

Chapter 19

Synthesis of Metallic Nanoparticles by Halotolerant Fungi



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19.1 Introduction

Halotolerance is the adaptation of living organisms to conditions of high salinity. Halotolerant organisms tend to live in hypersaline environments, along with halophilic organisms. However, they are different from halophilic organisms in that they do not require elevated concentrations of salt to grow. Microbial diversity studies of hypersaline environments including saltern ponds worldwide (Gunde-Cimerman et al. 2000; Butinar et al. 2005; Diaz-Munoz and Montalvo-Rodriguez 2005; Cantrell et al. 2006; Nayak et al. 2012), the Great Salt Lake (Baxter et al. 2005), the Dead Sea (Buchalo et al. 1998; Kis-Papo et al. 2003a, b; Wasser et al. 2003; Bodaker et al. 2010; Nazareth et al. 2012), saline lakes in Inner Mongolia (Pagaling et al. 2009), African soda lakes (Jones and Grant 1999), deep-sea brines (van der Wielen et al. 2005), salt pans or salt marshes (Setati 2010), Mono Lake in California (Steiman et al. 2004), coastal environments of Arctics (Gunde-Cimerman et al. 2005), saline soils of Soos in the Czech Republic (Hujšlova et al. 2010), and many others have led to the isolation of halophiles and halotolerant microorganisms.

Fungi hypersaline environments are mostly halotolerant (Gunde-Cimerman et al. 2009; Zajc et al. 2012) rather than halophilic. Thus, they constitute a relatively large and constant part of hypersaline environment communities. Well-studied examples include the yeast *Debaryomyces hansenii* and black yeasts *Aureobasidium pullulans* (Gunde-Cimerman et al. 2009). Surprisingly, more and more cases are being reported of the isolation of halophilic and halotolerant microorganisms from low-salinity environments (Abdel-Hafez et al. 1978; Tiquia et al. 2007; Gunde-Cimerman et al. 2009; Tiquia 2010; Gonsalves et al. 2012). Halophilic and halotolerant fungi

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are often found in unexpected environments such as domestic dishwashers and polar ice, and even on spider webs in desert caves (Gunde-Cimerman et al. 2009).

Halotolerant fungi represent a versatile reservoir of bioactive metabolites and potential source of halotolerant genes that could be used in biotechnology (Tiquia 2010; Tiquia and Mormile 2010). They are a valuable resource of enzymes and other metabolites with stability in harsh conditions of pH, temperature, and/or ionic strength (Margesin and Schinner 2001; Oren 2010). Hence, their use as biocatalysts in the presence of novel nanomaterials is attractive. Combining these bioactive molecules with various nanomaterials like thin layers, nanotubes, and nanospheres results in novel compounds harboring both biological properties of biomolecules and physicochemical characteristics of nanomaterials. While the biosynthesis of nanoparticles in bacteria is well understood (Rai et al. 2012), very few fungal (halotolerant) species have been investigated so far for nanoparticle biosynthesis. In general, the microbiology of fungi is much less investigated, mainly because fungi are difficult to characterize, as their structure complicates the microscopic and mechanistic studies that are required for nanoparticle characterization in it. However, fungi may have some advantages over bacteria for bioprocess, including nanoparticle biosynthesis. Fungi also harbor untapped biological diversity and may provide novel metal reductases for metal detoxification and bioreduction. This review focuses on halotolerant fungi that have been exploited for nanomaterial synthesis; the mechanisms in the nanomaterial fabrication; and possible applications.

19.2 Synthesis of Nanoparticles by Halotolerant Fungi

Reports on nanoparticle synthesis by halotolerant fungi are mostly confined to metallic nanoparticles. These halotolerant fungi capable of synthesizing metallic nanoparticles include *Penicillium fellutatum* (Kathiresan et al. 2009); *Aspergillus niger* (Kathiresan et al. 2009); *Pichia capsulata* (Manivannan et al. 2010); *Yarrowia lipolytica* (Bankar et al. 2009; Pawar et al. 2012); *Rhodospiridium diobovatum* (Seshadri et al. 2011); *Schizosaccharomyces pombe* (Kowshik et al. 2002a, b); *Thraustochytrium* sp. (Asmathunisha and Kathiresan 2013); *Schwanniomyces occidentalis* (Mohite et al. 2016); and *Williopsis saturnus* (Mohite et al. 2017).

