7 Role of Enzymes in Synthesis of Nanoparticles

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Abstract

In the last few decades, nanotechnology has come up as an emerging and accelerated interdisciplinary field of science. Multifarious applications of the nano-sized objects are usually attributed to the size and shape, and thus, progressing with size-controlled synthesis of nanomaterials is important. Nanoparticles are obtained by either of the three modes of synthesis, i.e., physical, chemical and biological methods. Biological synthesis or the green synthesis of nanoparticles has received huge attention owing to the economics of production and biological compatibility over the other two methods. Several cellular and biomolecular products from microbes and plants have been successfully utilized to obtain nanoparticles derived using metals and non-metals. Enzyme-mediated synthesis of nanoparticles has provided an alternative approach for the synthesis of nanoparticles in a suitable way. In this chapter, we have compiled several plant and microbial enzymes utilized for the synthesis of nanoparticles.

Keywords

Green synthesis · Nanoparticles · Enzymes · Limitations · Factors

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7.1 Introduction

The application of nanoparticles (NPs) has increased many folds in recent times across different fields of industry including agriculture, health, bioengineering, textile, chemical, paints, etc. Therefore, several approaches are being undertaken to synthesize NPs in a more economic and convenient way. Since size, shape, morphology and stability are few important characteristics in affecting the utilities of NPs, the synthetic approaches should be flexible enough to accommodate the required changes as per needed. The synthesis of NPs can be broadly classified into two types such as top-bottom and bottom-up approaches. The top-bottom approach involves breaking down of suitable bulk material into smaller fine particles by size reduction techniques. Similarly, bottom-up approach involves assembling of atoms and nuclei to grow into required nano size (Gour and Jain 2019). The top-down approach involves different physical techniques like thermal ablation, milling and grinding, microwave (MW) irradiation, ultrasonication, etc. However, the physical approaches are associated with some drawbacks like high energy requirement, costlier and low yield (Shedbalkar et al. 2014). The chemical approach of nanoparticle synthesis involves different approaches including photochemical reduction and electrochemistry techniques. Though chemical approach techniques involve reduced energy during step reduction and production of homogenous particles having high preciseness in size and shape, the methods are not environment friendly and the so-obtained NPs are toxic, unstable and less biocompatible (Kharisov et al. 2016; Shah et al. 2015). Hence, the development of environment friendly technique in which the size, shape, surface charge, stability and characteristics of NPs is one of the most sought research areas in recent times. In this connection, green nanotechnology provides a promising and effective alternative route for NP synthesis. Nanoparticle obtained through biological technique is an environment friendly approach that exploits biological agents such as bacteria, fungi, algae, viruses and plants (Fig. 7.1). This route provides a nontoxic way for nanoparticle synthesis with diversity physico-chemical properties (Gahlawat and Choudhury 2019). Further, green synthetic routes are attractive, considering their potential to reduce the toxicity level exhibited by NPs.

Recently, enzyme-mediated synthesis of nanoparticle is one of the advancements in the field of nanotechnology. Besides, recently many plant- and microbial-derived enzymes have been reported for their ability to synthesize metallic NPs (Adelere and Lateef 2016). However, very few studies have reported the biochemical and molecular mechanisms of enzyme-mediated nanoparticle synthesis. Therefore, the present chapter reviews various enzymes from plants and microorganisms involved in the production of nanoparticles and their possible mechanisms for fabrication of nanoparticles.

