*Fusarium oxysporum* f. sp. *Lini*		7 d	9	

Peganum harmala (Zygophyllaceae)	Harmaline	Cell suspension culture	*Aspergillus flavus*	Fungal mycelium homogenate	15 d	1.69	Ebrahimi and Zarinpanjeh (2015)
	Harmine		*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*		1.68	
Melissa officinalis (Lamiaceae)	Hydroxycinnamic acid derivatives	Cell suspension culture	*Botrytis cinerea*	Fungal hydrolysate	72 h	3	Urdová et al. (2015)
Silybum marianum (Asteraceae)	Silymarin	Hairy root culture	*Trichoderma harzianum* strain KHB	Mycelia extract	120 h	1.7	Hasanloo et al. (2015)
Agastache rugosa (Lamiaceae)	Rosmarinic acid	Cell suspension culture	-	Yeast extract	–	18.5	Park et al. (2016)
Panax quinquefolius (Araliaceae)	Total ginsenosides	Cell suspension culture	*Trichoderma atroviridae*	Culture filtrate	5 d	3.2	Biswas et al. (2016)
Lantana camara (Verbenaceae)	Ursolic acid	Cell suspension culture	*Piriformospora indica*	Filter-sterilised culture filtrate	2 d	3.5	Kumar et al. (2016)
	Oleanolic acid					5.6	
	Betulinic acid					7.8	
Atractylodes lancea (Asteraceae)	Volatile oil	In vitro plantlet	*Gimaniella* sp. (AL12)	Exopolysaccharide mannan	20 d (weekly spray)	1.69	Chen et al. (2016)
Argemone mexicana (Papaveraceae)	Sanguinarine	Cell suspension culture	–	Yeast extract	48 h	>8	Guízar-González et al. (2016)
Gymnema sylvestre (Asclepiadaceae)	Gymnemic acid	Cell suspension culture	*Xylaria* sp. + *Polyancora globosa*	Consortium of dried powder of fungal mycelia	72 h	10.45	Netala et al. (2016)

(continued)

Table 9.1 (continued)

Plants (family)	Secondary metabolites enhanced	In vitro culture systems	Fungal species	Elicitor used for optimum result	Duration of elicitor treatment	Fold or % rise of secondary metabolites with compare to control	References
Ophiorrhiza mungos (Rubiaceae)	Camptothecin	Cell suspension culture	Commercial source	Carbohydrate fraction of yeast extract	10 d	13.3	Deepthi and Satheeshkumar (2016)
Linum album Kotschy ex Boiss (Linaceae)	Podophyllotoxins	Hairy root culture	*Piriformospora indica*	Mycelia extract	24 h	1.8	Tashackori et al. (2016)
	6-Methoxy podophyllotoxins					1.36	
	Lariciresinol					1.6	
Rauvolfia serpentina (Apocynaceae)	Ajmaline	Hairy root culture	Commercial source	Mannan from *Saccharomyces cerevisiae*	1 w	2.9	Srivastava et al. (2016)
Solanum khasianum (Solanaceae)	α-Solanine			Cellulase from *Aspergillus niger*	24 h	1.6	
Salvia castanea Diels f. *tomentosa* Stib (Lamiaceae)	Cryptotanshinone	Hairy root culture	Commercial source	Yeast extract	7 d	8.37	Li et al. (2016a)
	Tanshinone IIA					2.77	
Panax quinquefolius (Araliaceae)	Total ginsenoside	Root culture	*Alternaria panax*	Mycelia extract	8 d	3.2	Yu et al. (2016)
	Ginsenoside (Rg3)					4.9	
	Ginsenoside (Rh2)					5.5	
	Ginsenoside (Re)					5.9	
Calligonum polygonoides (Polygonaceae)	Catechin	Cell suspension culture	–	Crude yeast extract	48 h	4	Owis et al. (2016)
	Isoquercitrin					2.4	
	Astragalin					1.7	

Glycyrrhiza uralensis (Fabaceae)	Glycyrrhetinic acid	Adventitious root culture	*Aspergillus niger*	Mycelia with spores extract	7 d	1.8	Li et al. (2016b)
	Total flavonoids				7 d	3.47	
Catharanthus roseus (Apocynaceae)	Vincristine	Somatic embryogenesis	*Aspergillus flavus*	Mycelia extract	–	15.50%	Tonk et al. (2016)
	Vinblastine					7.88%	
Withania somnifera (Solanaceae)	Withaferin A	Cell suspension culture	*Piriformospora indica*	Cell homogenate	7 d	2.04	Ahlawat et al. (2016)

d day/days, *h* hours, *w* week, *sp* species
[a]Increased

suspension culture of *Taxus chinensis* (Yuan et al. 2002), podophyllotoxin (anticancer) in hairy root culture of *Linum album* (Kumar et al. 2013; Tashackori et al. 2016) and artemisinin (antimalarial) in hairy root culture (Wang et al. 2001, 2006) and shoot culture (Sharma and Agrawal 2013) of *Artemisia annua*. Interestingly, *Panax quinquefolius* cell suspension culture has been shown to produce two new ginsenosides following treatment with *Trichoderma atroviridae* culture filtrate that have so far been not found in the roots of any *Panax* species (Biswas et al. 2016).

The enhanced in vitro secondary metabolite production using fungal elicitors depends upon a number of factors which includes use of fungal species, type and method of fungal elicitor(s) preparation, concentration/dose of elicitor(s), duration of elicitor exposure, age of fungal culture, etc. Besides, the age/growth stage of plant cells in culture and the culture conditions including medium composition, growth regulators, etc. also plays important role on secondary metabolite elicitations.

The selection of fungal species is always a challenging task for successful elicitation of secondary metabolites. Elicitation with pathogenic fungi is a successful strategy for increased in vitro production of secondary metabolites in a number of plant species (Yu et al. 2016) by exploiting the natural ability of plant cells to produce secondary metabolites in response to pathogen. Yu et al. (2016) successfully used a pathogenic fungus, *Alternaria panax* Whetz, for elicitation of ginsenoside in adventitious root culture of *Panax quinquefolius*. However, in some cases, the viability of the cells in culture was decreased due to hypersensitive responses of the fungal species resulting in suppressed growth of cell culture and secondary metabolite production (Baldi et al. 2010). Unlike pathogenic fungi, the fungal endophytes, usually, don't cause strong hypersensitivity responses and, thus, are being increasingly used for production of secondary metabolites (Chen et al. 2016). Recently, elicitation of gymnemic acid in cell suspension culture of *Gymnema sylvestre* using two different endophytic fungi, *Polyancora globosa* and *Xylaria* species, isolated from the leaf of the same plant has been documented (Netala et al. 2016). Gadzovska-Simic et al. (2015) used a non-pathogenic fungus, *Fusarium oxysporum* f. sp. lini, of *Hypericum perforatum* and successfully elicited the total phenolics and flavonoids in its cell suspension culture.

Mostly fungal-derived preparations including mycelial extract, cell wall fragments, polysaccharides fractions of cell wall, culture filtrate, yeast cell extract, carbohydrate fractions of yeast extract, etc. have been employed for successful enhancement of secondary metabolite production in cell, tissue and hairy root cultures (Table 9.1). Reports on use of live fungal cells and other fungal elicitors, e.g. mannan for successful enhancement of secondary metabolite productions, are also available (Table 9.1). A single fungal elicitor can exhibit different responses for production of different secondary metabolites in the same plant species. For example, *Aspergillus flavus* has increased the yield of anthocyanin but failed to enhance the accumulation of hypericin in cell suspension cultures of *Hypericum perforatum* (Gadzovska-Simic et al. 2012). However, Gadzovska-Simic et al. (2015) has reported that the production of hypericin could be enhanced in the cell suspension culture of *Hypericum perforatum* using another fungal species, *Fusarium*

oxysporum, as an elicitor, suggesting that different elicitors are required for the production of different secondary metabolites, at least, in some plant species.

The method of elicitor preparation is also critical for the success of elicitation. Elicitors prepared from same fungus differently, e.g. autoclaved and filter-sterilised filtrates show various responses as observed in *Lantana camara* (Kumar et al. 2016), where enhancement of secondary metabolite production was more in cell suspension cultures treated with filter-sterilised elicitor compared with the autoclaved one. 'The elicitor moieties/binding sites in the culture filtrate are heat-sensitive and may have lost their elicitation capability on autoclaving' (Kumar et al. 2016).

Of the different factors influencing the production of enhanced secondary metabolites, the concentration/dose and duration of treatment of a fungal elicitor to the culture are important. A smaller dose of elicitor than optimum may not bind sufficient number of receptors in cells required for activation of secondary metabolite biosynthesis (Ahlawat et al. 2016), whereas higher dose may induce hypersensitive response leading to growth inhibition and cell death (Namdeo et al. 2002; Ahlawat et al. 2016), and eventually both affect the secondary metabolite production. Similarly, exposure to fungal elicitor for longer duration may result in degradation of induced secondary metabolites or its conversion to different compounds by activation of other biosynthetic pathways (Baldi et al. 2010). The concentration and duration of exposure of fungal elicitors is plant species specific. Thus, the selection of optimum concentration and exposure time of elicitors is empirical.

As several plant cultures are responsive to different elicitors, in a number of instances, dual elicitation strategy have also been evaluated. For example, augmentation of abiotic elicitors, either chemical or physical, such as salicylic acid (Yu et al. 2001); ferrous ion (Zhao et al. 2001a); copper sulphate (Baldi et al. 2008b); copper ions (Rhee et al. 2010); tetramethylammonium bromide (Zhao et al. 2001b); methyl jasmonate (Ahlawat et al. 2014); even sorbitol, an osmotic stress inducer (Shi et al. 2007); etc., to fungal elicitor has a synergistic effect on the production of in vitro secondary metabolites (Table 9.2). A combined approach of fungal elicitation and precursor feeding has also been reported to be useful for increased production of sanguinarine in cell suspension culture of *Papaver somniferum* (Verma et al. 2014a). The culture filtrate of *Trichoderma harzianum* has enhanced the production of sanguinarine. However, the highest production was observed when *Trichoderma harzianum* filtrate was used along with a precursor, shikimate. The improvement was maybe due to the ability of the shikimate in diverting the metabolic flux towards sanguinarine production (Verma et al. 2014a; Table 9.2).

Age/growth phase of cell suspension culture during elicitor treatment is vital for the production of secondary metabolites (Namdeo 2007). The optimum age of plant culture for elicitation varies among plant species and cultures (Ahmed and Baig 2014). Usually, the synthesis of secondary metabolites is optimum at a stage when cultures moves from exponential phase to stationary phase (Taticek et al. 1991). Mostly early stationary phase or late growth/exponential phase was found to be best for application of elicitor for optimum production of secondary metabolites (Namdeo 2007) as reported in the case of the production of psoralen in *Psoralea corylifolia* (Ahmed and Baig 2014) cell suspension cultures. It is assumed that in

Table 9.2 Secondary metabolites production in different in vitro culture systems using dual elicitation strategies

Plants (family)	Secondary metabolites enhanced	In vitro culture systems	Fungal species	Elicitor used for optimum result	Duration of elicitor treatment	Rise of secondary metabolites in fold compared to control	References
Taxus chinensis (Taxaceae)	Taxol	Cell suspension culture	Fungus isolated from inner bark of *Taxus chinensis*	Mycelia extract + Salicylic acid	50 h	7.5	Yu et al. (2001)
Cupressus lusitanica (Cupressaceae)	β-Thujaplicin	Cell suspension culture	–	Yeast extract + Ferrous ion	7 d	3–4	Zhao et al. (2001a)
Catharanthus roseus (Apocynaceae)	Ajmalicine	Cell suspension culture	*Aspergillus niger*	Mycelia homogenate + Tetramethylammonium bromide	–	4	Zhao et al. (2001b)
Salvia miltiorrhiza (Lamiaceae)	Total tanshinone	Hairy root culture	Commercial source	Polysaccharide fraction of yeast extract + sorbitol	9 d	10	Shi et al. (2007)
Withania somnifera (Solanaceae)	Withaferin A	Cell suspension culture	*Verticillium dahliae*	Cell extract + copper sulphate	4 d	13.8	Baldi et al. (2008b)
Angelica gigas Nakai (Apiaceae)	Decursinol angelate	Root culture	Commercial source	Yeast extract + copper ion	48 h	3.22	Rhee et al. (2010)
Papaver somniferum (Papaveraceae)	Sanguinarine	Cell suspension culture	*Trichoderma harzianum*	Culture filtrate + shikimate	5 d	4	Verma et al. (2014a)
Artemisia annua (Asteraceae)	Artemisinin	Hairy root culture	*Piriformospora indica*	Cell homogenate + methyl jasmonate	5 d	2.44	Ahlawat et al. (2014)

d days, *h* hours

this stage the cellular machinery is in its maximum operation status; thus, the elicitors work most efficiently to enhance secondary metabolite biosynthesis (Verma et al. 2014a). However, at the same time, examples of addition of elicitors at the beginning of exponential/log phase yielding maximum production of camptothecin in cell suspension culture of *Ophiorrhiza mungos* (Deepthi and Satheeshkumar 2016), thiophene in hairy root culture of *Tagetes patula* (Buitelaar et al. 1992) and hypericin in cell suspension culture of *Hypericum perforatum* (Gadzovska-Simic et al. 2015) are also available.

9.6 Future Prospects

In vitro culture systems have great potential for large-scale production of secondary metabolites. However, achievement of substantial yield to make the system economically viable is a challenging task. Fungal elicitation technique has shown great promises to overcome the yield problems. It is evident from the reports that several-fold increase in valuable secondary metabolites has already been achieved using fungal elicitors in different culture systems of various medicinal plants (Table 9.1). Scaling-up of these products is a major problem which needs to be addressed for the commercial success of these techniques. Attempts have been made for up-scaling of the secondary metabolite production using different types of bioreactors such as stirred tank (5-L) or balloon-type bubble (5-L) bioreactors (Verma et al. 2014a; Li et al. 2016b). However, reports on successful use of bioreactors for scaling-up of cell, tissue, organ and hairy root cultures for commercial scale production of secondary metabolites are limited (Weathers et al. 2010), considering the vast array of secondary metabolites in nature. Phyton Biotech, USA (www.phytonbiotech.com), is producing anticancer agents, paclitaxel and docetaxel, in industrial scale from cell suspension cultures of *Taxus chinensis* using Plant Cell Fermentation (PCF®) platform technology. The production cost of paclitaxel using PCF® is 20% cheaper compared to that extracted from the natural source (Mountford 2010; Wilson and Roberts 2012). Although the production cost of semisynthetic paclitaxel is relatively cheaper than PCF® (due to low yield), the former is considered environmentally detrimental (Muchiri and Walker 2012), whereas the latter is safe and sustainable (Wilson and Roberts 2012). The CBN Biotech in Korea (cbnbiotech.com/en/) is producing callus-mediated adventitious root cultures of *Panax ginseng* (mountain/wild ginseng) in a commercial scale using bioreactors (Murthy et al. 2008). In fact, different types of bioreactors are required for scaling-up secondary metabolite production in different culture types [cell suspension, plantlets, hairy root, etc.; Weathers et al. 2010]. This is due to the difference in the nature and properties of cultures involved in these systems. Thus, emphasis should be given for the development of suitable bioreactors with optimised parameters so that large-scale production of secondary metabolites can be achieved at commercial scale using different culture systems.

The successful use of fungal elicitors for increased production of secondary metabolites requires in-depth knowledge on plant-pathogen interaction, different secondary metabolite biosynthetic pathways and the gene regulatory network involved in their regulation. Our knowledge on metabolic pathway regulation is limited and complete genome sequence of most of the medicinal plants is not available (Goossens et al. 2003). However, recently, transcriptomic resources have been generated for a number of non-model medicinal plants (Xiao et al. 2013) including *Centella asiatica* (Sangwan et al. 2013), *Catharanthus roseus* (Van Moerkercke et al. 2013; Verma et al. 2014b), *Andrographis paniculata* (Garg et al. 2015) and *Oplopanax elatus* (Eom et al. 2017) using next-generation sequencing. These transcriptomic resources of different medicinal plant species will help us better understand the transcriptional and post-transcriptional regulations of different metabolic pathways in these medicinal plants (Devi et al. 2016; Wang et al. 2017). Genome-wide transcriptome analysis may enable us to identify transcription factors involved in the regulation of the pathway and enhance the production of bioactive compounds on a commercial scale by metabolic engineering.

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Chapter 10
Metal and Metal Oxide Mycogenic Nanoparticles and Their Application As Antimicrobial and Antibiofilm Agents

Siddhardha Busi and Parasuraman Paramanantham

Abstract In the recent decades, nanotechnology showed extensive expansion and contributing different application in various interdisciplinary fields. Nanoparticles are the leading edge of the rapidly growing field of nanotechnology. Nanoparticles are defined as solid particles at the size range of 10–100 nm with at least one dimension. These nanoparticles gain greater attention due to their surface area to volume ratio which makes nanoparticles more reactive. Among the nanomaterials, metal and metal oxide nanoparticles are considered as magic bullets that deal with a wide range of applications. Exploitation of different physiochemical methods in metal and metal oxide nanoparticle synthesis ends up numerous drawbacks such as energy intensive, costly, rely upon toxic chemicals, time-consuming, and produce hazardous waste. Hence, biological synthesis of metal and metal oxide nanoparticles is majorly accepted as alternative technology to overcome limits of physical and chemical methods. Among the biological systems, the synthesis of metal and metal oxide nanoparticles using diverse range fungi has gained significant importance due to their unique properties that facilitate fermentation and downstream process. Usually fungi obey certain mechanism to synthesis the metal and metal oxide nanoparticles. Herein, metal ions are trapped on the surface or inside of the fungi. The trapped metal ions undergo reduction to form nanoparticles in the presence of enzymes and/or organic polymers. Fungi undergo two different modes of nanoparticle synthesis like extracellular and intracellular. Under large-scale production, extracellular process has obvious advantage over an intracellular process to handle the expenses in downstream process. Keeping the inherent advantages of fungal-mediated metal and metal oxide nanoparticle synthesis, fungi are now being gradually employed as myconanofactories for the synthesis of myconanoparticles. Presently, myconanoparticles are gaining attention to employ as antimicrobial and antibiofilm agents. Emergence of antibiotic-resistant microorganism created challenging situation to invent novel therapeutics to eradicate them. Myconanoparticles are an alternative option to eradicate the antibiotic-resistant and biofilm-forming

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microorganisms. Fungi-mediated synthesis of metal and metal oxide nanoparticles is gaining importance as they are eco-friendly, and fabricated material derived from the fungi enhances the antimicrobial and antibiofilm efficacy.

Keywords Antimicrobial · Antibiofim · Myconanoparticles · Myconanotechnology

10.1 Introduction

As the nano-revolution developed to a promising stage, there is an extensive expectation to develop nanorange size material, which is safe, reliable, and eco-friendly. This extensive need triggers the field of nanotechnology to proliferate symbiotic relationship between other disciplines, such as material chemistry and biotechnology, to furnish the novel technology in the development of nanoparticles with precise shape and size (Verma et al. 2010). The word "nano" originated from the Greek work "nanos" which means dwarf and denotes a measurement on the scale of 1 billion (10^9) of a meter in size. Nanoparticles always exhibit in different properties than bulk material due the very small size and high surface area. Generally, nanoparticles are defined as microscopic solid particles with at least one dimension at a range of 10–1000 nm. The property such as surface to volume ratio is the most important feature of nanoparticles which allowed the nanoparticles to interact with other particles in feasible manner (Pantidos and Horsfall 2014; Zinjarde 2012). By accounting these intrinsic properties of the nanoparticles, great scientific interests emerged in the recent years.