Metallic nanoparticles have fascinated scientists for over a century and are now heavily utilized in biomedical sciences and engineering. They are a focus of interest because of their huge potential in nanotechnology (Tiquia-Arashiro and Rodrigues 2016a). Metallic nanoparticles have possible applications in diverse areas such as electronics, cosmetics, coatings, packaging, and biotechnology (Tiquia-Arashiro and Rodrigues 2016b, c). For example, nanoparticles can be induced to merge into a solid at relatively lower temperatures, often without melting, leading to improved and easy-to-create coatings for electronics applications. Typically, nanoparticles possess a wavelength below the critical wavelength of light. This renders them transparent, a property that makes them very useful for applications in cosmetics, coatings, and packaging. Metallic nanoparticles can also be attached to single

strands of DNA nondestructively, which opens avenues for medical diagnostic applications (Tiquia-Arashiro and Rodrigues 2016c).

19.2.1 Synthesis of Gold (Au) Nanoparticles

Gold nanoparticles (AuNPs) have attracted attention in biotechnology due to their unique optical and electrical properties, high chemical and thermal ability, and good biocompatibility and potential applications in various life sciences-related applications including biosensing, bioimaging, and drug delivery for cancer diagnosis and therapy (Jiang et al. 2013). Covalently modified gold nanoparticles have attracted a great deal of interest as a drug delivery vehicle. Their predictable and reliable surface modification chemistry, usually through gold-thiol binding, makes the desired functionalization of nanoparticles quite possible and accurate. Recently, many advancements have been made in biomedical applications of AuNPs with better biocompatibility in disease diagnosis and therapeutics (Tiquia-Arashiro and Rodrigues 2016b). AuNPs can be prepared and conjugated with many functionalizing agents, such as polymers, surfactants, ligands, dendrimers, drugs, DNA, RNA, proteins, peptides, or oligonucleotides. Overall, AuNPs is a promising vehicle for drug delivery and therapies.

Yarrowia lipolytica, a halotolerant yeast (Gunde-Cimerman and Zalar 2014), is known to synthesize AuNPs (Apte et al. 2013a; Nair et al. 2013; Tiquia-Arashiro and Rodrigues 2016d). Melanin (a dark-colored pigment from this yeast) plays an important role in its ability to synthesize nanoparticles. Since the inherent content of melanin in *Y. lipolytica* is low, the yeast is induced to overproduce melanin by incubation with a precursor, L-3,4-dihydroxyphenylalanine (L-DOPA). This process mediates the rapid formation of AuNPs. The AuNPs display antibiofilm activity against pathogenic bacteria (Apte et al. 2013a). They also display effective antifungal properties (Apte et al. 2013b). In addition to AuNPs, some strains of *Y. lipolytica* (e.g., *Y. lipolytica* NCIM 3589) can also synthesize CdO and CdS nanostructures in a cell-associated and extracellular manner (Pawar et al. 2012).

19.2.2 Synthesis of Silver (Ag) Nanoparticles

Silver nanoparticles (AgNPs) are already being commercially used as antimicrobial agents. For example, silver NPs are currently found in surgically implanted catheters to reduce the infections caused during surgery, in toys, personal care products, and silverware. The reason for using silver for antimicrobial applications is because silver possesses antifungal, antibacterial, anti-inflammatory, and anticancer effects (Li et al. 2010, 2014; Chernousova and Epple 2013; Tiquia-Arashiro and Rodrigues 2016c; Zhang et al. 2016).

The halotolerant fungus *Penicillium fellutanum* (De Hoog et al. 2005) can produce AgNPs at a faster rate by extracellular means (Kathiresan et al. 2009). Silver nanoparticles are synthesized within 10 min of silver ions being exposed to the *P. fellutanum* culture filtrate. The increase in color intensity of culture filtrate corresponds to the increase in number of nanoparticles formed by reduction of silver ions (Wang et al. 2009). The AgNPs obtained from this assay has a good monodispersity, with maximum synthesis occurring at pH 6.0, temperature of 5 °C, 24 h of incubation time, and silver nitrate concentration of 1 mM and 0.3% NaCl. Most of the AgNPs generated by *P. fellutanum* are spherical in shape with size ranging from 5 to 25 nm. In this study, the enzyme nitrate reductase is secreted by the *P. fellutanum* biomass and is involved in the reduction of the silver ions (Wang et al. 2009).