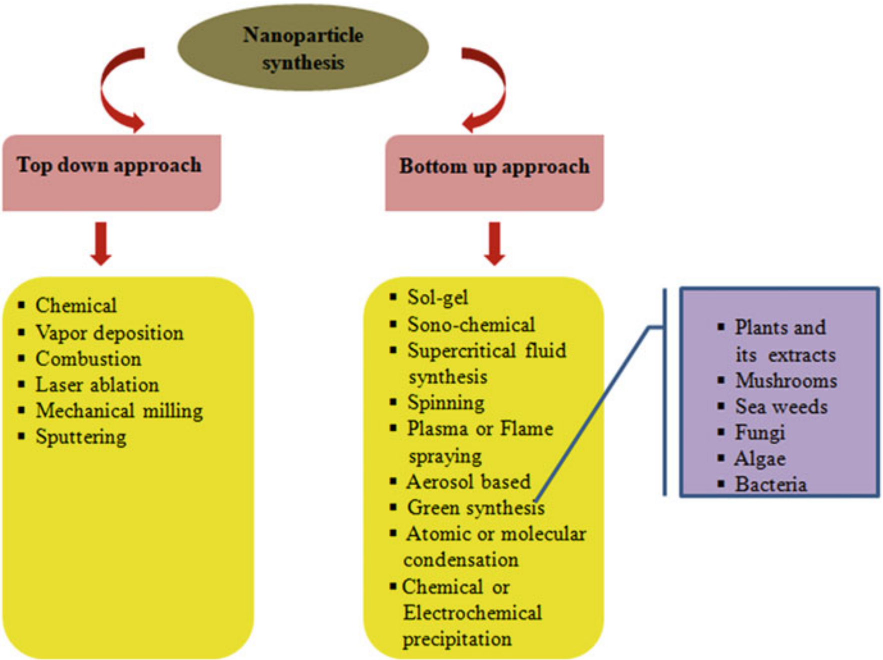

Fig. 7.1 Different approaches for nanoparticle synthesis

7.2 Biogenic Synthesis of Nanoparticle

In green or biogenic synthesis, nanoparticles are synthesized using biological agents such as bacteria, fungi, algae or plants (Ovais et al. 2018). Biogenic methods of nanoparticle synthesis are more suitable as compared to physical and chemical methods which are not eco-friendly and not scalable easily. The plants are composed of an array of complex phytochemicals of different chemical classes such as alcohols, phenols, terpenes, alkaloids, saponins and proteins and can act as a both reducing and capping agents in the biosynthesis of nanoparticles. Similarly, microbes are endowed with different metabolic enzymes which can act as both reducing and stabilizing agents for NP synthesis (Kaushik et al. 2010).

7.2.1 Plants

Biosynthesis of NPs using plant extracts follows a bottom-up approach in which involves synthesis of NPs by using reducing and stabilizing agents (Kalpana and Rajeswari 2018). The phytoconstituents encompass several groups of chemical groups, and hence, the mechanism of biosynthesis of nanoparticles may vary substantially. Therefore, the mechanism of nanoparticle synthesis using plant extracts has not been elucidated completely. However, metallic nanoparticle synthesis using plants and phytoextracts includes three main phases: (1) activation phase: reduction of metal ions and nucleation of the reduced metal atoms occur; (2) growth phase: the small adjacent nanoparticles spontaneously coalesce into particles of a larger size and (3) termination phase: this phase involves in the determination of the final shape of the nanoparticles (Singh et al. 2016).

Synthesis of nanoparticles using phyto components have generated keen interest in the scientific research community since they are used for the bioreduction of metal ions to form nanoparticles in a more rapid, safer, cost-effective and environmentally safer way. The green approach of nanoparticle synthesis using plant extracts provides a more flexible control over the size and shape of the nanoparticles along with facilitating easy purification. Generally, the metallic nanoparticles are synthesized by incubating the metal salts with the plant extracts. The presence of the various phytoconstituents induces the reduction, and the process is often peaked by several compounds present in the plant cells (phytocompounds) and other reducing agents (Asmathunisha and Kathiresan 2013). The advantages of plant extracts over other biomaterials for synthesis of nanoparticles include easy availability, safety in handling, cost-effective, single-step synthesis process, presence of different secondary metabolites as reductants, rapid rate of synthesis, eco-friendly and stable nanoparticles, size and shape of nanoparticles and suitability for large-scale production (Vijayaraghavan and Ashokkumar 2017).