According to the current market status, approximately 1600 nano-based products are available in the market in various commodities like antimicrobial agents, processing additives, pH control, carrier for flavors and aromas, color additives, ultraviolet (UV) filter, and clarification agent. Gold and cerium nanoparticles are commonly employed for medicinal applications; other nanoparticles such as silver, titanium, silica, and iron are used in food processing and packing industries. Moreover, industeries like cosmetics, sports clothes, electronics, energy, and fuel are exploiting the nanoparticles like titanium, zinc, and cerium oxide for their use. Nevertheless silicate and aluminosilicate, iron, and titanium oxide nanoparticles are popular in the industries like nutraceuticals and pharmaceuticals, and these nanoparticles also play a major role in efficient wastewater treatment process (De la Calle et al. 2016). Among different kinds of nanoparticles, metal and metal oxide nanoparticles are gaining outstanding attention due to their crucial physicochemical properties. Metallic nanoparticles alone have various applications in diverse industries such as cosmetics, electronic, biomedical, food, building material, and painting and as decontaminants (Song et al. 2016). For example, in the case of nanoparticles, relatively low temperature is required for the merging of nanoparticles into the solid matter without melting, which eases the coating process for electronic applications like capacitors. Nevertheless, metallic nanoparticles also serve several applications in the biomedical industries (Thakkar et al. 2010). On the other hand, metal oxide

nanoparticles are considered as one of the important classes of nanomaterials owing to their unique physical and chemical characteristics associated to their nanorange size, which offers versatility. As like metallic nanoparticles, metal oxide nanoparticles are also metallic nanoparticle metal oxide, nanoparticles also play versatile roles such as constituent in catalysis, diagnosis, drug delivery, semiconductors, sensing, and solid oxide fuel cells. Additionally in recent years, much attention is given to the metal oxide nanoparticles to develop antimicrobial agents (Raghunath and Perumal 2017).

At present, several synthesis methods are available to prepare the metal and metal oxide nanoparticles such as physical, chemical, enzymatic, and biological. In the physical methods, different techniques are employed that include plasma arcing, ball millings, thermal evaporate, spray pyrolysis, ultrathin films, pulsed laser desorption, layer-by-layer growth, and diffusion flame, whereas the chemical methods of nanoparticle synthesis are techniques like electrodeposition, sol-gel process, chemical solution deposition, chemical vapor deposition, Langmuir-Blodgett method, coprecipitation method, and wet chemical method (Kuppusamy et al. 2016). High radiation, high concentration reductant, and stability agent used in the physical and chemical methods of synthesis of nanoparticles create adverse effect to the environment and human health (Patil and Kim 2016). To overcome this assimilation of physical and chemical method, the other two methods such as enzymatic and biological have been proposed. Enzymatic method of nanoparticle synthesis has been considered as eco-friendly method but, due the certain hindrance such as stability and expensive, creates situation to use other methods. In recent years, biological synthesis of metal and metal oxide nanoparticles gains significant importance due to the availability of more biological entities and eco-friendly procedures.

The major goal of the biological synthesis of nanoparticles is to reduce the use of toxic chemicals to maintain the equilibrium of the environment (Siddiqi and Husen 2016b). In the biological synthesis of nanoparticles, both eco-friendly and green chemistry-based approaches are used by employing bacteria, fungi, actinomycetes, yeast, and algae (Patil and Kim 2016; Prasad et al. 2016). The nanoparticles that are synthesized by biological approaches are fabricated with biogenic surfactant or capping agent that facilitates the stability and biocompatibility and is nontoxic in nature (Tanzil et al. 2016; Prasad 2014). Among all the mentioned microorganisms, fungi are given greater attention due to the fungal mycelial mesh that can withstand flow pressure, agitation, and other conditions in the bioreactors as compared to other microorganisms. Moreover, the fungal-mediated nanoparticle synthesis owing other advantages like fungi is fastidious to grow, easy to handle, and simplified methods for nanoparticle synthesis, has no necessity of further downstream processing, and is comparatively quick as that of intracellular synthesis (Balakumaran et al. 2015; Prasad et al. 2016; Prasad 2016, 2017). Currently, fungal-medicated metal and metal oxide nanoparticles are considered as an effective antimicrobial agent (Aziz et al. 2016).

The enormous increases in incidence of multidrug-resistant microorganisms and continuing emphasis on healthcare expenses create strong necessity to formulate novel antimicrobial agent (Fatima et al. 2016). On the other hand, most of the microorganisms are capable of forming biofilm, which make difficult situation to

treat the microbial infections. In general, biofilm can be defined as any group of microorganisms that adhere to the surface and gather the other cells to the surface by self-producing matrix composed of extracellular polymeric substance. The pathogens including *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Serratia marcescens* are the organisms that often form biofilm. It has been reported that all the abovementioned microorganisms possess a quorum-sensing controlled ability to produce biofilm. The microbial cells that grow inside the biofilm matrix are resistant to series of encounters such as phagocytes, antibodies, and antibiotics that fail the host defense mechanisms (Packiavathy et al. 2014). In the recent year, oral biofilm is noted as the most prevalent polymicrobial infection that is associated with an array of microbial community such as oral bacteria, archaea, viruses, fungi, and protozoa. This condition leads to the formation of dental caries and periodontal and peri-implant diseases (Szafrański et al. 2017). Herein fungal-mediated metal and metal oxide nanoparticles have the scope for solving the problems arising with biofilm formers and multidrug-resistant microorganisms. Presently, metal and metal oxide nanoparticles synthesized from fungus are focused toward their application in antibiofilm activity due to their unique antimicrobial activities. This chapter deals with the brief knowledge about mycometal and metal oxide nanoparticle, mechanisms that are involve in synthesis of myconanoparticles, different metal and metallic myconanoparticles, and their antimicrobial and antibiofilm activities.

10.2 Scope of Myconanotechnology

Myconanotechnology is a combinational technology of two different disciplines of science such as mycology and nanotechnology. Myconanotechnology can be defined as fabrication of nanoparticles by fungi and its metabolite and their vast area of application especially in the field of medicine. The combination of these two disciplines of science consider as an effective due to the wide range of diversity in fungi (Rai et al. 2009). Recently researches of nanotechnology are focusing toward metal and metal oxide nanoparticles because of their different chemical, physical, and optical properties. The term nano becomes a fascinating word that hopes for the future development of many scientific applications. Currently, myconanotechnology is a rapid growing field of research; numerous methods for the synthesis of metal and metal oxide nanoparticles have been formulated in an eco-friendly manner. These biological methods induced increased interest in using fungi as tool for metal and metal oxide nanoparticle synthesis. Biosynthesis of silver, gold, platinum, cobalt, titanium, cadmium sulfide, zirconium, and cadmium telluride nanoparticles by fungi has been reported (Alghuthaymi et al. 2015). Among nanoparticles, cadmium sulfide nanoparticle is considered one of the earliest metallic nanoparticles synthesized by yeast (Dameron et al. 1989). When compared with all microorganisms, fungi are most preferred for industrial applications due to their outstanding advantages like withstanding capacity in the flow of pressure, agitation, and other

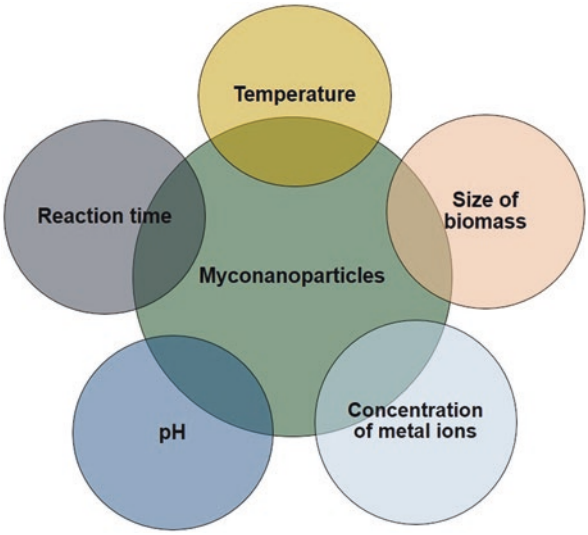

Fig. 10.1 Advantages and limitations of myconanoparticles

bioreactor conditions with less limitations as conveyed in Fig. 10.1 (Balakumaran et al. 2015). Current state-of-the-art, fungi-mediated metal and metal oxide nanoparticles like silver, gold, and platinum have great interest due to their numerous advantageous properties in different fields of application particularly as antimicrobial agent (Prasad 2016, 2017). As discussed earlier development of resistance of microorganisms against antibiotics becomes major concern in global medical sector. The recent scientific reports are emphasizing that the development of resistance by the microorganisms against nanoparticles was not evident (Saglam et al. 2016). Therefore, myconanotechnology is considered as a novel approach for the improvement of green biosynthesis of nanoparticles for medical applications.

10.3 Mechanisms Involving Metal Myconanoparticle Synthesis

The actual mechanism of formation of metal and metal oxide nanoparticles by all of these microorganisms particularly fungi is still an open question, even though several possible mechanisms have been proposed, but no particular convincing mechanism has been identified (Rai et al. 2009; Alghuthaymi et al. 2015). Most accepted mechanisms were bioreduction of metal salts to elemental metal that might be stabilized by organic molecules secreted by fungi. Another reliable proposed mechanism is biosorption where metal ions of the aqueous medium are attached to the surface of the cell wall of the organisms mostly by electrostatic interaction. This mechanism was explained based on the nature of certain microorganisms that when they are exposed to the foreign material, they produce enzymes or the metabolite

that reduces the metal ion which leads to the formation of nanoparticles. For example, napthoquinones and anthraquinones are the products of fungi, which act as reducing agent (Siddiqi and Husen 2016a). For intensity, cell walls and cell sugar of fungi play a major role in the reduction of metal ions where it begins with trapping of metal ions to positively charged groups in enzymes present in the cell wall by electrostatic interaction. Enzymatic reduction of metal ions inside cells leads to the formation of metal and metal oxide nanoparticles (Rai et al. 2009). Nitrate reductase action of fungi also argued as mechanisms of fungus in metal and metal oxide nanoparticle synthesis. In the ferric ion reduction to iron nanoparticles, it has been emphasized that for the metal ion reduction, not only the enzymes were necessary but also an electron shuttle (Siddiqi and Husen 2016a; Alghuthaymi et al. 2015).

10.4 Types of Myconanoparticle Synthesis

In the synthesis of metal and metal oxide myconanoparticles, two different distinct methods were proposed which directly depends on the metabolic nature of the fungi on metal ion reduction such as extracellular and intracellular. In the extracellular synthesis of metal and metal oxide myconanoparticles, fungi secrete the metabolites and/or enzymes to the outside of the cell wall that act as a reducing agent in metal ion reduction and develop the myconanoparticles (Fatima et al. 2016). Extracellular synthesized metallic nanoparticles of different size from diverse fungal species are listed in Table 10.1. The steps involved in the extracellular synthesis metallic myconanoparticles were briefed in the Fig. 10.2, whereas in intracellular synthesis of metal and metal oxide myconanoparticles, the metal ions transport into the fungal cell to form nanoparticles in the presence of enzymes (Li et al. 2011). Based on the above aspect, different metal and metal oxide myconanoparticles have been reported.

10.5 Extracellular Synthesis of Myconanoparticles

10.5.1 Silver

Silver myconanoparticles show effective antimicrobial activity more than their bulk counterpart against gram-positive and gram-negative bacteria. Nevertheless, they are even much effective against multiresistant strains such as methicillin-resistant *Staphylococcus aureus* (Li et al. 2011). This intrinsic nature of silver nanoparticle gains much interest in the nanobiotechnology and leads to the discovery of several methods for the biological synthesis silver nanoparticles. In the recent year, Sarsar et al. (2015) reported myconanoparticles of silver from *Penicillium atramentosum* KM. The obtained silver myconanoparticle from this study was 5–25 nm range in size with uniform shape. The antimicrobial activity of synthesized

Table 10.1 Extracellular synthesized metallic nanoparticles of different size from diverse fungal species

S. no.	Name of the metal	Name of the organisms	Size of the nanoparticles (nm)	Reference
1	Silver	*Penicillium atramentosum* KM	5–25	Sarsar et al. (2015)
2	Silver	*Fusarium oxysporum*	5–13	Husseiny et al. (2015)
3	Silver	*Cladosporium cladosporioides*	–	Balaji et al. (2009)
4	Silver	*Rhizopus aqueous*	10	AbdelRahim et al. (2015)
5	Silver	*Aspergillus fumigatus*	5–25	Bhainsa and D'Souza (2006)
6	Silver	*Fusarium semitectum*	10–60	Basavaraja et al. (2008)
7	Silver	*Fusarium oxysporum*	5–15	Ahmad et al. (2003)
8	Silver	*Colletotrichum* sp. ALF2-6	–	Azmath et al. (2016)
9	Silver	*Penicillium fellutanum*	25	Kathiresan et al. (2009)
10	Gold	*Nigrospora oryzae*	–	Kar et al. (2014)
11	Gold	*Epicoccum nigrum*	5–50	Sheikhloo and Salouti (2011)
12	Gold	*F. oxysporum* f. sp. *cubense* JT1	22	Thakker et al. (2013)
13	Gold	*Fusarium solani*	20–50	Gopinath and Arumugam (2013)
14	Gold	*Penicillium rugulosum*	–	Mishra et al. (2012)
15	Gold	*Fusarium acuminatum*	8–28	Tidke et al. (2014)
16	Gold	*Cladosporium oxysporum* AJP03	75	Bhargava et al. (2016)
17	Gold	*Aspergillus niger*	10–30	Soni and Prakash (2012)
18	Cadmium	*Fusarium oxysporum*	–	Ahmad et al. (2002)
19	Cadmium	*Phanerochaete chrysosporium*	–	Chen et al. (2014)
20	Cadmium	*Helminthosporium solani*	5.5	Suresh (2014)
21	Cadmium	*Saccharomyces cerevisiae*	2–3	Bao et al. (2010)
22	Cadmium	*Fusarium oxysporum*	15–20	Syed and Ahmad (2013)
23	Zinc	*Aspergillus fumigatus* TFR-8	1.2–6.8	Raliya and Tarafdar (2013)
24	Zinc	*Saccharomyces cerevisiae*	–	Sandana Mala and Rose (2013)
25	Iron	*Aspergillus oryzae* TFR9	–	Tarafdar and Raliya (2013)
26	Iron	*Alternaria alternata*	–	Mohamed et al. (2015)

(continued)

Table 10.1 (continued)

S. no.	Name of the metal	Name of the organisms	Size of the nanoparticles (nm)	Reference
27	Platinum	*Fusarium oxysporum* sp. lycopersici	10–100	Riddin et al. (2006)
28	Platinum	*Fusarium oxysporum*	–	Govender et al. (2009)
29	Lead	*Aspergillus*	5–20	Pavani et al. (2012)
30	Silica	*Fusarium oxysporum*	–	Bansal et al. (2005)
31	Zirconia	*Fusarium oxysporum*	–	Bansal et al. (2004)

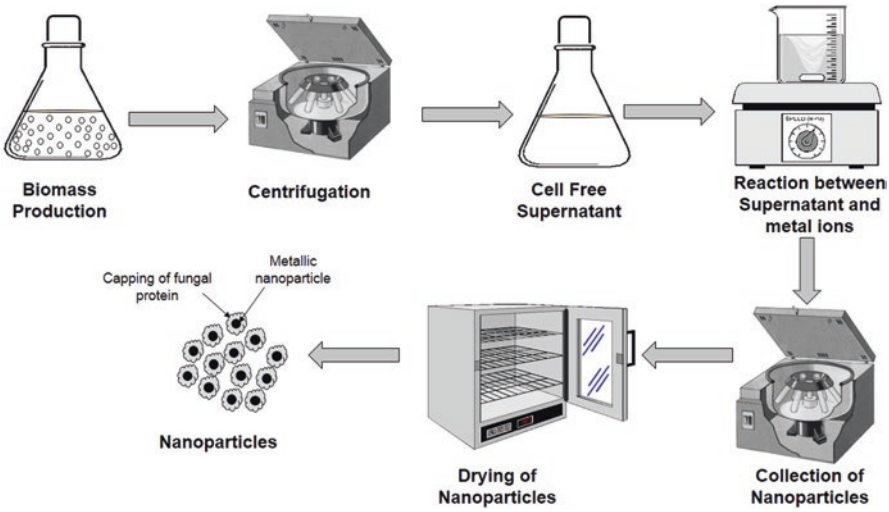

Fig. 10.2 Schematic representation of steps involved in the extracellular synthesis metallic nanoparticles

myconanoparticle was investigated against bacterial cultures such as *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Aeromonas hydrophila*, and *Enterobacter aerogenes*. The result showed that gram-positive *B. cereus and S. aureus* along with gram-negative *A. hydrophila* are highly susceptible to synthesized myconanoparticle. Similarly, the fungal strain *Fusarium oxysporum* was investigated for the synthesis of silver myconanoparticles. The authors reported the fungal strain of current study having capacity to synthesis the 5–13 nm range of silver myconanoparticles. Moreover, the myconanoparticles of present work were tested for the antimicrobial efficacy which reveals that *Escherichia coli* and *S. aureus* are more susceptible to silver myconanoparticles (Husseiny et al. 2015). Balaji et al. (2009) have demonstrated the formation of silver nanoparticles in the presence of *Cladosporium cladosporioides*. They found that the fungal extract is able to accumulate crystalline silver myconanoparticles in 24 h of incubation with metal ions. The formation of nanoparticles was confirmed by the appearance of a characteristic peak at 415 nm in the UV-visible spectrum (Balaji et al. 2009).

AbdelRahim et al. (2015) employed an isolate of *Rhizopus aqueous* mycelial extract to produce silver myconanoparticles by reacting with silver ions at 40 °C on an orbital shaker (180 rpm) for 3 days. The mono-distributed spherical-shaped particles of 10 nm size obtained and FTIR analysis showed that they also contained protein. Similarly, *Aspergillus fumigatus* was used for the fabrication of silver myconanoparticles (Bhainsa and D'Souza 2006). Herein 72-h cell filtrate mixed with the 1 mM of silver ions formed the silver myconanoparticles at 24 h of incubation time with the size range of 5–25 nm, and the XDR spectrum confirmed the crystalline nature of the nanoparticle. Biosynthesis of silver nanoparticles using *Fusarium semitectum* was reported by Basavaraja et al. (2008). Cell filtrate of *F. semitectum* was treated with 1 mM of silver ions and placed on a rotary shaker at 27 °C. The cell filtrate treated with silver ions accumulated silver myconanoparticles with average size range of 10–60 nm in most spherical shape. Extracellular biosynthesis and characterization of silver nanoparticles using *Fusarium oxysporum* have been reported by Ahmad et al. (2003). The authors demonstrated that silver myconanoparticle synthesis by the *F. oxysporum* cell-free filtrate undergone reduction of metal ions by enzymatic process. The size of the nanoparticles was in the rage of 5–15 nm in dimensions and stabilized in solution by proteins secreted by the fungus. Silver myconanoparticles have been synthesized from 1 mM silver nitrate and the culture filtrate of *Colletotrichum* sp. ALF2-6 inhabitant of *Andrographis paniculata* (Azmath et al. 2016). In this study, the formation of silver myconanoparticles was examined periodically using UV-visible spectrophotometer. The maximum absorption at 420 nm confirmed the formation of nanoparticles, and FTIR analysis showed possible biomolecules involved in reducing the metal salt and stabilization of nanoparticles. Investigation of antibacterial efficacy of the synthesized myconanoparticles from this study showed bactericidal activity against selected human pathogens. Silver myconanoparticles have been reported from *Penicillium fellutanum* isolated from coastal mangrove sediment that obtained myconanoparticles in the average size of 25 nm (Kathiresan et al. 2009).