Other halotolerant fungi capable of synthesizing AgNPs include *Pichia capsulata* (Manivannan et al. 2010), *Aspergillus niger* (Zomorodian et al. 2016), *Yarrowinia lipotyca* (Bankar et al. 2009), *Thraustochytrium* sp. (Asmathunisha and Kathiresan 2013), and *Schwanniomyces occidentalis* (Mohite et al. 2016).

19.2.3 Synthesis of Cadmium Sulfide (CdS) Nanoparticles

Cadmium sulfide (CdS) is a II–VI semiconductor which has been synthesized by microorganisms (Prasad and Jha 2010; Kowshik et al. 2002a). It is insoluble in water but soluble in dilute mineral acids. CdS has a bandgap energy of 2.42 eV (Zhang et al. 2007), at room temperature, and it shows great potential for uses in photochemical catalysis, solar cells, nonlinear optical materials, and various luminescence devices (Ma et al. 2007; Tiquia-Arashiro and Rodrigues 2016c). CdS nanocrystals have generated great interest due to their unique size-dependent chemical and physical properties (Zhao et al. 2006). Thus, extensive research has focused on the synthesis of various CdS nanostructures. In the classical studies by Dameron et al. (1989), it was shown that the halotolerant yeasts *Schizosaccharomyces pombe* can produce CdS nanocrystallites when challenged with cadmium in solution. Short chelating peptides of general structure (γ -Glu-Cys) $_n$ -Gly control the nucleation and growth of CdS crystallites to peptide-capped intracellular particles of diameter 20 Å. These quantum CdS crystallites are more monodisperse than CdS particles synthesized chemically. X-ray data indicate that, at this small size, the CdS structure differs from that of bulk CdS and tends towards a six-coordinate rock-salt structure (Dameron et al. 1989).

19.2.4 Synthesis of Lead Sulfide (PbS) Nanoparticles

Semiconductor PbS nanoparticles have attracted great attention in recent decades because of their interesting optical and electronic properties (Bai and Zhang 2009). *Rhodospiridium diobovatum* synthesizes PbS nanoparticles intracellularly with the help of nonprotein thiols (Seshadri et al. 2011). The nanoparticles are in the range

of 2–5 nm. Elemental analysis by energy dispersive X-ray (EDAX) reveals that the particles are composed of lead and sulfur in a 1:2 ratio, and that they are capped by a sulfur-rich peptide. Quantitative study of lead uptake through atomic absorption spectrometry reveals that 55% of lead in the medium accumulated in the exponential phase, whereas a further 35% accumulated in the stationary phase; thus, the overall recovery of PbS nanoparticles is 90%. The lead-exposed *R. diobovatum* displayed a marked increase (280% over the control) in nonprotein thiols in the stationary phase. A sulfur-rich peptide is suggested to be the capping agent. In the presence of lead, *R. diobovatum* produces increasing amount of nonprotein thiols during the stationary phase, which are possibly involved in forming the nanoparticles (Seshadri et al. 2011). *Schizosaccharomyces pombe* is another halotolerant fungus capable of synthesizing PbS nanoparticles (Kowshik et al. 2002b).

19.2.5 Synthesis of Zinc Oxide (ZnO) Nanoparticles

Zinc oxide (ZnO) NPs have unique optical and electrical properties. As a wide bandgap semiconductor, they have found more uses in biosensors, nanoelectronics, and solar cells. These NPs are being used in the cosmetic and sunscreen industry due to their transparency and ability to reflect, scatter, and absorb UV radiation and as food additives. Furthermore, zinc oxide NPs are also being considered for use in next-generation biological applications including antimicrobial agents, drug delivery, and bioimaging probes (Jayaseelan et al. 2012). The potential ability of the halotolerant fungi *Pichia kudriavzevii* (Cai et al. 2014) in the synthesis of zinc oxide nanoparticles (ZnO-NPs) was explored recently (Boroumand Moghaddam et al. 2017). The ZnO nanoparticles synthesized by *P. kudriavzevii* possess hexagonal wurtzite structure with an average crystallite size of ~10–61 nm. They are less toxic and displayed antioxidant and antibacterial activities and show strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical effect with a dose-dependent activity. The synthesized ZnO nanoparticles also displayed antibacterial activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli* and *Serratia marcescens*) bacteria.