Several plants and their extracts or bioactive constituents had been explored for the preparation of Ag nanoparticles (AgNPs) in which silver salt (mostly silver nitrate) gets reduced to AgNPs. The reaction is as follows:

$$\mathrm{Ag^{+}NO_{3}^{-} + Plant\ constituents\ (OH, C = H) \rightarrow Ag^{o}\ nanoparticle}$$

Similarly, gold nanoparticles (AuNPs) are prepared by bioreduction of chloroauric acid ($HAuCl_4$) to AuNPs by plant extracts and the reduction reaction is as follows:

$$\mathrm{H^{+}Au^{3} + 4Cl^{-} \cdot 4H_{2}O + Plant\ molecule\ (OH, COOH) \rightarrow Au^{o}\ nanoparticles}$$

Similarly, the platinum nanoparticles (PtNPs) are synthesized utilizing the plant extracts that reduces the aqueous chloroplatinic acid hexaydrate ($H_2PtCl_6{\cdot}6H_2O$) solution as follows:

$$\mathrm{H_{2}Pt + 6Cl^{-} \cdot 6H_{2}O + Plant\ molecule\ (OH, COOH, etc.) \rightarrow Pt^{o}\ nanoparticles}$$

The reduction of palladium chloride ($PdCl_2$) to nanoparticles by plant biomass follows the below equation:

$$\mathrm{Pd + Cl_{2}^{-} + Plant\ molecule\ (-C = C, -C = O) \rightarrow Pd^{o}\ nanoparticles}$$

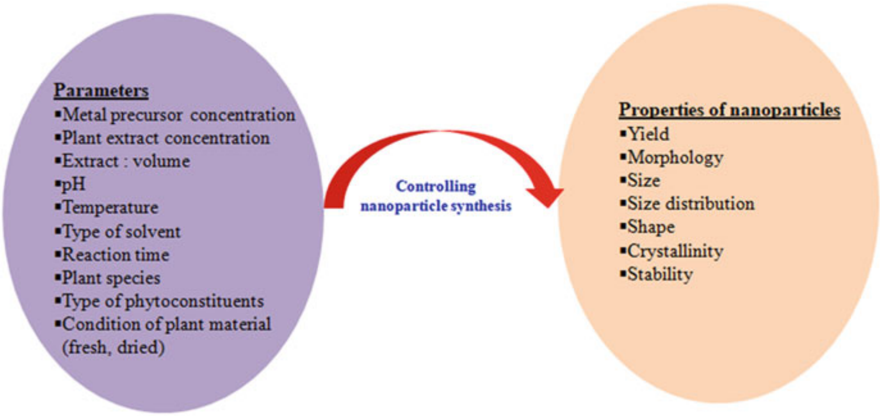

Fig. 7.2 Parameters controlling plant-based synthesis of nanoparticle

Further, several other nanoparticles such as copper nanoparticle, ZnO nanoparticle, titanium dioxide nanoparticle and iron nanoparticle are also synthesized using their metal salts and plant extracts (Vijayaraghavan and Ashokkumar 2017).

Although nanoparticle synthesis using phytoextracts is a surging approach, still few challenges are there, which need to be taken into consideration. Multiple factors affect the plant-mediated synthesis of NPs such as type, source and concentration of plant extracts, ratio of the reagents and experimental parameters like temperature, pH, time, yield and product characterization (Shah et al. 2015; Peralta-Videa et al. 2009) (Fig. 7.2).

7.2.2 Microorganisms

Microorganisms are ubiquitous to almost all environments. Their role in the sustainability of all organisms is extremely important and which is why the wide variety of applications by the use of microorganisms empathizes with its applicability. Their application varies from pharmaceutical sectors to environmental sustainability, further to their use in food industries. Their role in the synthesis of nanoparticles is rather wide and usually attributed to the secretion of metabolites and macromolecules which reduce the metal salts to ionic forms (McDevitt et al. 2011).