10.5.2 Gold

Gold nanoparticles are having rich historical background which showed the usage of gold nanoparticles is raised in the time of ancient Roman where they used gold nanoparticles for the decoration of stain glasses (Li et al. 2011). In recent year biological synthesis of gold nanoparticles received greater attentions due to an essential need for the development of eco-friendly technologies in material science. Kar and coworker have demonstrated biological synthesis of gold nanoparticles by employing *Nigrospora oryzae*. In this study, the fungal culture filtrate was treated with 1 mM metal salt solution and observed for the synthesis of myconanoparticles. Here authors conform the formation of gold myconanoparticles using series of methods such as visible color change, UV-visible spectroscopy, dynamic light scattering (DLS), atomic force microscopy (AFM), and transmission electron microscopy

(TEM). The FTIR result revealed that the presence of possible functional groups is responsible for the bioreduction and capping of the synthesized gold myconanoparticles. Furthermore, the investigation on efficacy of the synthesized myconanoparticles as vermifugal agent against a model cestode *Raillietina* sp. was conducted. (Kar et al. 2014). Sheikhloo et al. (2011) used the fungus *Epicoccum nigrum* isolated from Andalian gold mine in northwest of Iran and reported first time for the synthesis of gold nanoparticles. The myconanoparticles synthesized using *Epicoccum nigrum* had smallest particle size range from 5 to 50 nm.

Thakker and coworkers reported the synthesis of gold myconanoparticles by *F. oxysporum* f. sp. *cubense* JT1 extracellularly. It can be achieved when *F. oxysporum* f. sp. *cubense* JT1 mycelium was reacted with auric chloride solution; the myconanoparticles were produced within 60 min. The average size of the synthesized nanoparticles was 22 nm, and FTIR analysis showed that the nanoparticles were capped with fungal proteins. Authors also emphasized that the gold myconanoparticles possess significant antimicrobial efficacy against *Pseudomonas* sp. (Thakker et al. 2013). The recent research documented that *Fusarium solani* culture filtrate is able to reduce the gold metal ions and leads to the formation of gold myconanoparticles spherical in shape with size range of 20–50 nm. Authors account that use of *F. solani* has several advantages over industrial application due its fast growth rate, rapid capacity of metallic ions reduction, and capping of nanoparticles with protein as stabilizer. Moreover, extracellular biosynthesis of gold myconanoparticles has great application from the view of large-scale synthesis, time consumption, eco-friendly, nontoxic, and feasible downstream processing (Gopinath and Arumugam 2013). Investigation on gold myconanoparticle biosynthesis by the cell filtrate extracted from *Penicillium rugulosum* showed extracellular synthesis of gold myconanoparticles by bioreduction process (Mishra et al. 2012). Similarly, gold myconanoparticles were synthesized by extracellular biosynthesis process using the fungus *Fusarium acuminatum*. The result has been achieved by treating metal salt solution with fungal cell filtrate under room temperature for 24 h. The formation of gold myconanoparticles was confirmed by visual observation, UV-visible spectrophotometry, zeta potential, and TEM analysis. The nanoparticle synthesized in this work was found to be capped with protein and spherical in shape, polydispersed in the range of 8–28 nm size. Authors claimed the formulated technology of the present study was easy, better, and cheaper technology for the biosynthesis of gold myconanoparticles (Tidke et al. 2014).

A systematic study on the gold nanoparticles biosynthesis has been reported with the fungus *Cladosporium oxysporum*. The employed fungus show promises for the industrial application due to its easy handing, large protein secretion, high biomass yield, and accessibility for a scale-up. The work also emphasized that the soil fungus *Cladosporium oxysporum* AJP03 has high metal tolerance capacity and potential extracellular synthesis of gold nanoparticles with average size of 75 nm (Bhargava et al. 2016). The filamentous fungus *Aspergillus niger* was found to be a potential biological agent for the synthesis of gold myconanoparticles. Gold myconanoparticles of 10–30 nm diameter were synthesized by treating the mycelia-free culture filtrate of the *Aspergillus niger* with gold metal salt solution. Authors

reported the larvicidal efficacy of these gold myconanoparticles against three mosquito species such as *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. The test revealed that the gold myconanoparticles synthesized by *A. niger* were more effective against the *C. quinquefasciatus* larvae than the *A. stephensi* and *A. aegypti.* Authors concluded the gold myconanoparticle synthesis by fungus can be more rapid and eco-friendly approach for mosquito control using the current approaches (Soni and Prakash 2012).

10.5.3 Cadmium

Development of nanoscale semiconductor particles is a problem of great importance that makes researcher seeking inspiration from biological synthesis. Fungal-mediated cadmium sulfide (CdS) nanoparticles have been synthesized extracellularly by a pure enzyme. Utilization of certain enzymes like reductase secreted by fungi created exciting possibility to design précised nanoparticles. Ahmad and coworker reported mycological synthesis of CdS by fungus *Fusarium oxysporum*. The colloidal solution of cadmium sulfide nanoparticles was highly stable in time without showing any aggregation even after 1 month of storage (Ahmad et al. 2002). A recent study revealed that cadmium sulfide myconanoparticles were synthesized extracellularly by using white rot fungus *Phanerochaete chrysosporium*. The biological synthesis of myconanoparticles was achieved by incubating *P. chrysosporium* in a solution containing cadmium nitrate tetrahydrate; the formation of myconanoparticles was confirmed by visible color change in the mixture. The FTIR result revealed that the secretion of cysteine and proteins plays an important role in the formation and stabilization of cadmium sulfide myconanoparticles (Chen et al. 2014). Recently, engineered nanoparticles gained significant attention due to their profound applications. It creates the expectations on biological synthesis technique to avoid the adverse effects from the conventional chemical method. An investigation has been made to synthesize small and monodispersed cadmium selenide nanoparticles by employing plant pathogenic fungus, *Helminthosporium solani*, upon incubating with solution of cadmium chloride and selenium tetrachloride under ambient condition. The myconanoparticles from this work showed the particles are monodispersed sphere with an average diameter of 5.5 nm and are hydrophilic and highly stable (Suresh 2014). Bao and coworkers demonstrated a simple and efficient biological synthesis method to develop easily harvest technology for biocompatible cadmium telluride nanoparticles using yeast cells. The study yielded with cadmium telluride nanoparticles with uniform size of 2–3 nm and protein capped facilitates them to highly soluble in water and increase biocompatibility (Bao et al. 2010). Due to the increasing demand on semiconductor nanoparticles with characteristic features such as biocompatibility, simple synthetic methods and eco-friendly approaches gained significant attention using biological sources like fungi. Recently, *Fusarium oxysporum* is reported as potential fungus for the

biological synthesis of biocompatible cadmium telluride nanoparticles with average size ranging from 15 to 20 nm (Syed and Ahmad 2013).

10.5.4 Zinc

Potential application of zinc nanoparticles is electronic, antibacterial, personal care products, coating, paints, and healthcare that enhanced demand in the development of comfortable technique to synthesize biocompatible nanomaterials (Bhuyan et al. 2015). Nevertheless, zinc is an important element in the biological system for a variety of metabolic processes such as carbohydrate, lipid, nucleic acid, and protein synthesis and acts as an integral component of many enzyme structures. Study has been conducted to investigate zinc oxide nanoparticle synthesis from zinc nitrate using extracellular secretion of *Aspergillus fumigatus* TFR-8. The synthesized nanoparticles from this study showed size range of 1.2–6.8 nm at least one dimension with spherical and hexagonal in structure (Raliya and Tarafdar 2013). Additionally, nanoparticles in the quantum regime have significant attention due the unique characteristics with excellent applicability in bioimaging. Attempt has been made with commercially available yeast such as *Saccharomyces cerevisiae* for the biological synthesis of zinc sulfide nanoparticles in quantum regime. The test result emphasizes that the yeast has inherent sulfate metabolizing system and efficacy process to assimilate sulfate (Sandana Mala and Rose 2013).

10.5.5 Iron

Metallic nanoparticles with antimicrobial properties represent a promising alternative for the convectional antibiotics. Development of comfortable and environmental friendly approaches for the synthesis of metallic nanoparticles biologically becomes a significant approach in the field of nanoscience and nanotechnology. Recent report showed biological synthesis of iron nanoparticles by employing *Aspergillus oryzae* TFR9 extract treated with solution of iron chloride. The authors are claiming that fungal-mediated iron nanoparticle production will be more feasible than that of chemical methods due to eco-friendliness, easy availability, and low-cost biomass production (Tarafdar and Raliya 2013). Similarly, Mohamed and coworker demonstrated that *Alternaria alternata* is capable of reducing iron metal ion and synthesized iron myconanoparticles. Additionally, iron myconanoparticles showed antibacterial activity against gram-positive and gram-negative bacteria (Mohamed et al. 2015).

10.5.6 Other Metal and Metal Oxide Nanoparticles

Riddin et al. (2006) reported extracellular synthesis of highly stable and crystalline platinum myconanoparticles using the fungus *Fusarium oxysporum* sp. lycopersici. The fungal filtrate was treated with chloroplatinic acid solution; the color of the cell filtrate that changed from yellow to dark brown indicated the formation of myconanoparticles. The extracellular myconanoparticles of varying size 10–100 nm with different shapes such as hexagons, pentagons, circular, squares, and rectangles were synthesized. Similarly, comparative study has been conducted with fungus *Fusarium oxysporum* enzyme hydrogenase under two different platinum metal salts such as chloroplatinic acid and platinum chloride (Govender et al. 2009). The authors argued that octahedral chloroplatinic acid was too large to bind with the active region of the enzymes, and optimum condition for the formation nanoparticles in the reaction mixture should be pH 9 under 65 °C. Platinum chloride underwent a two-electron reduction on their molecular surface of the enzyme. The optimum condition for the nanoparticle synthesis is pH 7.5 under 38 °C.

Study has been conducted due the current demand to formulate mycological technologies in material science. Herein biological synthesis of lead nanoparticle using *Aspergillus* species was demonstrated. The extracellular synthesis of lead nanoparticles in the range of 1.77–5.8 μm was observed, whereas due to the presence of myconanoparticles inside the cell surface, size ranges from 5 to 20 nm (Pavani et al. 2012). Bansal and coworkers synthesized silica nanoparticles by using fungus *Fusarium oxysporum*. The extracellular protein-mediated hydrolysis of the anionic complexes results in calcination at 300 °C for the crystallization of silica (Bansal et al. 2005). A report on biological synthesis of zirconia myconanoparticles by the fungus *Fusarium oxysporum* was demonstrated by Bansal et al. (2004). The extracellular hydrolysis of metal anions by cationic protein is the mechanism responsible for the synthesis of zirconia nanoparticles.

10.6 Intracellular Synthesis of Myconanoparticles

The intracellular mode of myconanoparticle synthesis consists of transporting ions into the microbial cells to form nanoparticles by the enzymes (Li et al. 2011). The myconanoparticles synthesized from extracellular synthesis method are much faster than that of intracellular. On the other hand, nanoparticle synthesized using intracellular methods is smaller in size as compared to the extracellular synthesis of myconanoparticles (Yadav et al. 2015). There are few reports available in the intracellular (Table 10.2). Hence, several works has been carried out on the intercellular myconanoparticle synthesis.

Table 10.2 Intracellular synthesized metallic nanoparticles of different size from diverse fungal species

S. no.	Name of the metal	Name of the organisms	Size of the nanoparticles (nm)	Reference
1	Silver	*Phoma* sp.3.2883	71.06	Chen et al. (2003)
2	Silver	*Verticillium*	25	Mukherjee et al. (2001)
3	Silver	*Fusarium oxysporum*	25–50	Korbekandi et al. (2013)
4	Gold	*Alternaria fumigatus*	–	Bathrinarayanan et al. (2013)
5	Gold	*Phoma macrostoma*	100–200	Sheikhloo and Salouti (2012)
6	Gold	*Penicillium chrysogenum*	5–100	Sheikhloo and Salouti (2011)
7	Cadmium	*Candida glabrata*	–	Dameron et al. (1989)
8	Cadmium	*Schizosaccharomyces pombe*	1–1.5	Kowshik et al. (2002)
9	Lead	*Rhodosporidium diobovatum*	2.5	Seshadri et al. (2011)
10	Nickel	*Hypocrea lixii*	–	Salvadori et al. (2015)

10.6.1 Silver

Chen and coworkers demonstrated the intracellular synthesis of silver myconanoparticles using filamentous fungus *Phoma* sp.3.2883 via adsorption and accumulation. The TEM analysis showed the adsorbed silver particles on the mycelium and the average size of the nanoparticles was 71.06 nm. The reduction of absorbed silver particles was confirmed by X-ray photoelectron spectroscope. The authors reported the frozen mycelium of *Phoma* sp.3.2883 is effectively used in the silver nanoparticles (Chen et al. 2003). In another study by Mukherjee et al. (2001), the fungus *Verticillium* was used for the intracellular synthesis of silver myconanoparticles. The exposure of the fungal biomass to the metal ions solution resulted in the intracellular reduction of metal ion and formation of silver myconanoparticles of average size 25 nm. The reduction of metal ions was occurring due to the presence of enzymes in the cell wall of fungus, and proliferation of fungal cells confirms that high concentration silver metal ions were not toxic to the fungal cells. Similarly, Korbekandi and coworkers conducted the study to optimize the production of silver myconanoparticles by *Fusarium oxysporum*. The optimized procedure from this study enhanced the production of silver myconanoparticles with size range of 25–50 nm, and other analysis reports support the hypothesis that nanoparticles are synthesized by intracellular method (Korbekandi et al. 2013).

10.6.2 Gold

Intracellular synthesis of gold myconanoparticles by employing *Alternaria fumigatus*. In this study, the authors investigated the production of stable gold myconanoparticles when the metal salt solution was treated with *A. fumigatus* biomass, which acted as a reducing agent. The production of myconanoparticles was confirmed by the change in color from yellow to pinkish violet at room temperature for 72 h of incubation (Bathrinarayanan et al. 2013). Since nanomaterials are growing edge of the rapid developing field of myconanotechnology, work has been demonstrated for biosynthesis of gold myconanoparticles using the fungus *Phoma macrostoma*. The gold myconanoparticles were obtained by incubating metal salt solution with fungal biomass for 48 h with the particle size ranging from 100 to 200 nm in spherical, triangle, and rod shape (Sheikhloo and Salouti 2012). Similarly, a study has investigated fungus *Penicillium chrysogenum* isolated from Ahar copper mine for the biological synthesis of gold myconanoparticles. The TEM result revealed that intracellular formation of gold nanoparticles in spherical, triangle, and rod shapes with the size range of 5–100 nm (Sheikhloo and Salouti 2011).

10.6.3 Other Metal and Metal Oxide Nanoparticles

The cadmium myconanoparticle synthesis was achieved using the yeast *Candida glabrata* and *Schizosaccharomyces pombe* that were cultured in the presence of cadmium salt. These cadmium sulfide crystallites are more monodispersed as compared to cadmium sulfide synthesized chemically (Dameron et al. 1989). Similarly, cadmium sulfide nanoparticles were synthesized intracellularly by employing *Schizosaccharomyces pombe* strain treated with 1 mM cadmium salt solution. Hexagonal lattice structure with 1–1.5-nm-sized nanoparticles was obtained from the present work (Kowshik et al. 2002). Other works reported the intracellular synthesis of stable lead sulfide myconanoparticles using marine yeast *Rhodosporidium diobovatum*. The structure of synthesized nanoparticles was examined by TEM analysis, and the size of particles is ranging from 2 to 5 nm where lead sulfide myconanoparticles were capped by a sulfur-rich peptide (Seshadri et al. 2011). Recently, the investigation was carried out with dead biomass of the fungus *Hypocrea lixii* to convert nickel ions into nickel oxide myconanoparticles in aqueous solution (Salvadori et al. 2015).

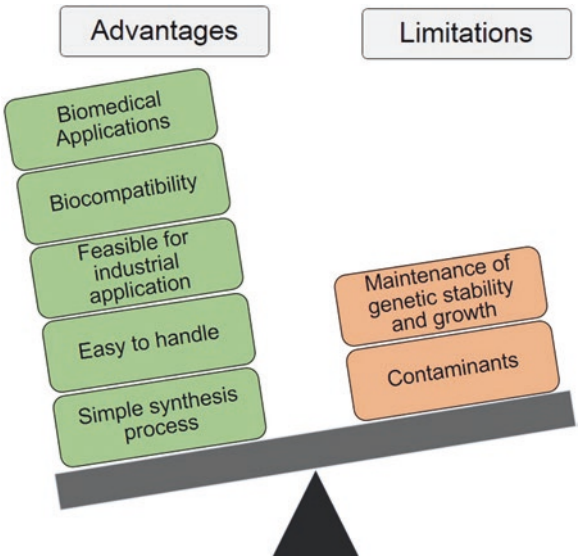

Fig. 10.3 Schematic representation of factors affecting myconanoparticle synthesis

10.7 Factor Affecting Mycological Synthesis of Metal and Metal Oxide Nanoparticles

It has been well documented that the growth and development of the fungal cultures are directly coordinated by the environmental conditions. In fungi certain behavior changes; synthesis of metabolites and enzymes is also influenced by the environmental conditions. Biotechnology is a technology where biological entities cultured in the laboratory are conditioned by mimicking the same environment for the exploitation of them. Maintaining specific environmental condition in laboratory condition is quite a difficult process, which needs several experiments to optimize the growth conditions such as temperature, size of biomass, concentration of metal ions, time, pH, and agitation for each fungus (Fig. 10.3).

10.7.1 Temperature

Temperature is an important factor that affects the mycological synthesis of metallic nanoparticles. Moreover, temperature also plays a major role in determining the size, shape, and yield of the nanoparticles (Shah et al. 2015). For example, syntheses of silver nanoparticles at a reaction temperature of 4 °C with fungus *Neurospora crassa* cell-free extract produced particles with average size of 2–9 nm. However, when the reaction temperature was increased to 25 °C, the average size of the nanoparticles was ranging from 2 to 22 nm (Quester et al. 2016). Similar investigation was conducted to determine the effect of temperature in the yield and biogenic

synthesis of gold nanoparticles using the fungus *Fusarium acuminatum* and found that 37 °C is an optimum temperature (Tidke et al. 2014). Additionally, Singh and coworkers optimized reaction temperature for the maximum yield of silver myconanoparticles by employing *Penicillium* sp. isolated from the healthy leaves of *Curcuma longa* (turmeric). The parametric optimization showed 25 °C is the ambient temperature for the enhanced yield of silver nanoparticles from *Penicillium* sp. (Singh et al. 2014). From the above information, it can be concluded that the rate of reaction and the nanoparticle formation becomes faster under increase in temperature, whereas the average size of the nanoparticles decreases with increasing temperature.

10.7.2 Size of Biomass

The concentration of biomass is considered as one of the major factors in the biological synthesis of nanoparticles. Fungal-mediated nanoparticle preparation directly depends on the metabolite secreted by the fungi, which act as reducing and capping agent for the synthesis and stabilization of nanoparticles. Hence, the size of biomass is an essential parameter to be optimized for the betterment of myconanoparticle synthesis. A study by Singh et al. (2014) determines that the change in the concentration of fungal biomass in the reaction mixture could effectively influence the rate of the reaction and yield of the nanoparticles. Herein extracellular synthesis of silver nanoparticles was conducted by treating 5–20 g of wet biomass with the difference of 5 g of endophytic fungi *Penicillium* sp. in 1 mM of metal ion solution. The maximum and faster yield was observed in 15 and 20 g of wet biomass without any agglomeration because presence of enzymes is sufficient for the production of silver nanoparticles (Singh et al. 2014). Similarly, the effect by varying the amount of *Fusarium oxysporum* biomass in the reaction was studied in the fungal-mediated silver nanoparticle synthesis. The effect of fungal biomass concentration was investigated by employing 5–20 g of wet biomass with a difference of 5 g of fungus *F. oxysporum*. Herein the optimum fungal biomass is determined as 20 g, which is responsible for the amalgamation of nanoparticle and bioreduction of silver ions (Khan and Jamme 2016). Furthermore, the effect of biomass concentration on the extracellular production of silver nanoparticles was evaluated by employing various concentration of biomass of the fungus *Aspergillus niger*. The test result revealed that 20 g of biomass concentration is optimum concentration for the synthesis of myconanoparticles using *A. niger* (Khan et al. 2016).