19.3 Nanoparticle Formation Mechanisms in Fungi

The nanoparticles are produced either intracellularly or extracellularly. In case of intracellular synthesis, the nanoparticles are produced inside the cells by the reductive pathways. The synthesis occurs when the metal (e.g., Au³⁺) concentration available is high and when the membrane integrity is compromised to allow for metal ion diffusion within the cell. In extracellular synthesis, the nanoparticles are produced extracellularly when the cell wall reductive enzymes or soluble secreted

enzymes are extracted outside the cell and are involved in the reductive process of metal ions. The extracellular biosynthesis of metallic nanoparticles is similar to the enzymatic machinery required for metal detoxification. Overall, nanoparticle biosynthesis is essentially a reduction process followed by a stabilization step (capping). There is no evidence available yet showing that fungi use biosynthetic nanoparticles for their metabolism. The biosynthesis of metal NPs by fungi is a function of heavy metal toxicity resistance mechanisms, whereby toxic metals are converted to nontoxic species and precipitated as metal clusters of nanoscale dimension and defined shape (Narayanan and Sakthivel 2010). Resistance mechanisms include redox enzymes that convert toxic metal ions to inert forms, structural proteins that bind protein. It is proposed that such mechanism works to coordinate synthesis.

One of the enzymes involved in the biosynthesis of metal nanoparticles is the nitrate reductase which reduces the metal ions (Me^{1+}) to the metallic form (Me^0). This enzyme is a NADH- and NADPH-dependent enzyme. Some fungi are known to secrete cofactor NADH- and NADH-dependent enzymes that can be responsible for the biological reduction of Me^{1+} to Me^0 and the subsequent formation of nanoparticles. This reduction is initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier during which the metal ions gain electrons and are therefore reduced to Me^0 . Synthesis of silver nanoparticles using α -NADPH-dependent nitrate reductase and phytochelatin in vitro has been demonstrated by Anil Kumar et al. (2007). The silver ions are reduced in the presence of nitrate reductase, leading to the formation of a stable silver hydrosol 10–25 nm in diameter and stabilized by the capping peptide. Nitrate reductase is suggested to initiate nanoparticle formation by many fungi including *Penicillium* species, while several enzymes, α -NADPH-dependent reductases, nitrate-dependent reductases, and an extracellular shuttle quinone, are implicated in silver NP synthesis for *Fusarium oxysporum*. Jain et al. (2011) indicated that silver NP synthesis for *Aspergillus flavus* occurs initially by a 33 kDa protein followed by a protein (cystein and free amine groups) electrostatic attraction which stabilizes the nanoparticle by forming a capping agent (Soni and Prakash 2011). Several researchers supported nitrate reductase for extracellular synthesis of metallic NPs (Vigneshwaran et al. 2006; Wang et al. 2009; Deepa and Panda 2014; Siddiqi and Husen 2016; Boroumand Moghaddam et al. 2017).

Cadmium sulfide nanoparticle synthesis by yeast involves sequestration of Cd^{2+} by glutathione-related peptides followed by reduction within the cell. Ahmad et al. (2002) reported that cadmium sulfide nanoparticle synthesis by *Fusarium oxysporum* was based on a sulfate reductase (enzyme) process. The nanoparticle formation proceeds by release of sulfate reductase enzymes by *F. oxysporum*, conversion of sulfate ions to sulfide ions that subsequently react with aqueous Cd^{2+} ions to yield highly stable CdS nanoparticles. While the reduction of sulfate to sulfite is known in sulfate-reducing bacteria (which are strictly anaerobic), this is the first report on the secretion of sulfate-reducing enzymes by a fungus. The extracellular synthesis of AgNP by *P. chrysosporium* is attributed to laccase, while intracellular gold nanoparticle synthesis was attributed to ligninase (Sanghi et al. 2011).