Bacteria are omnipresent and are phylogenetically diverse (Sathyavathi et al. 2014; Rohwerder and Müller 2010). The adaptability of the bacteria ranges from highly acidic mine drainage to extreme sub-zero temperature regions (Saeed et al. 2020; Klaus-Joerger et al. 2001; Deobagkar et al. 2015; Alghuthaymi et al. 2015; Feroze et al. 2020). The presence of unique metabolic features in bacteria possess is exploited for the biosynthesis of metallic nanoparticles. Though their exposure to harsh environments leaves them to nothing except cell death; however, with time these unicellular organisms have developed strategies to survive (Gajbhiye et al. 2009; Duran et al. 2015). Several studies have proved that the mineralization of

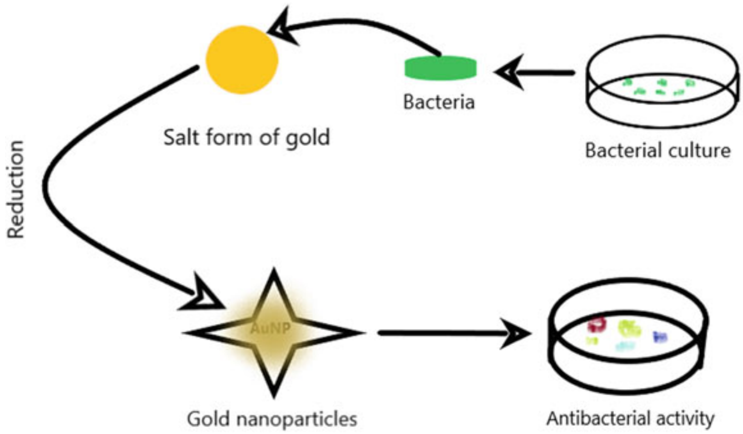

Fig. 7.3 Mechanism of nanoparticle synthesis by bacteria and its application

various metals can be achieved by the use of bacteria (Mishra and Sardar 2012; Kisailus et al. 2005). In a study by Saeed, Iqbal and Ashraf on the effect of bacteria-mediated silver nanoparticles on human pathogens, the silver nanoparticles were able to exhibit antibacterial activity against *Staphylococcus aureus* exhibiting resistance to methicillin (MRSA) and few other drug-resistant strains as well. The zone of inhibition they observed was ranging from 10 to 28 mm (Yang et al. 2016). In another study by Klaus-Joerger et al. (2001), bacterial cells were exploited for the accumulation of biosynthesized nanoparticles. They reviewed the properties of the nanoparticles and concluded that the use of bacteria-mediated nanoparticles can be utilized for structured materials (Zomorodian et al. 2016). Deobagkar et al. (2015) studied the highly resistant *Deinococcus radiodurans* bacteria to synthesize silver nanoparticles. The bacterium was able to accomplish the objective under optimized conditions. The effect of the biosynthesized nanoparticles was tested against for antibacterial and antifouling activity. Further they were also able to inhibit cell proliferation of cancer cell lines (Gholami-Shabani et al. 2015) (Fig. 7.3).

Fungi are an excellent source for the synthesis of nanoparticles. Their capacity is understood from the tolerance levels to different lanthanides and transition metals. The large-scale production of macromolecules especially enzymes makes fungi as one of the suitable biological agents for the synthesis of different metallic nanoparticles (Khan and Ahmad 2014). Feroze et al. (2020) studied the antibacterial activity of fungal nanoparticles synthesized using silver nitrate (Fig. 7.4). They adopted the method of well diffusion to assess the antibacterial effect of the biosynthesized nanoparticles against some of the notorious pathogens. Their synthesized nanoparticles also suggested the efficacy of wound healing and as an anti-inflammatory agent (Kumar et al. 2007). Gajbhiye et al. (2009) in their study on the combinatorial assessment of nanoparticles with fluconazole showed several pathogenic fungi that were inhibited in the presence of silver nanoparticles (Duran et al. 2014).