10.7.3 Concentration of Metal Ions

The concentration of metal ions is another important parameter that affects nanoparticle formation in fungal-mediated synthesis. The variation in concentration of metal ions is believed to be the main contributing factor for the synthesis of fungal-mediated nanoparticles. In the recent study, different concentrations of silver ions ranging from 0.2 to 2 mM were evaluated for the optimum concentration of metal ions for the enhanced production of silver nanoparticle using fungus *Sclerotinia sclerotiorum*. The optimization study showed that 2 mM concentration of silver metal ions is the optimum concentration for the significant production of silver nanoparticle by *S. sclerotiorum* (Saxena et al. 2016). In a similar study by Kumari and coworkers, *Trichoderma viride* fungal filtrate was used to produce gold nanoparticles. In the synthesis process, two different concentrations (250 and 500 mg/L) of gold metal ions were used to evaluate the specific concentration of metal ions for efficient synthesis of nanoparticles. The results emphasize that 250 mg/L optimum concentration of metal ion for production of gold nanoparticles using *T. viride*. The authors concluded that at highest concentration of gold salt, the cell filtrate concentration becomes less, resulting in insufficient capping and stabilizing action of reducing agent (Kumari et al. 2016).

10.7.4 pH

The pH value of the reaction mixture has great influence in the formation of nanoparticles. A variation in pH results in change of charge of fungal metabolite that affects their ability to bind and reduce metal cations and anions in the course of fungal-mediated metal and metal oxide nanoparticle synthesis. Moreover, the change in the pH also affects the shape, size, and yield of the nanoparticles (Makarov et al. 2014). The reports have shown that differing the pH of the reaction is inclined to produce difference in shape and size of nanoparticle synthesis. In general, larger-sized nanoparticle tends to produce lower acidic pH values as that of higher pH values (Shah et al. 2015). The study demonstrates change of pH in the cell filtrate that affects the synthesis, size, and shape of the nanoparticles in the course of fungal-mediated silver nanoparticle synthesis using the fungus *Trichoderma viride*. The study reported that the pH of the medium has a great effect on the control synthesis, size, and shape of the nanoparticles (Chitra and Annadurai 2013). The comparative study has been conducted to investigate mycological synthesis of silver nanoparticles by employing fungus *Neurospora crassa* under two different pH values such as pH 6.5 and pH 10 in the reaction mixtures. The authors showed that pH 10 is the optimum pH value to maintain the same size in a range of 2–9 nm even after storage of 10 months. Furthermore, the optimum reaction conditions such as optimal pH in fungal-mediated silver nanoparticle synthesis improve the storage time of particles without losing its original size and without any sign of aggregation (Quester et al.

2016). In a similar study, silver nanoparticles were synthesized using *Penicillium* sp. with different pH ranging from 4 to 8 with the difference of 1 to determine the influence of pH on silver nanoparticle production. The authors reported that at pH 7 maximum nanoparticle production was achieved, whereas decreases in pH to 4 showed null production of silver nanoparticles. Hence, the test report concluded that protein secreted by *Penicillium* sp. in the reaction mixture for the capping of silver nanoparticles is stable at pH 7 other than acidic pH (Singh et al. 2014).

10.7.5 Reaction Time

In a recent report by Kumari et al. (2016), the reaction time influences the morphology of the gold nanoparticles in fungal-mediated metal nanoparticle synthesis. The study was performed by using fungus *Trichoderma viride* to reduce the gold metal ions with different time intervals such as 24, 48, and 72 h. Herein, after 24 h 100% of the particles synthesized were small spheres with size range of 7–24 nm; at 48 h of incubation, mixed population of spheres, triangles, and prisms of larger size such as 7–120 nm were observed, whereas in 72 h of incubation, particles are formed predominantly triangles and prisms of 20–400 nm size. The results suggest that smaller size of nanoparticle at 24 h of incubation was due the initiation of nucleation, which gradually showed increase in the size and different shapes of nanoparticles in 48 h and 72 h of incubation due the growth of crystals. The study concluded that during the period of crystal growth, spheres might have fused to form triangles in which further fusion yielded larger nanoparticles (Kumari et al. 2016). Furthermore, a study by Khan and Jamme (2016) demonstrated that time is the major factor that is involved in the yield of silver nanoparticle in the biological synthesis. In this study, the synthesis of silver nanoparticles by *Aspergillus terreus* was optimized with different time intervals such as 45, 55, and 75 h of incubation. An incubation period of 55 h was determined as optimum time for the enhanced synthesis of silver nanoparticles by *A. terreus*.

10.8 Antimicrobial and Antibiofilm Application of Myconanoparticles

Nowadays, myconanoparticles are considered as an emerging antimicrobial agent to substitute traditional antibiotics that facilitate to avoid the formation of resistance unlike antibiotics. Over the past few decades, metal and metal oxide nanoparticles are emphasized for their unique electromagnetic, optical, and catalytic properties. Among nanoparticles, noble metal nanoparticles like silver, gold, and platinum are predominately used for the biomedical applications. All the nanoparticles that are mentioned above were investigated for their antimicrobial efficacy. Among

Table 10.3 The fungus involved in the metal and metal oxide nanoparticle synthesis and their antimicrobial activities

S. no.	Name of the organisms	Nanoparticles	Antimicrobial activity toward	Reference
1	*Aspergillus niger*	Silver	*P. aeruginosa*, *S. aureus*, *L. monocytogenes*, *M. luteus*, *K. pneumonia*	Kathiresan et al. (2010)
2	*Trichoderma viride*	Silver	*S. aureus* (MRSA), *S. boydii*, *A. baumannii*, *S. sonnei*,	Elgorban et al. (2016)
			S. typhimurium	
3	*Bipolaris tetramera*	Silver and gold	*B. cereus*, *S. aureus*, *E. aerogenes*	Fatima et al. (2015)
4	*Aspergillus clavatus*	Silver	*P. fluorescens*, *E. coli*	Verma et al. (2010)
5	*Schizophyllum radiatum*	Silver	*E. coli*, *K. pneumonia*, *S. aureus*,	Metuku et al. (2013)
			S. paratyphi, *B. stearothermophilus*,	
			B. subtilis	
6	*Aspergillus versicolor*	Silver	*S. aureus*, *S. pneumoniae*, *P. aeruginosa*, *K. pneumonia*	Netala et al. (2016)
7	*Aspergillus niger*	Silver	*Staphylococcus* sp., *Bacillus* sp., *E. coli*	Jaidev and Narasimha (2010)
8	*Aspergillus Terreus*	Silver	*S. aureus*, *P. aeruginosa*, *E. coli*	Li et al. (2011)
9	*Yarrowia lipolytica*	Silver	*Salmonella paratyphi*	Apte et al. (2013)
10	*Fusarium oxysporum*	Gold	*Pseudomonas* sp.	Thakker et al. (2013)
11	*Trichoderma viride* and *Hypocrea lixii*	Gold	*E. coli*, *S. sonnei*, *P. syringae*	Mishra et al. (2014)
12	*Candida albicans*	Gold	*S. aureus*, *E. coli*	Ahmad et al. (2013)
13	*Aspergillus terreus*	Gold and silver	*S. aureus*, *B. subtilis*	Balakumaran et al. (2015)
14	*Penicillium atramentosum* KM	Silver	*B. cereus*, *S. aureus*, *M. luteus*, *S. typhimurium*, *A.hydrophila*, *E. aerogenes*	Sarsar et al. (2015)
15	*Fusarium oxysporum*	Silver	*E. coli*, *S. aureus*	Husseiny et al. (2015)
16	*Alternaria alternata*	Iron	*B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*	Mohamed et al. (2015)

nanoparticles, silver nanoparticles are extensively studied for the antimicrobial activity followed by gold and platinum. In biomedical application, gold nanoparticles are most often preferred as drug carrier in medical applications, whereas platinum nanoparticles are employed in theranostic application (Yadav et al. 2015). Several studies have demonstrated that metal and metal oxide nanoparticles could cause potential lethal effect to antibiotic-resistant and biofilm-forming bacteria (Table 10.3).

These studies suggest that metallic nanoparticles are inhibiting the bacterial growth by hyperosmotic shock. Even though metal ion is required for the bacterial growth, due to the overdoses of metal ion near cell membrane, selectively abolish the enzyme activity, disturb the membrane, or damage DNA (Lemire et al. 2013).

In recent years, silver nanoparticles gained significant attention as antimicrobial agent. The mechanism of the antimicrobial action of silver nanoparticles is unrevealed, but several reports are partially elucidating the mechanism action of silver nanoparticle. Breakdown of silver nanoparticles leads to release of ionic silver which inactivates the bacterial enzymes by interacting with thiol group, and it also involves in the inhibition bacterial DNA replication, damaging the bacterial cytoplasm membranes, reducing the intracellular adenosine triphosphate (ATP), and causing bactericidal activity (Jung et al. 2008). Kathiresan and coworkers investigated the extracellular biosynthesis of silver nanoparticles using *Aspergillus niger* and demonstrated its antimicrobial activity with the bacterial cultures such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Micrococcus luteus*, and *Klebsiella pneumoniae*. The result confirmed the efficacy of the biosynthesized myconanoparticles for antimicrobial activity against bacterial pathogens (Kathiresan et al. 2010). In the recent year, the work has reported the green synthesis of silver nanoparticles by employing fungus *Trichoderma viride* and evaluated its antibacterial activity against human pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA), *Shigella boydii*, *Acinetobacter baumannii*, *Shigella sonnei*, and *Salmonella typhimurium*. The study concluded that the green syntheses of silver nanoparticles are possessed antibacterial activity against all the enlisted human pathogens. Furthermore, authors argue that gram-negative bacteria showed more susceptibility than gram-positive bacterium to green synthesized silver nanoparticles from the study (Elgorban et al. 2016). Fatima and coworkers reported mycofabricated silver and gold nanoparticles were synthesized using the novel phosphate-solubilizing fungus *Bipolaris tetramera* that is isolated from rhizospheric soil. The nanoparticles were assessed for their antibacterial efficacy against *Bacillus cereus*, *Staphylococcus aureus*, and *Enterobacter aerogenes*. The test result indicated that the nanoparticles exhibited greater bacterial growth inhibition against *B. cereus* followed by *S. aureus* (Fatima et al. 2015). Verma et al. (2010) demonstrated the biological synthesis of silver nanoparticles using *Aspergillus clavatus* isolated from stem tissues of *Azadirachta indica* and evaluated their antimicrobial potential against *Pseudomonas fluorescens* and *Escherichia coli.* The results propounded that the antimicrobial activity of silver myconanoparticles against test microorganisms is found very promising.

Nevertheless, numerous studies were conducted to determine the efficacy of the myconanoparticles toward antibacterial activity. The work study with white rot fungus, *Schizophyllum radiatum*, for the biological synthesis of silver nanoparticles and antimicrobial efficacy of the nanoparticles was performed. The antibacterial activity of mycofabricated silver nanoparticles was investigated against various pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Bacillus stearothermophilus*, and *Bacillus subtilis*. The result

reported that the highest antimicrobial activity was observed against *B. subtilis* and *S. paratyphi* followed by *B. stearothermophilus*, *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Salmonella typhi* and least was observed against *Klebsiella pneumoniae* (Metuku et al. 2013). Netala and coworker reported the biosynthesis of silver nanoparticles using endophytic fungus, *Aspergillus versicolor*, isolated from the ethnomedicinal plant *Centella asiatica*. Antimicrobial activity of the biosynthesized silver nanoparticles was evaluated against bacterial pathogens like *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The strong antibacterial activity of silver nanoparticles against enlisted pathogen was observed. The maximum inhibition was observed against *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Further, the result revealed that silver nanoparticles showed strong growth inhibition against gram-negative bacteria to that of gram-positive bacteria (Netala et al. 2016). In other study by Jaidev and Narasimha (2010), biological synthesis of silver nanoparticles that were synthesized using the fungus *Aspergillus niger* and their antibacterial activity was studied. The silver nanoparticles showed extensive antibacterial activity against both gram-positive and gram-negative bacteria such as *Staphylococcus* sp., *Bacillus* sp., and *E. coli*. In another study, the biosorption of silver in the form of nanoparticles by the fungus *Aspergillus terreus* was demonstrated. The myconanoparticles characterized by transmission electron microscopy exhibited polydispersed spherical-shaped silver nanoparticles with a diameter range of 1–20 nm and stabilized in the solution. Authors argue that reduced nicotinamide adenine dinucleotide (NADH) was found to be a major reducing agent for the biological synthesis, and formation of silver nanoparticles due to the involvement of an enzyme mediated extracellular reduction process. Moreover, antimicrobial activity of the mycofabricated silver nanoparticles was evaluated against pathogenic bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The result indicated that the synthesized silver nanoparticles could efficiently inhibit various pathogenic organisms (Li et al. 2012). An environmental friendly process for the synthesis of bioinspired silver nanoparticles employing the psychrotrophic yeast *Yarrowia lipolytica* has been demonstrated. The experimental result showed the distribution of spherical silver nanoparticles with average diameter of 15 nm. Further, antimicrobial and antibiofilm activity of the melanin-mediated silver nanoparticles against a representative pathogen *Salmonella paratyphi* was evaluated. Test result showed that the synthesized silver nanoparticles possess significant antimicrobial activity toward *S. paratyphi*. Nevertheless these nanoparticles are also reported as potential antibiofilm agent (Apte et al. 2013).

On the other hand, fungal-mediated gold nanoparticles were also reported as potential antimicrobial agent. The metal gold is often considered the most inert of all the metal. Gold nanoparticles gain greater attention due to their stability under atmospheric condition, resistance to oxidation, and biocompatibility. These intrinsic properties of gold nanoparticles tempted researcher to develop the various methods for the synthesis of gold nanoparticles. Among all the reported methods, biological synthesis of gold nanoparticles encounters major importance because of their simple

step synthesis process and less cost, highly stable, nontoxic, and eco-friendly natures. Hence, for the biologically synthesized gold nanoparticles, prior attention has been given to employ them for the biomedical application, especially antimicrobial activity and drug delivery. The study has been conducted to demonstrate the biological synthesis of gold nanoparticles using the fungus *Fusarium oxysporum* and investigated their antimicrobial activity against *Pseudomonas* sp.. The study proved that rapid extracellular synthesis of biogenic gold nanoparticles by a fungus *F. oxysporum* has the ability to inhibit the growth of *Pseudomonas* sp. effectively (Thakker et al. 2013). In a study, the gold nanoparticles obtained in the reduction of gold salt by fungus *Trichoderma viride* and *Hypocrea lixii* with narrow size distribution were demonstrated as antimicrobial agent against bacterial culture *Escherichia coli*, *Shigella sonnei*, and *Pseudomonas syringae*. The result elucidated that dose-dependent antimicrobial activity of biosynthesized gold nanoparticles is against all three pathogens (Mishra et al. 2014). Ahmad and coworkers investigated the biological synthesis and antimicrobial activity of gold and silver nanoparticles using cell-free extract of the fungus *Candida albicans*. The authors have achieved the synthesis of gold and silver nanoparticles with size in the range of 5 nm and 30 nm. Further, the antimicrobial activity of the synthesized gold and silver nanoparticles was tested against the bacterial culture *Staphylococcus aureus* and *Escherichia coli*. The authors claim that the synthesized nanoparticles are effective growth inhibitors against test pathogens and reported silver nanoparticles possess greater bactericidal activity than that of gold nanoparticles (Ahmad et al. 2013). Similarly, Balakumaran and coworker have conducted a demonstration to prove the biological synthesis of gold and silver nanoparticles by fungus *Aspergillus terreus* and superior antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The authors revealed that the synthesized silver nanoparticles are spherical in shape, whereas gold nanoparticles are in different morphologies with a size 10–50 nm. Moreover, the myco-derived silver nanoparticles showed superior antimicrobial activity than the standard antibiotic (Balakumaran et al. 2015).

10.9 Future Prospects

Understanding the availability of huge diversity or prospecting of microorganisms in the biosphere of Earth, significant work has been required to screen the efficient exploitation of microorganism group for the biological synthesis of metallic nanoparticles. This addresses the utilization of biological or environmental friendly material to facilitate a convenient method for nanoparticle synthesis. Various methods need to be developed for the microbial synthesis of metallic nanoparticles which created the situation to rely completely on the microorganisms. Nevertheless, microbial synthesis of metallic nanoparticle has certain limitation when it has been employed for large-scale industrial synthesis. Majorly, microorganism required certain optimum environment for the better production rate. Hence, it is necessary to develop the synthesis protocol along with the optimization of reaction conditions

like temperature, pH, incubation period of the culture, amount of biomass, metal salt concentration, etc. at which the maximum yield can be achieved. Moreover, to maintain the stability of the metallic nanoparticles and to avoid the aggregation of nanometals are still a challenge to the nano-based research. Most often the biological synthesis of metallic nanoparticle protein of/from the microorganisms involves in the stabilization of the metallic nanoparticles. Hence, the role of microbial protein in the biological synthesis of metallic nanoparticles has yet to be elaborated in convincing manner. On the other hand, bionanotechnology still fails to elucidate the mechanisms that are involved in the biological synthesis of metallic nanoparticles. It is highly necessary to figure out the exact mechanisms, which lead to the determination of specific gene and protein involved in the synthesis process that ease the several methods involve in the purification and optimization of reaction condition. Until date several attempts were taken to synthesize bimetallic nanoparticle using microbial sources and surface functionalizing of metallic nanoparticles with different biomolecules such as antibodies, antibiotics, proteins, and other bioactive molecules. These attempts are required for the better utilization of biological synthesized metallic nanoparticle in the biomedical applications.

10.10 Conclusion

Nanotechnology and nanomedicine are two major science fields showing their magnificent growth in the recent era of biomedical science. Nanoparticles become burgeoning topic for the researchers that reasoning for the tremendous improvement of nanoparticle synthesis and application prospects. There is an increasing interest of employing nanoparticles for biomedical application as carrier for drug molecules and diagnostic agent such as antibodies, protein, etc., and most often metallic nanoparticles alone are used as antimicrobial agents. Since the utilization of nanoparticle for biological application, it required to fulfill certain criteria like nontoxicity and biocompatibility and undergo simple excretion pathway like other waste materials from human biological system, simple synthesis process, cost-effective, and eco-friendly. Chemically synthesized nanoparticles possess contrast properties unlike mentioned above. On the other hand, microbial synthesis of nanoparticles owing all necessary feature as mentioned will suit for the biomedical applications. Utilization of microorganism especially fungi in nanoparticle synthesis can be classified into extracellular and intracellular synthesis based on the site in which nanoparticles are developing. Several external factors such as temperature, pH, reaction time, amount of biomass, metal salt concentration, etc. accelerate the rate and yield in nanoparticle synthesis process. Research currently accomplish the utilization of myconanoparticles in the antimicrobial applications, which facilitate the eco-friendly and biocompatible approach to humankind. These all give clear vision for future myconanotechnological approaches on an industrial scale and commercial application in biomedical and healthcare sectors.