Fungi have several advantages over bacteria for NP biosynthesis. They secrete larger amounts of extracellular proteins with diverse functions. The so-called secretome in fungi include all the secreted proteins into the extracellular space (Girard et al. 2013). The high concentration of the fungal secretome has been used for industrial production of homologous and heterologous proteins. For instance, the expression of a functionally active class I fungal hydrophobin from the entomopathogenic fungus *Beauveria bassiana* has been reported (Kirkland and Keyhani 2011). The tripeptide glutathione is a well-known reducing agent involved in metal reduction and is known to participate in cadmium sulfide (CdS) biosynthesis in yeasts and fungi (Chen et al. 2009). However, the knowledge of the fungal secretome is still largely underexplored especially for halophilic and halotolerant fungi. The large and relatively unexplored fungal secretome is an advantage because of the role of extracellular proteins and enzymes it generates have in metal reduction and nanoparticle synthesis.

19.4 Potential Applications

Bionanoparticles have found uses in biomedical and environmental fields. In the biomedical field, these nanoparticles have been investigated for antimicrobial applications (Sondi and Salopek-Sondi 2004), biosensing (Yu et al. 2003; Mckindles and Tiquia-Arashiro 2012; Tiquia-Arashiro 2012), imaging (Boisselier and Astruc 2009), and drug delivery (Muller et al. 2013). In the environmental field, nanoparticles have been investigated for applications in bioremediation of diverse contaminants (Srivastava et al. 2012; Tiquia-Arashiro and Rodrigues 2016c), water treatment (Ma et al. 2012; Yu et al. 2013), improving plant resistance (McKnight et al. 2003; Rai et al. 2012), and production of clean energy (Wan et al. 2015; Tiquia-Arashiro and Rodrigues 2016c). Overall, bionanoparticles have attracted the attention of diverse researchers because their synthesis is more environmentally friendly and produces more homogeneously distributed nanoparticles, and some of them can be easily biodegradable. Although there are several studies investigating the application of fungal-based nanoparticles, they are still way less studied than bacterial-based nanoparticles. Researchers are still identifying the microbiological synthetic pathways of these bionanoparticles. It is expected that with the advancement of the understanding of bionanoparticle synthesis pathways, the application of bionanoparticles will expand to many more fields than biomedical and environmental and they will be potentially applied in diverse nanotechnological industries.

19.5 Conclusions and Future Perspectives

The interest for bionanoparticles has increased in the past years because they present very different properties and functions than synthetic nanoparticles and they tend to be more biocompatible than their inorganic nonbiological counterparts. The

“green” method for nanoparticle synthesis, which is rapidly replacing traditional chemical syntheses, is of great interest because of eco-friendliness, economic views, feasibility, and wide range of applications in several areas such as nanomedicine and catalysis medicine. The most obvious disadvantages of biological nanoparticles (BNP) are that they frequently do not withstand high or low temperatures, extreme pH values, high salt concentrations, presence of harsh chemicals and potential environmental conditions that could lead to their hydrolysis (Tiquia-Arashiro and Rodrigues 2016e, f). It is possible, however, that BNPs from extremophiles or extremotolerant microorganisms might overcome these issues. While a variety of prokaryotic and eukaryotic microorganisms have been investigated with respect to their nanoparticle synthetic abilities, the vast biodiversity encountered in the fungal halophilic/halotolerant world has been relatively less explored. Furthermore, most of the studies are related to the synthesis of silver nanoparticles, followed by those of gold and less on cadmium, lead, and zinc. One reason for this could be the relative ease with which the noble metal ions of gold and silver are reduced. Currently, there are no reports on synthesis of nanoparticles of platinum, bismuth, antimony sulfide, and titanium oxide by halotolerant fungi. As summarized in this review, biologically active products from halotolerant fungi represent excellent scaffolds for this purpose. However, there is a need to understand the mechanisms involved in the synthetic process. Another limitation of the studies is that the experiments have been conducted at laboratory scale and there are hardly any efforts for the scale-up of these processes. In the future, these shortcomings need to be addressed in an effective manner to harness the actual nanoparticle synthetic potential of the halotolerant to their full extent.

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