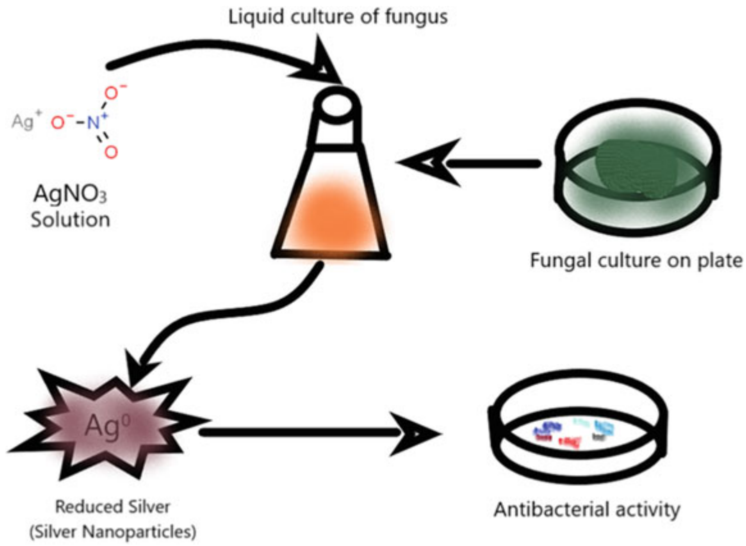

Fig. 7.4 Mechanism of nanoparticle synthesis by fungi and its application

7.3 Enzyme-Mediated NP Synthesis

Enzyme-mediated nanoparticle synthesis is considered as environmentally friendly, economic and easily scaled-up process. Enzyme-mediated nanoparticle synthesis is one of the most promising synthesis strategies in recent times in the field of nanobiotechnology. The enzymes may differently behave during the formation of nanoparticles like reducing and capping agent (Adelere and Lateef 2016; Duran et al. 2014).

7.3.1 Plant-Based Enzyme-Mediated Synthesis

Enzymes by their general characteristics modulate the synthesis but do not involve in the biochemical reactions itself. They may also sometimes serve as reducing and stabilizing agents. Duran et al. (2014) showed the involvement of sulphur-containing groups and disulphide bridge moieties present in enzymes during nanoparticle formation process. Similarly, sulphur moieties of denatured enzymes also help transform the metallic ions to form nanoparticles. Enzymes present in plants may act as catalysts modifying the reduction speed or acting simply as chemicals having a direct reducing activity towards the cation. Several studies have reported plant-derived enzyme-mediated synthesis of nanoparticles which are discussed in Table 7.1.

Table 7.1 Plant-derived enzyme-mediated nanoparticles

Enzyme	Source	Types of nanoparticle	Mechanism of synthesis	References
Amylase	Plants	Ag	Interaction of the thiol group (−SH) of cysteine with the metal ions leading to the reduction of metal ions to corresponding metal atom	Mishra and Sardar (2012)
Cysteine protease	*Calotropis procera*	Cu	Act as capping/stabilizing agent; bind to metal nanoparticles through the free amine groups or carboxylate ion	Dubey and Jagannadham (2003)
Curcain protease	*Jatropha curcas*	ZnS	As reducing and stabilizing agents. Cysteine or thiol residues present in curcain may be donating these sulphide (S^{-2}) ions to Zn ion	Hudlikar et al. (2012)
Peroxidase	*Armoracia rusticana*	Ag, Au	Reduction of $HAuCl_4$ by $NaBH_4$	Parashar et al. (2017) and Kumar et al. (2018)
Urease	*Canavalia ensiformis*	Au, Ag, Pt, ZnO	Urease acts as a reducing and stabilizing agent for the synthesis of nanoparticles	Sharma et al. (2013)

7.3.1.1 α-Amylase

α-Amylase is one of the most common enzymes used in in vitro nanoparticle synthesis. It acts as both reducing agent and capping agent in green synthesis of nanoparticle. Mishra and Sardar (2012) reported the synthesis of silver nanoparticles using α-amylase from aqueous solution of silver nitrate. The mechanism behind nanoparticle synthesis could be attributed to the interaction of the thiol group (–SH) of cysteine with the metal ions leading to the reduction of metal ions (Ag^+) to corresponding metal atom (Ag^o). α-Amylase is also involved in the synthesis of gold nanoparticles. The frees –SH group present in the amylase enzyme helps in the reduction of $AuCl_4^-$ to Au nanoparticles (Rangnekar et al. 2007).