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Chapter 11
Applications of Fungal Nanobiotechnology in Drug Development

Kanti Bhooshan Pandey and Brahm Kumar Tiwari

Abstract Nanotechnology is gradually being incorporated into drug industry sector. This technology is used to overcome the problem of drug delivery through existing approaches; however the cost factors and implementation issues have restricted this field and increased the need for basic as well technological innovations. There is an increasing interest in the use of fungi in these processes since they have potential to generate eco-friendly and relatively rapid and clean metallic nanoparticles. Fungal nanobiotechnology (FNBT) has resulted in development of nanodrugs and novel diagnostic/analytical tools for therapy and prevention of many chronic diseases such as cancer, HIV infections, and kidney diseases. In present chapter applications of FNBT in targeted drug delivery, bio-sensing, and development of drugs with enhanced efficiency and efficacy having lesser side effects have been discussed.

Keywords Nanobiotechnology · Nanoparticles · Drug delivery · Anticancer · Antibacterial · Antifungal · Biosensor

11.1 Introduction

The term coined by Prof. Norio Taniguchi "nanotechnology" in 1974 to illustrate the synthesis of materials at the nanometer level has now become one of the most fascinating technologies to design the products with several folds smaller but many folds higher effects (Taniguchi 1974). In recent years, almost all the areas connected directly or indirectly to the humans from food to environment have reflected positive influence of this innovative technology (Prasad et al. 2014; Mi et al. 2016; Eleftheriadou et al. 2016; Formoso et al. 2016; Scheinberg et al. 2017). To put the nanoscale into context of human biology, a typical hair is 80,000 nm wide,

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erythrocyte (red blood cell) 7000 nm, and key of life DNA is 2.5 nm wide (Alghuthaymi et al. 2015). Various nanoparticles are being successfully utilized as catalysts, biosensors, and semiconductors; however metal nanoparticles like gold (Au), silver (Ag), titanium (Ti), iron (Fe), etc. have gained much attention in recent years due to their fundamental and technological interests in biomedical applications especially in nanodrug/medicine development (Formoso et al. 2016; Rudramurthy et al. 2016; Prasad et al. 2017). Using nanoscale structures for biomedical applications is preferred due to increase in functional surface area to optimize the operational abilities with comparatively reduced size. In addition to this desired stability, resistance and support mobility are other important characteristics which make nanoparticles an ideal candidate for health preventive measures (Prasad et al. 2014; Saglam et al. 2016).

Nanobiotechnology is a new branch of nanotechnology, combining biology with physical and chemical means to generate nanoscale particles with specific functions and structures. The correlation of nanomedicine and public health has accelerated each other in number of ways. Nanodrug identifies cells/receptors of the concern disease, reaches to the target, and releases the medicine in sustainable way (Panchangam and Dutta 2015). This innovative way is used to overcome the problem of drug delivery through existing approaches; however the cost effectivity and implementation issues have restricted this field and have increased the need for basic as well as technological innovations. The conventional methods including chemical and physical techniques employed for synthesis of nanoparticles are much costly. Besides this, application of hazardous and poisonous chemicals in these techniques causes biological mischief (Boroumand Moghaddam et al. 2015). All these serious concerns raise the need to develop environment-friendly procedure or green synthesis of nanoparticles using biological means. Implementation of microorganisms such as bacteria, fungi, etc. with nanotechnology is the latest trend to synthesize nanoparticles with least hazard (Pantidos and Horsfall 2014; Boroumand Moghaddam et al. 2015; Prasad et al. 2016).

Among many microorganisms available for green synthesis, use of fungi in nanotechnology is considered as important due to their toleration and metal bioaccumulation capability (Sastry et al. 2003). Besides this, easiness in their scale-up, economic liability, and facility of employing biomass are other merits for utilization of green approach mediated by fungi to biosynthesize nanoparticles. Many species of fungi grow fast, and therefore their culture in laboratory is very simple (Castro-Longoria et al. 2011; Prasad 2016, 2017; Prasad et al. 2016).

Fungi are frequently used source of industrial enzymes due to their good capacity of protein production extracellularly. These fungi-derived industrial useful enzymes are mainly utilized in chemical and biomedical products, food, drinks, detergents, and baking (Saglam et al. 2016). The integration of nanotechnology with fungi to biosynthesize nanomaterials is known as fungal nanobiotechnology (FNBT) which has resulted in development of novel diagnostic and analytical tools and nanodrugs for therapy and prevention of many chronic diseases such as cancer, kidney diseases, multiple sclerosis, microbial infections, and chronic pain (Fig. 11.1) (Nithya and Ragunathan 2014; Gupta et al. 2012; Panchangam and Dutta 2015).

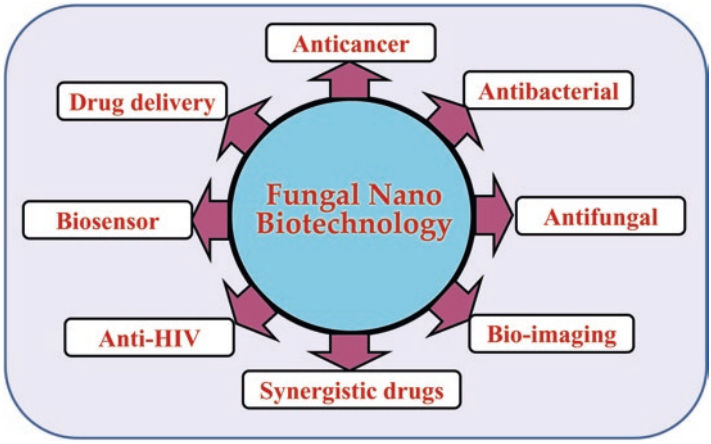

Fig. 11.1 Use of fungal nanobiotechnology in therapeutic applications

The successful and promising studies in these areas have provided a better understanding of fungi in nanobiotechnological applications (Prasad 2016, 2017). The present chapter deals with applications of FNBT in precise drug delivery and development of targeted drugs with enhanced efficiency and efficacy.

11.2 Advantages of FNBT and Applications in Drug Delivery

There is wide-spectrum application of FNBT in pharmaceutics to promote human health including development of analytical tools, nano-imaging, flourishing of nano-devices, improved drug delivery systems, and regulation of many toxicological issues (Bouwmeester et al. 2009; Castro-Longoria et al. 2011; Nithya and Ragunathan 2014). In the area of drug development especially drug delivery and therapy applications, FNBT has a great impact. Different types of metallic nanoparticles of dynamic medicinal effects can be biosynthesized by using many fungal species (Table 11.1). Silver nanoparticles (AgNPs) are one of the most studied metal nanomaterials; its wide-spectrum usage in health-protecting applications including against cancer and infections makes them very preferable and attainable particularly in biomedical field (Saglam et al. 2016). Nano-therapeutics obtained through FNBT is able to provide targeted drug delivery, improved drug solubility, extended drug half-life, and improved drug therapeutic drug index. Another most important property of these developed drugs is their reduced immunogenicity which has resulted in the potential to positively transform the treatment of many life-threating human diseases including cancer (Aliosmanoglu and Basaran 2012; Smith and Lodder 2013; Toffoli and Rizzolio 2013).

The unique properties of nanomaterials regardless of their sizes have been utilized in a wide range of applications. To synthesize metallic nanoparticles, the living

Table 11.1 List of major fungal species utilized in biosynthesis of therapeutically important metallic nanoparticles

Name of the fungus	Nanoparticles	Size (nm)	Medicinal activity	References
Alternaria alternata	Silver	20–60	Antifungal	Gajbhiye et al. (2009)
Alternaria clavatus	Silver	10–25	Antibacterial	Verma et al. (2010)
Aspergillus flavus	Titanium	62–74	Antimicrobial	Rajakumar et al. (2012)
Aspergillus fumigatus	Silver	15–45	Anti-HIV	Alani et al. (2012)
Alternaria niger	Silver	15–20	Antifungal, antibacterial	Kumar et al. (2008a) and Fateixa et al. (2009)
Alternaria terreus	Silver	1–20	Antifungal	Li et al. (2012)
Candida albicans	Gold	60–80	Anti-liver cancer	Chuhan et al. (2011)
Fusarium acuminatum	Silver	5–40	Antibacterial	Ingle et al. (2008)
Fusarium oxysporum	Silver	20–50	Antibacterial	Khosravi and Shojaosadati (2009)
Fusarium vasinfectum	Silver	3–30	Antibacterial	Joshi et al. (2013)
Ganoderma neojaponicum	Silver	5–8	Anticancer	Gurunathan et al. (2013)
Ganoderma spp.	Gold	20	Biocompatibility	Gurunathan et al. (2014)
Helminthosporium solani	Gold	2–70	Anticancer	Kumar et al. (2000b)
Mucor hiemalis	Silver	5–15	Antimicrobial	Aziz et al. (2016)
Penicillium brevicompactum	Gold	10–50	Anticancer	Mishra et al. (2011)
Penicillium purpurogenum	Silver	5–25	Antibacterial	Nayak et al. (2010)
Phoma glomerata	Silver	60–80	Antibiotics	Birla et al. 2009
Pleurotus cornucopiae var. citrinopileatus	Silver	20–30	Anticandidal	Owaid et al. (2015)
Pleurotus djamor var. roseus	Silver	90–370	Anticancer	Raman et al. (2015)
Pleurotus florida	Silver	15–25	Antibacterial	Bhat et al. (2011)
Pleurotus ostreatus	Silver	100	Antibacterial	Mirunalini et al. (2012)
Pleurotus ostreatus	Silver	4–15	Anticandidal, anticancer	Yehia and Al-Sheikh (2014)
Pleurotus sajorcaju	Silver	5–50	Antibacterial	Nithya and Ragunathan (2009)
Trichoderma viride	Silver	5–40	Antibacterial	Fayaz et al. (2010)
Usnea longissima	Usnic acid	50–200	Antifungal	Shahi and Patra (2003)

extracts of fungi, as extracellular or intracellular reductants, have been utilized by the researchers all over the world (Fig. 11.2). Reducing enzymes and the procedure of biomimeting mineralization are the possible mechanisms of nanoparticle biosynthesis using fungi (Boroumand Moghaddam et al. 2015). There is an array of advantage of nanoparticles obtained by fungal species such as gold nanoparticles (AuNPs).

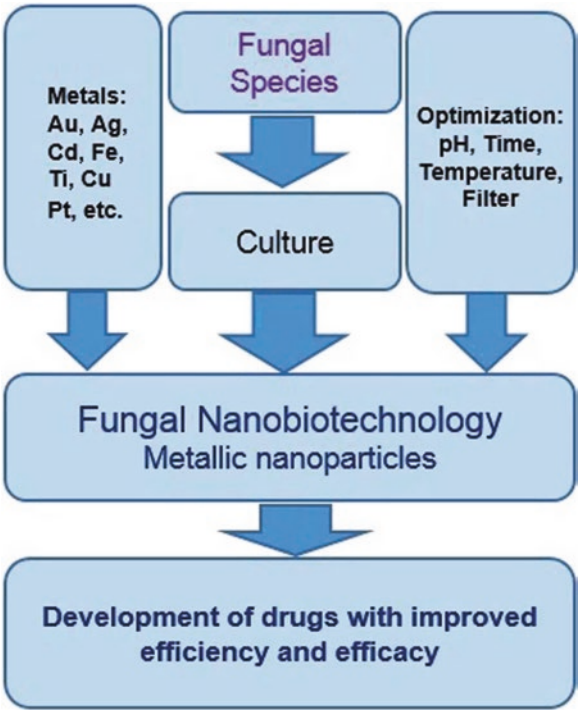

Fig. 11.2 Diagrammatic representation of fungal nanobiotechnology in drug development

AuNPs biosynthesized by utilizing fungi are less invasive and more contrast with nil photo bleaching, and the designed nano capsules help in efficient drug accumulation at the targeted site with sustained drug release even for a week (Ahmad et al. 2003; Duran et al. 2005).

Delivery of drugs at the targeted place in proper time and amount is a matter of major concern in designing of an effective drug. Many times it has been observed that the synthesized drug is capable enough against a particular disease; however its quick release and/or diminished dose makes them ineffective. Keeping this rationale in mind, achieving a controlled and targeted release of drugs via nano-carriers is attaining a highest impact (Boroumand Moghaddam et al. 2015). The metallic nanoparticles synthesized through FNBT are found to be very appropriate nano-conveyors since they fulfill the prerequisites of an ideal ligand such as passing the blood tissue obstacles and special endocytotic and transcytotic transfer mechanisms across cellular obstacles to reach targeted cells (Hafeli et al. 2009; Fadeel and Garcia-Bennett 2010). AgNPs obtained by utilizing *Aspergillus* and *Fusarium* species are able to pass through blood-brain barrier as most important epithelial joints of the skins due to their nano size. Their precise size also helps in restricting their expose area therefore reducing chances of poisoning. Likewise AuNPs obtained through biological technology by using *Penicillium* species may be another very proper nano-carrier in drug delivery due to their steadiness and tenability role (Giljohann et al. 2010). It has been predicted that nanoparticle-interceded targeted

delivery of many drugs especially in cancer therapy may result in improved efficiency and squad toxicities (Alghuthaymi et al. 2015; Boroumand Moghaddam et al. 2015). There are several advantages of fungal nanobiotechnology in drug development: inexpensive and wide spectrum, fewer side effects, easy biomass handling, both extracellular and intracellular biosynthesis of nanoparticles, good metal accumulation, sustainable and environment friendly, high wall-binding capacity, and feasible larger-scale production (Prasad 2016, 2017).

11.3 Applications of FNBT in Effective Drug Development

11.3.1 Anticancer

Development of effective and less toxic drugs with targeted action is the major area where FNBT has shown its significant utilization. Cytotoxicity is vital issue behind the chemosynthetic drugs available in the market for the treatment of various types of cancers. Nanoparticles synthesized by many species of *Penicillium* have shown very effective cure against carcinogenesis. Mishra and colleagues have evaluated the impact of AuNPs biosynthesized by supernatant, live cell filtrate and biomass of *Penicillium brevicompactum* on cancer cells and reported a significant inhibition of cell proliferation via induction of cell death (apoptosis); however the mechanism(s) involved behind this is/are under investigation (Mishra et al. 2011; Jeyaraj et al. 2013).

The study performed by Hsin et al. (2008) on NIH3T3 cells proposed that AgNPs may induce apoptosis in these cells through release of cytochrome c into the cytosol and translocation of Bax, a pro-apoptotic protein. The whole mechanism was mitochondria mediated. Mainly work has been done to study the mechanisms of action of titanium oxide nanoparticles (TiO_2NPs) which can be biosynthesized by using many species of *Aspergillus*. The studies done on human monoblastoid cells and bronchial epithelial cells have described that the anticancer effects of TiO_2NPs were mitochondria-mediated apoptosis and oxidative stress-induced cell death, respectively (Vamanu et al. 2008; Park et al. 2008). Zhao et al. (2009) conducted experiment on mouse JB6 cells (epidermal cells) and reported that TiO_2NPs induce death in cancerous cells via caspase-mediated signaling.

Angiogenesis is one of the key mechanisms involved in growth and development of cancer cells. The growth of new blood vessels from existing vessels is necessary to circulate oxygen and nutrients to the cancerous microenvironment (Nishida et al. 2006). It has been reported that anti-angiogenic therapy is the promising approach to control cell proliferation followed by metastasis (Riechelmann and Grothey 2017). AuNPs which can be biologically synthesized by *Helminthosporium* species have emerged as an effective candidate in therapy of various types of cancers including ovarian and as good nano-conveyors for targeted delivery (Ghosh et al. 2008; Tiwari 2012).

AgNPs synthesized by *Achillea biebersteinii* have been reported to possess dose-dependent cytotoxic effects on endothelial cells (Baharara et al. 2014). Will et al. (2011) reported the indirect effect of AgNPs on the microcirculation of developing chorioallantoic membrane of chick embryo, an effect associated with the partial preservation of the capillary diameters (Will et al. 2011). Later studies confirmed that AgNPs inhibit vascular endothelial growth factor (VEGF)-mediated formation of new blood microvessels via suppressing VEGF (Gurunathan et al. 2009; Sheikpranbabu et al. 2009). The studies have also reported that AgNPs synthesized by fungal species may also elicit the anticancer effect by inhibiting the vascular permeability in retinal cells (Sheikpranbabu et al. 2009).

11.3.2 Antibacterial

Bacterial infections are one of the most prevailed reasons behind many diseases in humans. In comparison to the numbers of pathogenic bacterial species, with their abilities to resist toward antibiotics and types of life-threating diseases caused by their infections, the available drugs/therapies against these microorganisms are a few and limited. Metallic nanoparticle-based antibiotics/antiseptics against bacterial infections have gain much emphasis in the last few years (Dastjerdi and Montazer 2010; You et al. 2012; Alghuthaymi et al. 2015; Formoso et al. 2016). AgNPs with a size range 5–40 nm, biosynthesized by using *Trichoderma viride*, have exhibited very effective augmentation in antibacterial activities with a variety of antibiotics against both Gram-positive and Gram-negative types of bacteria (Fayaz et al. 2010). A series of frequently used antibiotics including ampicillin, kanamycin, erythromycin, and chloramphenicol were tested for their efficiency after augmentation with AgNPs and found improved potentials against different strains of bacterial species (Dastjerdi and Montazer 2010; Fayaz et al. 2010; You et al. 2012). The researchers have reported that ampicillin exerted maximum augmentation when combined with AgNPs among all the above tested antibiotics (Fayaz et al. 2010). Duran and colleagues have reported that AgNPs biosynthesized by using *Fusarium oxysporum* can avoid or reduce the chance of infections caused by pathogenic bacteria such as *Staphylococcus aureus*, and moreover they can be integrated into textile fabrics (Duran et al. 2007).

Another study performed on an endophytic fungus, *Penicillium* species which was isolated from the leaves of *Curcuma longa* for biosynthesis of AgNPs, reports the successful application of synthesized nanoparticle as a weapon against *Staphylococcus aureus* and *Escherichia coli* in a facial way (Duran et al. 2007; Singh et al. 2014).

11.3.3 Antifungal

It is quite interesting to describe the development of antifungal drugs by using fungal species. Many studies performed in different laboratories in different environmental conditions have claimed that FNBT can be successfully applied in designing the drugs with enhanced effects against many diseases caused by pathogenic fungi (Gajbhiye et al. 2009; Sardi et al. 2013; Poulose et al. 2014). *Aspergillus niger* is the fungal species which is most studied/described for antifungal properties of biosynthesized nanoparticles by them. A vast study performed by Gajbhiye and coworkers in 2009 on antifungal activities of biosynthesized nanoparticles with combination of fluconazole, a widely referred antifungal drug against a series of fungal species including *Phoma glomerata*, *P. herbarum*, *Fusarium semitectum*, and *Trichoderma*, has documented that AgNPs biosynthesized by utilizing *Alternaria alternata* enriched the antifungal activity of fluconazole in most of the tested fungal strains (Gajbhiye et al. 2009).

Dar and Soytong (2014) proposed the ability of electrospinning technique to encapsulate the antifungal compounds. They also encapsulated the effective antifungal agents from *Chaetomium* species (Dar and Soytong 2014). El-Newehy and coworkers (2012) synthesized the nanofibers of polyvinyl alcohol and polyethylene oxide which were quite effective against many pathogenic fungi including *Penicillium* and *Aspergillus* spp. (El-Newehy et al. 2012). In continuation, another study done by Musarrat et al. (2010) to biosynthesize AgNPs by utilizing myceliafree water extracts of *Amylomyces rouxii* and testing it against microbial infections reported that the biosynthesized AgNPs showed significant antifungal effects against *Candida albicans* and *Fusarium oxysporum* infections (Musarrat et al. 2010). The AuNPs biosynthesized on the fungus surface, *Rhizopus oryzae*, exhibited very strong inhibition of *Saccharomyces cerevisiae* and *Candida albicans* (Das et al. 2009).