7.3.1.2 Glutathione

Glutathione (GHS) is considered as one of the most common antioxidants present in plant cells. It is a reducing agent and has highly reactive thiol group and hence can be used to convert the oxidation state of the metals. Along with the thiol group, GSH molecule also contains amine and carboxylate functional groups which may help in cross-linking to other molecules. Baruwati et al. (2009) reported the synthesis of AgNPs, PdNPs, PtNPs and AuNPs using glutathione under microwave irradiation conditions. The glutathione acts as both reducing and coating agent in synthesis of metal nanoparticles synthesis.

7.3.1.3 Protease

Cysteine protease isolated from medicinal plant *Calotropis procera* (Family Asclepiadaceae) has molecular weight and isoelectric point of 28.8 kDa and 9.32, respectively (Dubey and Jagannadham 2003). The enzyme has been used to fabricate copper nanoparticles from copper acetate. The proteinaceous material encapping the particles has possibly served capping/stabilizing agent. It has been reported that proteins attach the metal ions in the nanoparticles through the presence of free amine groups or carboxylate ions in the amino acid residues (Harne et al. 2012). Similarly another protease, e.g. curcain isolated from latex of *Jatropha curcas* plant has been reported for the synthesis of zinc sulphide (ZnS). The curcain enzyme present in the latex of *J. curcas* acted as both reducing and stabilizing agents. The cysteine or thiol residues present in curcain may be donating these sulphide (S^{-2}) ions to Zn ions and helps in green synthesis of ZnS NPs (Hudlikar et al. 2012).

7.3.1.4 Peroxidase

Horseradish peroxidise (HRP) obtained from *Armoracia rusticana* has been reported for the synthesis of Ag and Au nanoparticles. Parashar et al. (2017) have reported the green synthesis of AuNPs using $HAuCl_4$ and $NaBH_4$ and HRP at optimized condition. The reduction of $HAuCl_4$ was carried out by $NaBH_4$ and H_2O_2 further speeds up the reduction process. The formation of AuNPs was then mediated by HRP. In another study, Kumar et al. (2018) describe the synthesis of AgNPs using HRP. The enzymatic activity of HRP assisted in the formation of AgNPs, which was prevented upon the addition of an excess amount of hydrogen peroxide (H_2O_2).

7.3.1.5 Urease

Urease isolated from *Canavalia ensiformis* (jack bean plant) has been shown for the synthesis of Au, Ag and Pt nanoparticles. The enzyme acts as a reducing and stabilizing agent. The catalytic activity of urease is also exploited for the synthesis of ZnO core-shell nanostructures at ambient temperature. The exposed residues, i.e. Cys592 in the enzyme, was found to be responsible for the formation of metal and metallic alloy nanoparticles (Sharma et al. 2013). Zn^{2+} binds on the negative charge urease present on the surface through weak bond interaction at a pH of 9, thus forming of zinc hydroxide as an intermediate compound. Under the basic conditions, further dehydration of zinc hydroxide yields ZnO on the enzyme surface accelerated by the “salting out” effect (Makarov et al. 2002).

7.3.2 Microbial Enzyme-Mediated Synthesis

Microbial enzymes play an important role in the formation of metal salts leading to the synthesis of metal NPs. The enzymes act as reducing agents and work as an electron shuttles during the reduction of metals and synthesis of microbial NPs (Subbaiya et al. 2017). Therefore, optimization of conditional parameters for maximizing the activity of enzymes may enhance the synthesis of NPs. Few

Table 7.2 Microbial enzyme-based synthesis of nanoparticles

Enzyme	Microbial source	Type of nanoparticles	References
α-Amylase	*Aspergillus oryzae*	AgNPs	Mishra and Sardar (2012)
Aspartate protease	*Aspergillus saitoi*	AuNPs	Bharde et al. (2007)
Hydrolase	*Tethya aurantia*	Gallium NPs	Kisailus et al. (2005)
Hydrogenase	Sulphate-reducing bacteria (SRB)	Platinum nanoparticles	Riddin et al. (2009)
Laccase	*Pleurotus ostreatus*	AuNPs	El-Batal et al. (2015)
Laccase	*Trametes versicolor*	AgNPs	Duran et al. (2014)
Nitrate reductase	*Bacillus licheniformis*	AgNPs	Li et al. (2011a, b)
Nitrate reductase	*Rhodopseudomonas capsulata*	AuNPs	He et al. (2007)
Sulphite reductase	*Thermomonospora* sp.	AuNPs	Khan and Ahmad (2014)

microbial enzymes involved in NPs synthesis with their sizes are mentioned in Table 7.2.