The use of AgNPs as potent antifungal agent is becoming more widespread since technological advances in synthesizing them are being economical day by day. The use of AgNPs biosynthesized by applying FNBT against pathogenic fungi is moderately safer in comparison to synthetic fungicides (Oh et al. 2006). Ag and $AgSiO_2$ nanoparticles synthesized by γ-irradiation, on evaluation of their antibacterial and antifungal efficiencies, showed a strong antifungal effect against *Botrytis cinerea* (Oh et al. 2006). The study conducted by Fateixa and his colleagues (2009) has reported that Ag_2S nano-crystals on amorphous silica particles showed antifungal activity against *Aspergillus niger*. Later studies have also reported that metallic nanoparticles such as ZnO and $ZnTiO_3$ elicit great biocidal effects against *Aspergillus* with different efficiencies (Ruffolo et al. 2010; Jo et al. 2009).

11.3.4 Biosensor and Imagining

The use of biosensors in therapeutic applications is increasing day to day. Nanomaterials biosynthesized by FNBT have been reported as promising tools for development of biosensors with enhanced sensitivity. The optical and electronic properties of nanoparticles make them suitable candidate for their effective utilization in biosensor applications (Gou 2013; Boroumand Moghaddam et al. 2015; Faraz 2018). One of the most significant uses of biosensors is to locate the glucose with accuracy. Zheng et al. (2010) have reported AuNP-based glucose oxidase biosensors on the basis of augmentation of glucose oxidases by gold nanoparticles. Interestingly this AuNP-based biosensor showed a quality response in a range of 20–0.80 mM for glucose with a detection limit of 17 μM. This biosensor may be of important use in determining glucose in various biomedical samples including business glucose injections (Zheng et al. 2010).

In addition to the development of biosensors, the optical features of metallic nanoparticles biosynthesized by fungal species may also be utilized in biomedical techniques. The refractive indicator, photoluminescence, and plasmon resonance features are the characteristics of metallic nanoparticles which are useful in interaction of light of particles (Iskandar 2009; Zhu et al. 2012). AgNPs synthesized by using *Trichoderma viride* emitted photoluminescence in range of 320–520 nm when excited by laser, advocating the suitability of these nanoparticles in imaging techniques (Sarkar et al. 2010; Zhu et al. 2012). The size-based optical features of nanomaterials have been studied by many researchers (Podgaetsky et al. 2004; Sarkar et al. 2010). Studies investigating the cadmium telluride quantum dots (CdTeQDs) fabricated through extracellular synthesis by using yeast and *E. coli* documented that CdTeQD had high solvable ability in water. After spectro-studies it was concluded that CdTe QDs associated with folic acid may be utilized for in vitro imaging of cancer cells. In addition to this, they showed biocompatibility in cytotoxic assays (Bao et al. 2010a, b).

11.3.5 Other Biomedical Applications

Besides mentioned area of medical conditions, there are various fields concerned to human health where FNBT is utilized indirectly for discovery of novel drugs. Many metal nanoparticles biosynthesized by different species of fungi can be used to induce local interaction with tumor cells and thus may help in identification of biomarkers (Gou 2013; De Rosa and Caraglia 2013). Iron nanoparticles are used as one of the most accurate tools for cancer imaging (Toffoli and Rizzolio 2013; De Rosa and Caraglia 2013). Development of nanofiber scaffolds for regenerating central nervous cells, nanospheres as vaccine carriers for nasal vaccination, sequencing of

protons and nucleic acids unique to particular pathogenic microorganisms, and detection of various cancers and gene transfections via surface-functionalized nanoparticles are other important areas of medicine where fungal nanobiotechnology is being/may be utilized very successfully (Maite et al. 2000; Rosi and Mirkin 2005; Ellis-Behnke et al. 2006; Gupta et al. 2012; Sardi et al. 2013).

11.4 Conclusion

Development of drugs through nanobiotechnology has raised the expertise and use of various fields of science. Fungal science or mycology has shown an innovative way to develop drugs with enhanced therapeutic index due to its sustained availability, cost-effectiveness, and lowered side effects. Reported success of laboratory as well as community-based studies provides enough reason to believe that in forthcoming years, the benefits of nanodrugs and novel nanodiagnostic tools developed by utilizing fungal nanobiotechnology will provide a considerable impact on human health globally.

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Chapter 12
Mycosynthesized Nanoparticles: Role in Food Processing Industries

Lakshmishri Roy, Debabrata Bera, and Sunita Adak

Abstract Green synthesis of nanoparticles (NPs) is an evolving branch of nanotechnology. The use of fungi for the synthesis of NPs is referred to as mycosynthesis of metal NPs. Fungal endophytes have been recognized as important sources of a variety of structurally novel active secondary metabolites with anticancer, antimicrobial, and other biological activities. This mode of synthesis of metal nanoparticles is gaining more importance owing to its simplicity, rapid rate of synthesis of NP of attractive and diverse morphologies, and elimination of elaborate maintenance of cell cultures and eco-friendliness. Presently, the researchers are looking into the development of cost-effective procedures for producing reproducible, stable, and biocompatible metal NPs using fungal cultures. The present chapter is an exhaustive overview that assesses the role of fungi in the synthesis of nanoparticles, the mechanism involved in the synthesis, the effect of different factors on the reduction of metal ions in developing low-cost techniques for the synthesis, and recovery of nanoparticles. Finally, the application of nanoparticles in food processing industries, i.e., antimicrobial mechanisms, etc., has also been discussed.

Keywords Antimicrobial · Food processing · Nanoparticles · Mycosynthesis · Cost-effective

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12.1 Introduction

Myconanotechnology is the interface between "mycology" and "nanotechnology" and has considerable potential, partly due to the wide range and diversity of the fungi (Rai et al. 2009a). Mycofabrication can be defined as the synthesis of metal nanoparticles using fungi. The fungal system in recent times has emerged as "bionanofactories" synthesizing nanoparticles of silver, gold, platinum, cadmium, etc. Mycosynthesized nanoparticles have been observed to be of good monodispersity and dimensions. Physicochemical and biological mechanisms of accumulation of metal ions by fungi include extracellular binding by metabolites and polymers, binding to specific polypeptides, and metabolism-dependent accumulation (Volesky and Holan 1995; Prasad 2016, 2017).

Fungi have attracted more attention for research pertaining to biological production of metallic nanoparticles due to their metal toleration and bioaccumulation capability (Sastry et al. 2003); ease for scale-up studies for nanoparticle synthesis (e.g., utilizing a thin solid substrate fermentation technique); feasibility of fungal enzymes (Castro et al. 2012) that results into higher yield of nanoparticles (Sastry et al. 2003; Mandal et al. 2006; Gade et al. 2010); economic livability and facility of employing fungal biomass; short incubation and growth period required by a number of fungal species, thereby making their culturing and maintenance in laboratory very simple (Castro et al. 2011; Prasad et al. 2016); their high wall-binding and intracellular metal uptake capacities (Volesky and Holan 1995); ability to produce metal nanostructure via reducing enzyme intracellularly or extracellularly; and the procedure of biomimetic mineralization (Ahmad et al. 2003a; Duran et al. 2005; Prasad 2016, 2017).

In a nutshell the potential advantages of fungi as bionanofactories may be depicted as given in Figs. 12.1 and 12.2.

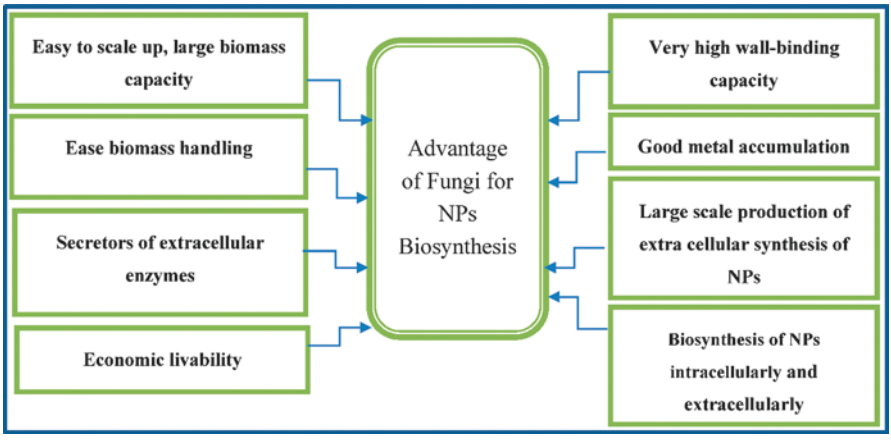

Fig. 12.1 Advantages of fungi to be used as biofactories for NP production. Thus, using these dissimilatory properties of fungi, it could be extensively used for the rapid and eco-friendly biosynthesis of metal nanoparticles

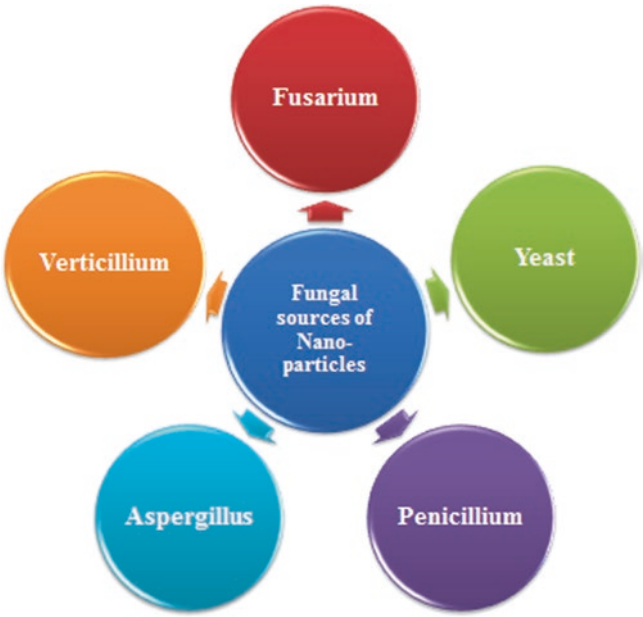

Fig. 12.2 Types of fungal sources for nanoparticle synthesis

12.2 Mycosynthesis of Nanoparticles (NPs)

Microbial synthesis of NPs is a green chemistry approach. Reports on fungal and other microbe-mediated biosynthesis of gold, silver, gold-silver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots (QDs), magnetite, and uraninite are available (Narayanan and Sakthivel 2010). These nanoparticles are biocompatible, less toxic, and of multifarious utilities in numerous fields. The exact mechanism of biosynthesis has not been clearly elucidated. Microbial interactions with nanomaterials may in certain instances produce nanostructured materials and enhance microbial growth (Kumar et al. 2007). This is because of their multiple attributes that include excellent performance, selective adsorption of metal ions, operation over a broad range of ecological conditions (pH, ionic strength, temperature), low cost, free availability, regeneration, high biosorption capacity, etc. (Mandal et al. 2006).

Mycosynthesis of nanoparticles is of considerable industrial significance because of the following distinctive features of fungal systems:

Are capable of growing on readily available and inexpensive substrates.
Produce stable nanoparticles which do not aggregate even upon prolonged storage and are thus characterized by longevity.
Enhanced tolerance toward a higher concentration of metal nanoparticles which are also well dispersed in the medium offering large advantage.

Mycelial and mesh-like characteristics of fungal species aid them to withstand flow pressure and other conditions in bioreactors or other chambers as compared to other microbes and plant materials (Narayanan and Sakthivel 2010).
Secrete a number of enzymes (Mohanpuria et al. 2007).
Easy to grow and maintain (Mukherjee et al. 2008a, b).
No stringent requirements for special equipments.
Biomass concentration remains much higher than bacteria.
Possess inherent ability of producing extracellular metabolites that serve as defensive agents supporting their own survival when exposed to different environmental stresses like toxic materials (such as metallic ions), predators, and temperature variations.
Can be effectively immobilized and employed.
Synthesis of metal nanoparticles.

The process becomes cost-effective and safe without requirement of any specific instruments (Mohanpuria et al. 2007) and offers distinct advantage for large-scale production, extraction, and recovery unlike the bacterial strains reported (Mukherjee et al. 2008a, b; Gaikwad et al. 2013; Prasad et al. 2016).

The fungal system shows the capability of both intracellular and extracellular synthesis of nanoparticles (Mandal et al. 2006; Riddin et al. 2006; Ingle et al. 2008). In the recent past, research work using the fungal system has been carried out using both aspects for synthesis of nanoparticles of gold, CdS, silver, silica, titania, zirconia, etc. (Mukherjee et al. 2001a, b; Ahmad et al. 2002; Chen et al. 2003; Bansal et al. 2004, 2005). Reports on a number of fungal species like *Verticillium*, *Phoma* sp., *Fusarium oxysporum*, *Aspergillus fumigatus*, *Trichoderma asperellum*, and *Mucor hiemalis* for myconanosynthesis have been explored (Mukherjee et al. 2001a, b, 2008a, b; Chen et al. 2003; Bansal et al. 2005; Aziz et al. 2016).

12.3 Mechanistic Aspects of Mycosynthesis of Nanoparticles

Mechanism of nanoparticle formation, in all microorganisms and plants, is still an open question, even though much research has been attempted (Meyer 2008; Kathiresan et al. 2009). Biogenic synthesis of metal nanoparticles involves bioreduction of metal salts to elemental metal which may be stabilized by organic molecules present in the microbes such as fungi and bacteria; and the alternative way could be biosorption where metal ions in the aqueous medium are bonded to the surface of the cell wall of the organisms.

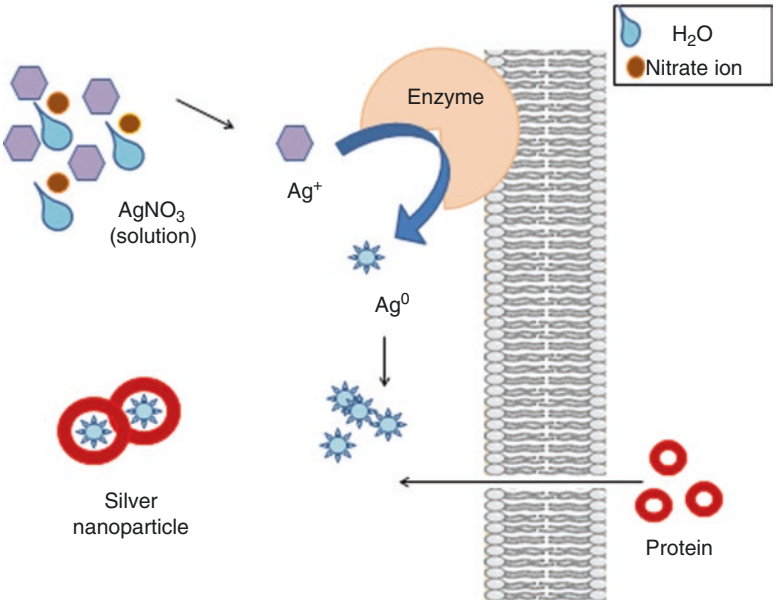

Fig. 12.3 Mechanism of extracellular biosynthesis of silver nanoparticles using fungi (Juibari et al. 2011)

12.3.1 Extracellular Synthesis

Several hypotheses for explanation of the mechanism of extracellular-mediated nanoparticles (Fig. 12.3) include:

Nitrate reductase was suggested to initiate nanoparticle formation by many fungi including *Penicillium* species, along with several enzymes like NADPH-dependent reductases, nitrate-dependent reductases, etc. In case of extracellular synthesis of silver nanoparticles due to the presence of these enzymes in fungal cytoplasm, reduction of silver metal ions into silver nanoparticles takes place. This finally leads to the formation of highly stable silver nanoparticles.

Jain et al. (2011) reported that AgNP synthesis for *Aspergillus flavus* occurs initially by a "33 kDa" protein followed by a protein (cysteine and free amine groups), which stabilizes the NPs by forming a capping agent (Jha and Prasad 2010). A number of researchers supported nitrate reductase for extracellular synthesis of NPs (Jain et al. 2011; Juibari et al. 2011; Bansal et al. 2004). Kumar et al. (2007) stated that the process of formation of silver nanoparticles requires 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 Ag0. 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.

Duran et al. (2005) had driven out the mechanism of biosynthesis of silver nanoparticles and stated that the synthesis of silver nanoparticles occurred in the presence of anthraquinone and NADPH-nitrate reductase. In this case, the electron required to fulfill the deficiency of aqueous silver ions (Ag^{+}) and convert it into Ag neutral (Ag0) was donated by both quinone and NADPH.

Edible mushroom *Volvariella volvacea* was also used for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles (Chertok et al. 2008). The morphology of these nanoparticles was greatly influenced by temperature and pH. All the nanoparticles synthesized by using this fungus were highly crystalline and photoluminescent when observed their XRD patterns. It has been suggested that the polysaccharides and oligosaccharides present in the broth might be responsible for the reduction of metal ions to metal nanoparticles.

Mukherjee et al. (2008a, b) 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. 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.

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 *A. 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 zirconium oxide nanoparticles (Bansal et al. 2004; Duran et al. 2005).

Chen et al. (2003) 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.

12.3.2 Intracellular Mechanism

Intracellular synthesis method involves a specific ion transportation system in the microbial cell. In this the cell wall of the microorganism plays an important role in biosynthesis of metallic nanoparticles. The hypothetical mechanism involves the electrostatic interaction forces found between the opposite charges, i.e., the negatively charged cell wall of microorganism and the positively charged metal ions. The enzymes present in the cell wall of microorganisms reduce these metal ions to nanoparticles which subsequently get diffused off through the cell wall (Fig. 12.4).

During the intracellular synthesis of gold nanoparticles, the gold metal ions firstly bind on the fungal cell surface. After that absorbed metal ions were reduced by enzymes present in the cell wall of fungi. These enzymes contain positively charged groups which lead to the aggregation of nano-shaped structures and finally formation of metal nanoparticles.

Other possibility is the migration of ions to cytoplasmic membrane to get reduced subsequently. The synthesis of silver nanoparticles requires the reduction of NADPH to NADP. Here, hydroxyquinone acts as an electron shuttle transferring the electrons generated during the reduction of nitrate to Ag ions converting them to Ag0.

The actual mechanism of mycosynthesis of nanoparticles, however, is still not fully understood. According to Mukherjee et al. (2001a, b), 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.

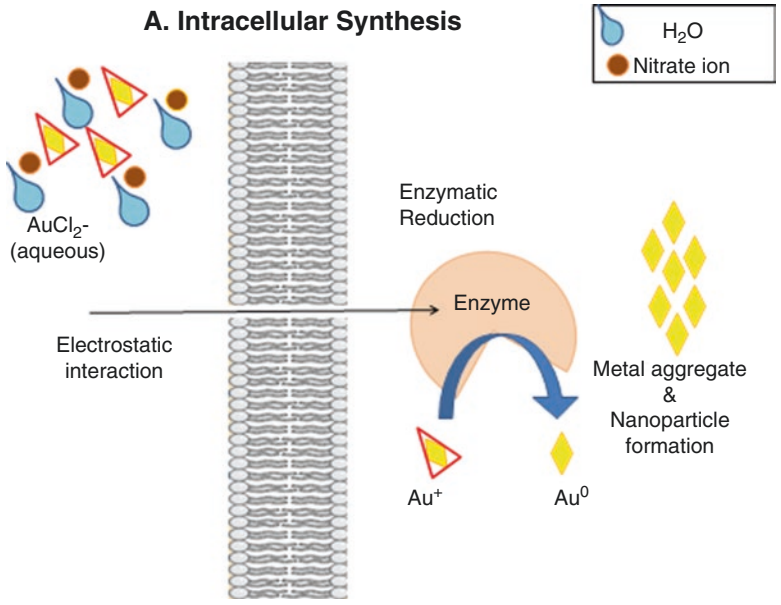

Fig. 12.4 A mechanism of intracellular synthesis of nanoparticles through fungi (Sarkar et al. 2010)

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. 2001a, b). 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.

12.4 Production of Myconanoparticles

The mycosynthesis of nanoparticles involves a series of steps that have been described in brief below:

12.4.1 Techniques of Isolation and Screening of Fungi Synthesizing Nanoparticles

12.4.1.1 Microbial Cultures Externally Synthesizing Nanoparticles

Different microbial sources are collected, milled, suspended in sterile normal saline, and subjected to screening process. Prepared Sabouraud dextrose agar (SDA)/potato dextrose agar (PDA) culture medium is supplemented with solution of metal ion (10 g/l) to final concentration equal to 100 mg/l of metal ion. One ml of the suspensions prepared from the collected samples are ten times diluted and spread on the surface of the metal ion-supplemented SDA/PDA plates (100 mg/l). The plates are incubated aerobically at 30 °C, and after 1 week, all colored colonies observed on the ion-supplemented media plates are picked up and transferred to metal ion-free PD or SD broth medium.

In the next step, all broth media are further incubated for 7–10 days at 25–30 °C in an incubator (150 rpm). During incubation periods several samples (0.5 ml) are aseptically withdrawn from culture flasks, centrifuged at 4000 × g for 20 min, and added to 4.5 ml metal ion solution (100 mg/l). After 60 min, the formation of specific colored colloid is checked in all reaction vessels. Red is the color of generated elemental SeNPs while blue for copper, brown for silver, yellow for gold, etc., and thus serves as a provisional marker to identify a culture supernatant of an isolate capable to form metal NPs.

12.4.1.2 Isolation of Microbial Cultures Intracellularly Synthesizing Metal Nanoparticles

The culture is grown in suitable liquid media incubated on shaker at optimal temperature. After incubation the flask is kept at static condition to allow the biomass to settle following which the supernatant is discarded and water is added for washing the cells. The flask is kept steady for 30 min to settle the biomass, post which the supernatant is again discarded. The biomass is then separated from the sterile distilled water by centrifugation for 10 min. The wet biomass is exposed to 50 ml of sterilized aqueous solution of metals at various dilutions and incubated on shaker at suitable temperature till visual color change is observed.

12.4.2 Identification of the Microbial Isolate

The identification of the isolate is carried out by 28S ribosomal deoxyribonucleic acid (rDNA) sequence analysis. Genomic DNA is obtained from fungal cells harvested from 72 h culture mediums and washed three times with sterile distilled water. The cells are disrupted by grinding with liquid nitrogen and the slurry subjected to phenol-chloroform DNA extraction procedure. DNA materials obtained are subjected to 28S rDNA polymerase chain reaction amplification for automated sequencing using the primers. Sequence similarity searches are done with the BLAST database (National Center for Biotechnology Information) and the sequence submitted to NCBI GenBank Nucleotide Sequence Database (accession number KC145152).

12.4.3 Culture Techniques and Optimal Conditions for Mycosynthesis of Nanoparticles

Biological synthesis approaches are novel routes for the production of nanoparticles and development of natural factories. Culture techniques and media vary depending upon the requirements of the fungal isolate involved; however the general procedure is more or less the same. Most of the important features of process conditions for synthesis of nanoparticles are constantly being searched by the researchers to get nanoparticles of desirable shape and size. The following parameters play an important role in biosynthesis of nanoparticles.

12.4.3.1 Sources for Production of Nanoparticles

For the synthesis of nanoparticles, it is important to select the best source on the basis of their intrinsic properties such as growth rate, enzyme production, and metabolic pathways which must be taken into account. Some of the microorganisms like bacteria, viruses, fungi, yeasts, and algae are known for the biosynthesis of metallic nanoparticles (Prasad et al. 2016).

12.4.3.2 Biomolecules Responsible for Biosynthesis

Biomolecules such as enzymes and proteins act as reducing and stabilizing agent for the biosynthesis of nanoparticles. Whole cells of microorganisms, crude enzymes which can be either in crude form or in purified form obtained from the microorganisms, are more often utilized. Nanoparticles synthesized mainly involve bioreduction process which also needs some coenzymes such as NADH, NADPH, FAD, etc. It is found that nanoparticle synthesis with the help of whole cell of fungi is much cheaper as compared to purified enzymes from the same fungus strain.

12.4.3.3 Optimal Reaction Conditions

Harvesting of microbial biomass is an important process subsequent to biosynthesis of nanoparticles. To avoid complications during synthesis process, it is important to avoid unwanted residual nutrients and metabolites. At industrial level, scale-up of production rate and yield is more important and needs to be optimized (including their exposure time, pH, temperature, etc.). Optimization of these factors can aid in controlling morphology and properties of desirable nanoparticles. Hence nowadays researchers have focused their attention to find optimal reaction conditions and actual mechanics that have been involved in the bioreduction process.

12.4.3.4 Favorable Conditions for Inoculum Growth

Growth conditions of microorganisms are important criteria while synthesizing nanoparticles. Enzyme production and growth of inoculum are the factors. So, the nutrients, pH, temperature, etc., should be optimized. Likewise when we are using whole cells and crude enzymes, harvesting time is also an important parameter so that it is necessary to monitor the enzyme activities during the time course of inoculum growth.

12.4.4 Factors Affecting Biosynthesis of Metal Nanoparticles

Major parameters affect the synthesis of nanoparticles including their size, shape, and monodispersity. Each of these depends on the physical and chemical parameters that mainly include the temperature, pH, presence of specific enzymes, type of biomass, exposure time to substrate and the substrate concentration, etc.

12.4.4.1 pH

pH is an important factor having efficient effect on the synthesis of metal nanoparticles. Gericke and Pinches (2006) carried out a research in which they demonstrated the change in shape of nanoparticles with variation in pH. They also found that *Verticillium luteoalbum* synthesizes nanoparticles of spherical shapes of size (<10.0 nm) at pH 3, but when pH is increased to 5, the shape obtained are hexagonal, triangular, and rodlike. Further increases in pH, that is, 7–9 nanoparticles with irregular and undefined shapes, were obtained. Similar work was carried out by Sanghi and Verma (2009); they studied the effect of pH on the fabrication of nanoparticles synthesized by *Coriolus versicolor*. The obtained results also suggested that reduction of metal ions was highly sensitive to pH.

12.4.4.2 Temperature

Temperature is an important physical parameter that also influences the synthesis of metallic nanoparticles. Dhillon et al. (2012) reported that the movement of ions and activity of microbial biomass were controlled by variation in temperature. It could be also suggested that temperature plays an important role on the growth of fungus as well as on metal uptake by the surrounding environment. Gericke and Pinches (2006) revealed that the formation of nanoparticles was directly affected by temperature. They found that at low temperature variety of nanoparticles formed after 1 h exposure to gold metal ion solution of spherical shapes. An increase in incubation time up to 24 h is likely to decrease in the number of smaller nanoparticles, whereas the large particles show well-defined shapes of bigger sizes. This is due to the segregation of smaller nanoparticles at high temperature. When temperature is increased up to 50 °C, there will be no difference observed in the shape and size of nanoparticles synthesized after 1 and 24 h exposure to metal ion solution. Further study on the effect of temperature on biosynthesis of metal nanoparticles suggested that the size of nanoparticles can be controlled by operating the reaction mixture at low temperature; however it would allow formation of nanoparticles at slower rate (Punjabi et al. 2015).

12.4.4.3 Concentration of Metal Ions

The concentration of metal ions also influences the synthesis of metal nanoparticles. Earlier reports have been suggested that the high concentration of fungus *Penicillium fellutanum* would inhibit the synthesis of nanoparticles. Kathiresan et al. (2009) demonstrated that at high concentration of silver ions, the size, shape, and monodispersity of the nanoparticles vary from the desired nano-size. As in the chemical reactions, the concentration of reactants decides the rate of reaction and also affects the size and shape of the synthesized particles. According to the study carried out by Gericke and Pinches (2006), they were synthesizing gold nanoparticles using *Verticillium luteoalbum*. The obtained results also suggested that when the concentration of $AuCl^{4-}$ was below 500 mg/L, the size of the gold nanoparticles was slightly narrow and uniform size ranges (>20 nm). The size of the synthesized nanoparticles increases with increase in the concentration $AuCl^{4-}$. In addition to this at high concentration of metal ions, the aggregation of smaller particles occursokay.

12.4.4.4 Exposure Time to Substrate

Synthesis of nanoparticles at different time intervals and their influence on synthesis process were also studied. It was found that as the incubation time increases, the shape and size of nanoparticles also vary with variation in the incubation period of reaction mixture. It has been also found that with increase in the incubation time, the synthesis of nanoparticles also increases. In one of the study, Gade et al. (2010) conducted a study in which they found that the majority of nanoparticles are synthesized after 1 h incubation to metal-containing solution. When incubation time increases up to 24 h, it shows the synthesis of nanoparticles of bigger sizes, and this is due to the clump formation or segregation of smaller-size nanoparticles. It has been also seen that when time increases up to 2–4 days, the synthesized nanoparticles show the alteration in their actual shape and size.

12.4.4.5 Type of Enzyme Used

The use of different types of enzymes for the bioreduction of metal ions into metal nanoparticles is greatly influenced by the type of enzymes secreted by the microorganisms. The main advantage to use these enzymes for the biosynthesis purpose is for the in vitro synthesis of nanoparticles by using the fungal mycelia; it also eliminates the need of optimization and harvesting process during the intracellular synthesis of nanoparticles. In case of fungi, it has been found that the enzymes secreted by them are also used for the synthesis of different metallic nanoparticles of various chemical composition, shapes, and sizes. In a study which was carried out by Ahmed et al. (Shakibaie et al. 2010), they found that the synthesis of silver nanoparticles does not take place in the presence of *Fusarium moniliforme* but it synthesizes when the reaction mixture is containing *Fusarium oxysporum* strain. The detailed

study of these two fungal metabolites and their protein assay suggested that the specific reductase enzyme (NADH-dependent reductase) was only produced by the fungal strain of *F. oxysporum*, while the other strain *F. moniliforme* does not produce this specific reductase enzyme. Obtained results also indicate that the synthesis of metal nanoparticles through *F. oxysporum* is due to the presence of specific nitrate reductase enzymes present in the cytoplasm or cell membrane. In another study Bansal et al. (2005) reported that *F. oxysporum* also synthesizes silica nanoparticles. The fungus *F. oxysporum* bioleached the silicates into silicic acid present in the zircon sand and then finally into silica nanoparticles.

12.4.5 Optimization

Vast emerging applications of nanoparticles (NPs) in distinct fields have led to an impending increase in demand for NPs. This ultimately leads to an enhanced demand, hence necessitating increased yield, for which optimization of the process is essential. The synthesis of NPs at nanoscale is still a challenge. Optimization of conditions during microbial culture production and post-induction becomes imperative in order to increase the shelf life of NPs with minimum investment. Statistical tools may also be employed for determining the optimal conditions.

12.4.5.1 Optimization of Process Conditions Pertaining to Culture Media is Carried Out for the Optimal Fungal Growth and Nanosynthesis

Different media compositions, namely, malt glucose yeast peptone broth, potato dextrose broth, protease production media, lipase assay medium, sucrose peptone yeast broth, and Sabouraud broth, have varied effects on the yield and quality of nanoparticles synthesized. Hence all the media are screened for the optimum and stable nanoparticle synthesis. The cell filtrate is used for protein estimation and synthesis of NPs.

It is well known that in different culture media conditions and compositions, microbial cell responds differently and secretes different metabolites and different kinds of proteins. Variation in pH and temperature on the nanoparticle synthesis is also significant. Also, it is known that the biological syntheses of several nanoparticles are enzyme-catalyzed reaction (Xie et al. 2007). More often enzymes, i.e., proteins, have multiple effects on the dispersion, including potential screening of the surface charges that helps to maintain the repulsion between the particles and bridging-type interactions.

OH^- ions are nucleophiles which play crucial role in maintaining the stability of certain nanoparticles by adsorbing on it and in synthesis of smaller size particles by providing electrons for reduction in metal ions. More nucleation regions are formed due to the availability of OH^- ions which helps in preventing the aggregates that are

formed through adsorbing on nanocrystals and maintains the smaller size (Gurunathan et al. 2009). At alkaline pH silver nanoparticles are stable and aggregates formed at lower pH. It indicates that, by controlling the pH of certain nanoparticle synthesis, it is easy to control the size.

It is hypothesized that the proton concentration affects conformational changes in the nitrate reducing enzymes present in the fungal filtrate, which may alter the morphology and size of the AgNPs. When the condition of the nanoparticle fabrication is alkaline, the synthesis will be faster than in acidic conditions.

With increase in temperature, the kinetic energy of the nanoparticles in the solution also increases; as a result, the collision frequency between the particles also rises, and this leads to the higher rate of agglomeration. This is because the surface potential of nanoparticles is inversely proportional to temperature which leads to the formation of aggregates as the particle demonstrates growth rate over a range of ionic strengths. Reaction temperatures cause variation in the interaction of particles via electrostatic and van der Waals forces.

Likewise the exact mechanism of light-mediated synthesis is not established. There may be photosensitization of aromatic amino acids (photosensitizer) of filtrate protein that may absorb the light energy and transfer to the reactants, while itself doesn't undergo any changes. In case of high-intensity light, heat generates, which may also accelerate the rate of synthesis of SNPs or photolysis. Cultural (culture medium, quantity of biomass, filtrate volume, and salt concentration) and physical conditions (pH, temperature, and light intensity) also are found to affect the maximum yield, rate of synthesis, and size of nanoparticles. Thus optimization of the process parameters ultimately leads to the rapid and large-scale production of NPs at industrial level.

12.4.5.2 Optimization of Post-induction Conditions, i.e., pH and Temperature, is Determined for Enhancing the Shelf Life

For large-scale synthesis and stability of fungal synthesized nanoparticles, studies have been conducted by suspending the fungal biomass in distilled water having different pH maintained by buffering system. Effect of temperature on the rate of synthesis of SNPs is studied by transferring fungal biomass into distilled water and incubated at its optimum temperature overnight, and later they are exposed to different temperatures.

12.5 Factors Controlling the Size and Shape of Biologically Synthesized Metallic Nanoparticles

The shape and size of nanoparticles depend on the volume of fungal biomass extract used (Philip 2009):

(i) Different shapes for the NPs can be obtained by varying the content of the extract in the reaction medium. In case of gold nanoparticle synthesis, lower

extract contents yield spheres and triangles, while higher extract contents yield flower-like NPs.

(ii) By tuning the experimental parameters: like the reaction time, the concentration of $HAuCl_4$, and the pH (Das et al. 2010). Interestingly, it is possible to favor one shape at the expense of another by just a slight tuning of the above-mentioned parameters.

(iii) Nature of the biomass used and on the species for a given microorganism. There is a variety of oxide- and carbonate-based NP biosynthesis such as those made, for instance, of zinc oxide (ZnO) (Azizi et al. 2014), copper oxide, magnetite (Fe_3O_4) (Mahdavi et al. 2013), silica (SiO_2) and titania (TiO_2) (Bansal et al. 2005), zirconia (ZrO_2) (Bansal et al. 2005), and calcium carbonate ($CaCO_3$) (Li et al. 2011). From a chemical composition point of view, the biosynthesis of iron oxide is more diverse compared to the previously mentioned oxides (Bharde et al. 2006).

(iv) By challenging the biomass with various conditions: the biosynthesis of quantum dots (QDs) of CdTe can be achieved by fungi. It is possible to tailor the optical properties of CdSe QDs, produced by challenging the biomass of the yeast *Saccharomyces cerevisiae* by aqueous solutions of $NaSe_2O_2$ and $CdCl_2$, through the screening of the following experimental parameters: time of addition, concentration and inoculating duration of $NaSe_2O_2$, and concentration and inoculating duration of $CdCl_2$. For instance, by varying the latter-inoculating duration of $CdCl_2$ from 14 to 44 h, the color spans from green to red, yellow being the one of the sample obtained at the in-between inoculating duration. Similar studies have been reported by several coworkers (Mao et al. 2006).

12.6 Recovery Methods

12.6.1 Extracellular

Fermented broth is incubated at optimal temperature until precipitate is observed at the bottom. After removal of the biomass, the solution is then centrifuged at 10,000 rpm for 20–30 min in non-refrigerated centrifuge. Supernatant is discarded and pellet composed of the nanoparticles is collected and dried.

12.6.2 Intracellular

Fermented broth incubated at optimal temperature is taken and the biomass is separated. Biomass is then subjected to cell disruption techniques, and the requisite separation techniques are employed for the recovery of nanoparticles.

12.6.3 Separation Techniques

Several separation techniques can be used for the nanoparticles (da Silva et al. 2011; Farre et al. 2011; Pycke et al. 2011; Bandyopadhyay et al. 2012). Techniques, including capillary electrophoresis (CE), chromatography, and field-flow fractionation (FFF), among others (Magnuson et al. 2011), are employed for separation of nanoparticles. The use of high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography, and CE with FFF has been reported as efficient separation techniques for nanomaterials from several kinds of samples (Magnuson et al. (2011)).

12.6.3.1 Chromatography

In chromatography, compounds can be separated based on their charge (weak/strong cation or ion-exchange chromatography [IEC]), molecular mass (size-exclusion chromatography[SEC]), hydrophobicity/polarity (reversed-phase HPLC, hydrophobic interaction chromatography), and specific characteristics (affinity chromatography), depending on the type of materials in the stationary phase (Williams et al. 2002; Lead and Wilkinson 2006; Tiede et al. 2008). Luykx et al. (2008) reported the use of SEC and IEC to measure nanomaterials in several different matrices.

HPLC allows the separation of several different types of components (Luykx et al. 2008; Magnuson et al. 2011). Hydrodynamic chromatography is also a very efficient technique to separate NPs in samples based on their hydrodynamic radius (Tiede et al. 2008).

12.6.3.2 Field-Flow Fractionation (FFF)

FFF is a technique similar to HPLC and can be used to separate nanomaterials based on thermal or hydraulic gradients, electrical forces, and sedimentation (Hassellov et al. 2008; Luykx et al. 2008; Bolea et al. 2010). The general principles of the FFF technique are described in details (Schimpf et al. 2000). It is a flexible elution technique where simultaneous separation and measurement can be done across a broad macromolecular colloidal particulate, ranging from about 1 nm to more than 100 nm (Giddings 1993). A major advantage of this method is the lack of a stationary phase, thus restraining the interaction between the sample and the equipment surfaces (Giddings 1993; Schimpf et al. 2000). FFF can be coupled with fluorescence, MS, and light-scattering techniques for the quantitative detection of nanomaterials in complex systems (Hassellov et al. 2008).

Sedimentation FFF (SdFFF) is suitable for separation and characterization of emulsions. It is an elution-based analytical technique, which provides higher solution separation of nanomaterials in gentle, low-shear conditions.

Asymmetric flow FFF (AF4) is another technique for nanomaterial characterization. Bouby et al. (2004) reported the characterization of Fe_3O_4/hydroxide colloids by using a combined AF4 and laser-induced breakdown technique with trace detection limit of 1 mg/L. This combination can be ideal for measuring NMs in samples.

12.7 Techniques for Characterization of Nanoparticles

The availability of advanced instrumentation techniques for the characterization of metal nanoparticles has proven to be very useful for gaining an insight into various morphological and structural features. The stability of the particles can also be ascertained by these techniques. Characterization is an important step for the identification of the nanoparticles by their size, shape, chemical composition, surface area, and dispersity. Thorough characterization studies of the nanoparticles aid in lucid understanding and control of the synthesis and applications of the nanoparticles. Different techniques available for the characterization of nanomaterials have been represented in the Fig. 12.5. The methods may be:

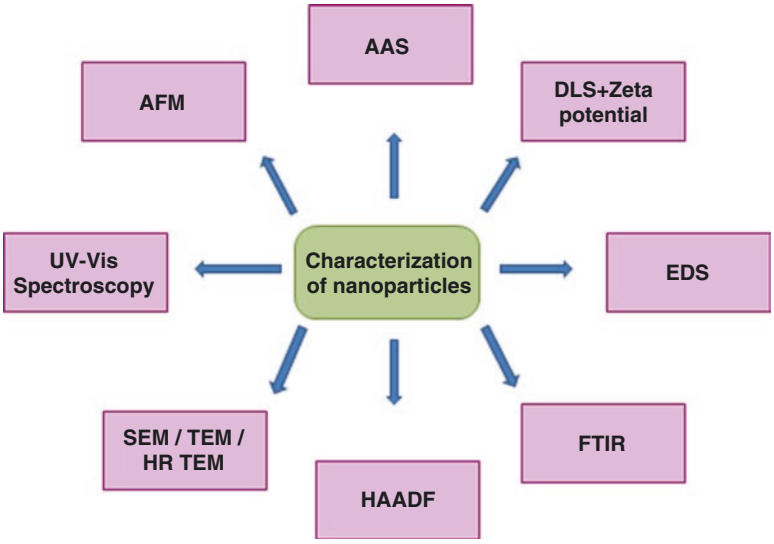

Fig. 12.5 Techniques for characterization of nanoparticles

12.7.1 For Determination of the Size, Shape, and Conformity of the Nanoparticles Synthesized

12.7.1.1 X-ray Diffraction (XRD)

X-ray diffraction data provides information about crystallinity, crystallite size, orientation of the crystallites, and phase composition and aids in molecular modeling to determine the structure of the material (Joshi et al. 2008). XRD is mainly used for the crystal analysis and phase identification of the synthesized nanomaterials. It also determines the overall oxidation state of the particle as a function of time (Hergt and Dutz 2007).

Advantages: Simplicity of sample preparation, rapidity of measurement, analyzes mixed phases, and determines sample purity.

Limitations: Requirement of homogenous and powdered material, peak overlays lead to unclear data.

12.7.1.2 Electron Microscopy

Electron microscopy is used for the surface and morphology characterization of the molecule. Similarly scanning electron microscope and transmission electron microscope are also used for the morphological characterization at nanometer to micrometer scale.

12.7.1.3 Scanning Electron Microscope (SEM)

The SEM images the sample surface by scanning it with a high-energy beam of electrons. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, signals in the form of secondary electrons, backscattered electrons, and characteristic X-rays are generated that contain information about sample's surface topography, composition, etc. (Joshi et al. 2008).

Advantages: SEM is its two-dimensional imaging, ease of sample preparation, and provision of digital data forms.

Limitations: Improper sample preparation can lead to confusion between artifacts and actual data. Obvious limitations are the size, cost, and maintenance.

12.7.1.4 Transmission Electron Microscope (TEM)

In TEM the crystalline sample interacts with electron beam mostly by diffraction rather than by absorption. The intensity of diffraction depends on orientation of planes of atoms in a crystal. This produces a variation in the electron intensity that reveals information of the crystal structure. Along with distribution and dispersion,

exfoliation, intercalation, and orientation of nanoparticles can also be visualized using a TEM micrograph (Joshi et al. 2008).

It is found that the TEM has much higher resolution as compared to the SEM. SEM gives the information of the morphological characteristics of the molecules at submicron level and the elemental information at the micron level. Due to the high resolution, TEM is widely used for the identification of the exact shape and size of the nanoparticles. Advantages of SEM and TEM include giving two-dimensional imaging, easy to sample preparation, and data in digital forms.

Advantages: High-quality, detailed, and powerful magnification of element and compound structures.

Limitations: Laborious sample preparation, artifacts from sample preparation, and definitely large and expensive.

12.7.1.5 High-Resolution Transmission Electron Microscope (HRTEM)

It is an imaging mode of TEM that allows imaging of crystallographic structure of samples at an atomic scale. In HRTEM electron wave after interacting with sample undergoes phase change and interacts with image wave in the imaging plane. Thus, individual atoms and crystalline defects can be imaged clearly using HRTEM (Joshi et al. 2008).

12.7.1.6 Atomic Force Microscopy (AFM)

AFM is ideally used for the qualitative estimation of surface roughness and also gives the complete visualization of the surface of synthesized nanoparticles. It gives very high three-dimensional spatial imaging resolution of the synthesized nanomaterials. The surface of the sample is scanned using a probe, and the oscillation amplitude is used to measure the surface characteristics of the sample (Mukherjee et al. 2001a, b; Joshi et al. 2008).

Advantages: AFM provides higher resolution than SEM. It gives true atomic resolution compared with scanning tunneling microscopy and transmission electron microscopy.

Limitations: AFM cannot scan images as fast as SEM and image artifacts, and it gives a single-scan image size. All these technologies are very reliable and useful for the complete analysis and detailed characterization of synthesized nanoparticles.

12.7.1.7 Zeta Potential Measurement

It is used for the analysis of the stability of the synthesized nanoparticles. The value of zeta potential is as high as the nanoparticles are more stabilized.

12.7.1.8 Dynamic Light Scattering (DLS)

It is mainly used for the qualitative detection of nanoparticles and also characterizes the surface charge and size of the nanoparticles. It is a well-established technique for measuring the size of molecules and particles. The fluctuation in the intensity of the scattered light from laser-illuminated particles is size dependent, and hence, the size of particles can be analyzed. Thus, the size and size distribution of particles can be studied by DLS (Joshi et al. 2008). With the help of this, we can also analyze the polydispersity index of the synthesized nanoparticles (Mukherjee et al. 2002).

Advantages: Offers measurement of particle sizes of 1 nm, precision of ±1%, repeatable analysis, no sample preparation, and liquid sample.

Limitations: Offers low resolution of polydisperse samples and multiple light scattering.

12.7.2 For Functional Group Identification of Synthesized Nanoparticles

UV-visible spectroscopic analysis, EDX analysis, and FTIR analysis techniques are different techniques employed for the evaluation of nanoparticles.

12.7.2.1 UV-Visible Spectrophotometer

Detection of the formation and presence of nanoparticles in the fungal medium can be done using UV-vis spectroscopy. It is also used for the identification and characterization of metallic nanoparticles. This is the most widely applicable technique because of its simplicity and reliability. Two hundred to eight hundred nanometer light wavelength is generally used for the identification of nanomaterials of size ranges 2–100 nm. It is well known that, for monodispersed NPs, only one plasma band is obtained. The increase in its intensity is an indication of the advanced degree of reaction with increase in the number of particles. Metal nanoparticles scatter optical light because of collective resonance of the conduction electrons in the metal known as surface plasmon resonance (SPR). This SPR peak is shown in UV absorption spectra by these nanoparticles. The magnitude of peak, wavelength, and spectral bandwidth associated with nanoparticles are dependent on size, shape, and material composition (Joshi et al. 2008).

Advantages: UV-vis spectroscopy is a rapid means of analysis. It provides very high precision and accuracy. It is useful for a wide variety of chemicals and can be used both quantitatively and qualitatively.

Limitation: It is nonselective for compounds that absorb at the same wavelength (Waghmare et al. 2011).

12.7.2.2 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR gives data of proteins and other compounds present in the mixture that interact with metal ions. The identification of functional groups leads to determination of the reducing agent and the capping agent responsible for synthesis and stability of nanoparticles (Jeevan et al. 2012). FTIR is mainly used for the detection of the organic functional groups attached to the surface of nanoparticles. It is also useful for the analysis of surface chemistry of the synthesized nanomaterials.

Advantages: Identifying and detecting changes in protein secondary structures, can resolve between similar components.
Limitations: Overlapping peaks make it difficult to distinguish, difficult to quantify, better results with solid components.

12.7.2.3 Energy-Dispersive Spectroscopy (EDS)

EDS is used for the analysis of elemental composition of the metal nanoparticles. It gives the complete information regarding elemental knowledge of the nanomaterials. This technique is used in conjugation with SEM. The characteristic X-rays are used to identify composition of sample by a technique known as energy-dispersive X-ray (EDX), thus giving an overall mapping of sample (Joshi et al. 2008).

Advantages: It improves quality control and helps in process optimization and identification of contaminant and gives higher production yield.
Limitations: Quantitative analysis requires standards of known composition and that fluorescence of emitted x-rays limits the precision.

12.8 Applications of Mycosynthesis of Nanoparticles in Food Processing Industries

The developing field of nanotechnology has opened up new avenues of progress in various technologies. Nanoparticles or nanomaterials are finding their application in different areas like polymer, paints, nutraceuticals, pharmaceuticals, cosmetics, food and beverage, agriculture, surface coatings, etc. (Bhattacharya and Gupta 2005; Ingale and Chaudhari 2013). Nanoparticles' multifaceted application is due to their unusual physicochemical and optico-electrical properties. These special properties arise due to confinement of electrons in particles of smaller dimensions compared to bulk electron delocalization, which is known as quantum confinement (Gade et al. 2010). Same properties have been also reported in nanoparticles derived from biological entities like bacteria, fungus, yeast, and extracts of microbes and plants (Prasad et al. 2016; Prasad 2014).

Metal nanoparticles are reported to have antimicrobial activity against many pathogenic organisms. Silver nanoparticles are found to be the forerunner in this aspect with wide antimicrobial activity against methicillin-resistant *Staphylococcus*

aureus, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Acinetobacter baumannii*, and *Proteus vulgaris*. Impregnating silver nanoparticles in textile fabrics and wound dressings has been successful in controlling infection by pathogens (Rai et al. 2009a, b; Aziz et al. 2016). In similar line of research reported, active role of silver nanoparticles externally synthesized using fungus, in increasing antifungal activity of fluconazole and susceptibility of multidrug-resistant bacteria against antibiotics, respectively (Gajbhiye et al. 2009; Gopinath et al. 2015; Aziz et al. (2016). Growth of many microbes like *E. coli*, *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* has been reported to be inhibited by copper nanoparticles as well (Gade et al. 2010; Yadav et al. 2017). Apart from antibacterial textiles, ultrahydrophobic fabrics, suits against biohazards, face masks, etc., have been developed using silver nanoparticles (Ramaratnam et al. 2008; Aziz et al. 2015). Nanoparticle-based delivery systems for drugs and genes prove better than their conventional counterpart. Metal nanoparticles as drug delivery system showed high stability, carrier capacity, and variable route of administration (Gelperina et al. 2005).

Gold nanoparticles with unique optical properties are promising candidates for development of sensors. Nanoparticles based on iron oxides have been used in different imaging techniques for in vivo diagnostic studies (Glomm 2005; Gao et al. 2008). For solar energy applications, magnetotactic bacteria which produced magnetite and greigite are used in optical coatings (Joerger et al. 1999).

As bioremediation tools also, nanoparticles can be used in wastewater treatment (Das et al. 2009). Potent activity of the S-layer of *Bacillus sphaericus* JG-A12 has been reported in bioremediation of radioactive wastes (Duran et al. 2007). Application of nanoparticles in food can be found at different stages, involving processing, packaging, and safety. Even at the agricultural level, nanoparticles are used in form of nanopesticides and nanoherbicides (Prasad et al. 2014, 2017). They are also being used as nanosupplements and nano-delivery systems to enhance nutritional aspects.

12.8.1 Food Processing

Food processing involves conversion of raw materials into consumable and marketable form having longer shelf life. Preservation, toxin and pathogen removal, and nutritional value as well as shelf-life improvement are some of the targeted aspects covered in food processing. Antimicrobial activity of nanoparticles is employed for disinfection process. Silver-based nanoparticles or nanocomposites like silver-containing zeolites have been approved by US FDA to be used as food contact disinfectant. Subsequent formation of reactive oxygen species (ROS) causes oxidative stress and subsequent cell damage (He and Hwang 2016).

On contrary less reactive nanomaterials have been developed to act as antioxidants. SiO_2-gallic acid nanoparticles show scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radicals (Deligiannakis et al. 2012). Such antioxidant-based nanoparticles used as edible coating have been reported to control browning of

fresh-cut fruits. One such example is ZnO-coated active packaging which improved shelf life of "Fuji" apples (Li et al. 2011).

Development of nanocoating as carrier of functional ingredients such as antioxidants, enzymes, flavors, and anti-browning agents has been done employing chitosan-based nano-silver or silver zeolite-incorporated edible film (Berekaa 2015). Immobilization of porcine triacylglycerol lipase on nanoscale SiO_2 improved the hydrolytic efficiency of olive oil as well as enzyme stability (Bai et al. 2006). Thus, nanoparticles can be successfully used for enzyme immobilization. Several inorganic nanoparticles based on silver, iron, calcium, magnesium, selenium, and silica have been used as preservatives and additives to improve taste and flavor of food. TiO_2 and SiO_2 have been approved by FDA as food color additives (He and Hwang 2016).

Yet another application could be on microbe-based food processing like fermentation, as many nanoparticles were found to enhance the microbial reaction rates (Zhang et al. 2011).

12.8.2 Food Packaging

Food packaging is one of the earliest commercial applications of nanotechnology. This helps to preserve the food along with making it marketable. Nanocomposites designed based on nanoclays, carbon nanoparticles, nano-metals, and oxides can improve various properties like mechanical strength, heat resistance, and barrier against ultraviolet radiation, oxygen, carbon dioxide, moisture, etc., of food package materials. Various edible nanocoatings have been developed with better moisture, lipid, and gas barrier properties that could be used for coating of fruits, vegetables, chocolate, bakery items, meats, etc. (Wesley et al. 2014).

Silver, copper, titanium oxide, and carbon nanotube-based packaging can provide antibacterial property and prevent the growth of pathogens. Chitosan-based nanocomposite films of silver showed potential antimicrobial effect (Rhim et al. 2006).

Another mode of active packaging employs entrapping enzyme in between polymer films which act as oxygen scavenger. Further, nanotechnology-based release of preservatives present in food package is being designed by researchers in the Netherlands (Sekhon 2010). The use of carbon nanotubes in packaging to pump out carbon dioxide or absorb undesirable flavors is also being developed (Sinha et al. 2006). Nano-based tracking technologies are being designed which often include an ingestible BioSilicon which could be placed in foods for monitoring purposes and pathogen detection (Wesley et al. 2014).

12.8.3 Food Safety

Another potential use of nanotechnology deals with development of nanosensors for detection of contaminant and pathogens in food system. Various gold, silver, silicon, magnetic, and carbon nanotube-based nanosensors have been developed to detect *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Mycobacterium avium*, etc. The

detection techniques based on optical or electrical properties employ voltammetry, spectroscopy, epifluorescence microscopy, and amperometry having a detection range of 15 CFU/ml to 1×10^9 CFU/ml (Inbaraj and Chen 2016). A disposable immune-sensing strip may be employed to detect the presence of *E. coli* O157:H7 in milk. These strips worked better in the presence of gold NPs imparting high sensitivity to the technique (Lin et al. 2008).

Microbial toxins can be also successfully detected using gold, zinc oxide, magnetic, and carbon nanotube-based nanosensors. Staphylococcal enterotoxin B, aflatoxin, and mycotoxin can be sensed using chemiluminescence (CL) immunosensors, immunoelectrode, and indium-tin-oxide glass plate detector (Sonawane et al. 2014). Tang et al. (2011) developed a sensitive electrochemical immunosensor by immobilizing BTX-BeBSA conjugate on GNP-decorated amine-terminated polyamidoamine dendrimers (GNPePAADs) for rapid screening of brevetoxin B (BTX-B) produced by *Karenia brevis* in food samples.

Different food contaminants like melamine, carbofuran, etc. can be detected using nanosensors based on colorimetric or electrochemical probes (Sonawane et al. 2014). Nanosensors can also track any adulteration in food, such as pork adulteration in meat products. Mostly gold NPs have been successfully used for this purpose. In this case the detection is based on color change following incubation of gold NP-DNA conjugates in the meat samples. Aggregation of gold NPs in the presence of pork DNA-containing sample leads to color change from red to purple gray, making it a simple detection tool (Ali et al. 2012).

12.9 Current Status and Recent Advancements of Mycosynthesis of Nanoparticles

The fungi are now known to be efficient tool for synthesis of nanoparticles by both intra- and extracellular methods. The fungal system has shown its compatibility over other groups of organisms as the handling of fungal biomass and its downstream processing is much simpler. A number of metallic nanoparticles including silver, gold, titanium, silica, zirconium, and platinum have been successfully synthesized using the fungal system. The fungal-derived nanoparticles have depicted a wide range of applications in different fields of science including medicines, pharmaceutical industry, agriculture, electronics, etc. But there are certain areas which need to be worked out before exploring the complete potential. An exact mechanism of synthesis of nanoparticles is yet to be discovered. Understanding the exact mechanism involved in the synthesis of nanoparticles and the effect of different factors on the reduction of metal ions will help in developing low-cost techniques for the synthesis and recovery of nanoparticles. Thus, sketching different practicalities and reducing agent involved in the synthesis of nanoparticles would help in understanding the fungal system as one of the most efficient systems for harnessing nanoparticles.

12.10 Conclusion

The last decade has witnessed tremendous developments in the field of microorganism-produced nanoparticles and their applications. However, more efforts are essential to improve the production efficiency and control particle size and morphology. The following are the key areas identified that need to be further addressed:

Reduction of synthesis time: Microbe-mediated synthesis of nanoparticles is quite a slow process (several hours and even a few days) in comparison to physical and chemical approaches. Minimization of synthesis time will make this biosynthesis route much more enticing.

Size and monodispersity: Evaluation of nanoparticle synthesis is determined by particle size and monodispersity.

Stability of nanoparticles: Degradation of microbially synthesized nanoparticles after a certain period of time has been reported. Thus, the stability of nanoparticles deserves further enhanced study (Xiang et al. 2007; Hergt et al. 2005; Hergt and Dutz 2007).

The control of particle shape in chemical and physical synthesis of nanoparticles is still an ongoing area of research. Hence, biological processes with the ability to strictly control particle morphology are perceived to be advantageous over the other processes. Variation of parameters, like microorganism type, growth stage (phase) of microbial cells, growth medium, synthesis conditions, pH, substrate concentrations, source compound of target nanoparticle, temperature, reaction time, and addition of nontarget ions, could be plausible strategies to obtain sufficient control of particle size and monodispersity. Biosynthesis methods are advantageous also because nanoparticles are sometimes coated with a lipid layer that confers physiological solubility and stability, which is critical for biomedical applications and is the bottleneck of other synthetic methods. Research is currently carried out manipulating cells at the genomic and proteomic levels. With a better understanding of the synthesis mechanism on a cellular and molecular level, including isolation and identification of the compounds responsible for the reduction of nanoparticles, it is expected that short reaction time and high synthesis efficiency can be obtained.

Biosynthesis of nanoparticles by microbes is thought to come under the purview of “green chemistry” procedures. Microbe employment for nanoparticle synthesis can be classified into intracellular and extracellular synthesis according to the location where they are formed. Rate of intracellular particle formation and control of the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as pH, temperature, substrate concentration, and exposure time to substrate. Research is currently executed by manipulating microorganisms at the genomic and proteomic levels. With the recent progress and the ongoing efforts in improving particle synthesis efficiency and exploring their biomedical applications, it is hopeful that the implementation of these approaches on a large scale and their commercial applications in medicine and healthcare are likely to open up more vistas in the coming years.

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