7.3.2.1 α-Amylase

Rangnekar et al. (2007) studied the biosynthetic process of gold nanoparticles. In their study, the conversion of chloroauric acid was converted to gold nanoparticles by the catalytic action of an amylase enzyme (Rangnekar et al. 2007; Duran et al. 2015). Mishra and Sardar (2012) also synthesized nanoparticles from silver salt using amylase enzyme. The observations made by them was accumulated to the active role of the enzyme amylase which could form the silver nanoparticles from the salts of silver (Mishra and Sardar 2012).

7.3.2.2 Hydrolases

The importance and role of hydrolases in the synthesis of nanoparticles was realized by Ramezani et al. (2010). They reviewed the properties of this enzyme in fungi and reported that the fungal specie *Verticillium* sp. could utilize the enzyme hydrolases to convert $[Fe\ (CN)_6]_3$ and $[Fe(CN)_6]_4$. The enzyme hydrolases was also explored by Kisailus et al. (2005) in their study on gallium salts which was capable of transforming the salts of gallium to gallium (III) nitrate. The particular enzyme was also found to be forming the crystal forms of the metallic nanoparticle at very low temperature.

7.3.2.3 Sulphite Reductase

Zomorodian et al. (2016) synthesized silver nanoparticles using three Aspergillus species. Their UV-Vis spectroscopic analysis showed the absorption at 430 nm which coincided with that of silver nanoparticles. They concluded that the formation of silver bio-nanoparticles was regulated by nitrate reductase enzyme. Gholami-Shabani et al. (2015) synthesized gold nanoparticles by adopting a cell-free extract from the bacterium *E. coli.* The cell-free extract contained the enzyme sulphite reductase which could exhibit antifungal activity against *Aspergillus fumigatus* and *Fusarium oxysporum* and two other fungal species. Their synthesized nanoparticles were able to show a MIC of 31.25 μg/ml to 250 μg/ml. The utility of this enzyme was also shown by Khan and Ahmad (2014). They described a protocol for the purification of the enzyme sulphite reductase that helped in rendering the gold nanoparticles to disperse into the solution. In another study conducted by Kumar et al. (2007), the role of sulphite-reductase was elaborated. Their study demonstrated that even fungal species (*Fusarium oxysporium*) could produce the enzyme sulphite reductase for the synthesis of gold nanoparticles.

7.3.2.4 Laccases

Duran et al. (2014) utilized the enzyme laccases in a semi-purified form obtained from a fungus *Trametes versicolor*. The authors concluded that the enzyme used for the synthesis of silver nanoparticles was interaction of silver ions with the T1 catalytic site of the enzyme laccases. Lateef and Adeeyo (2015) carried out a study on the efficiency of biosynthesized nanoparticles using laccase enzyme. Their study reports the efficiency of the laccase enzymes in the synthesis of nanoparticles could inhibit few pathogenic bacteria at a higher rate.

7.3.2.5 Nitrate Reductase

Multiple studies reported the involvement of nitrate reductase enzyme in the production of AgNPs by *Bacillus licheniformis* (Kalimuthu et al. 2008; Kalishwaralal et al. 2010). NADH-dependent nitrate reductases enzymes require cofactors like NADH for production of metal NPs. Different studies demonstrated the role of NADH and NADH-dependent enzymes (nitrate reductase) in *Bacillus licheniformis* for the possibility of forming Ag^0 (Duran et al. 2011; Li et al. 2011a, b). Similarly, in another study bioreduction of Au is initiated via electron transferring from NADH by NADH-dependent reductase enzymes present in *Rhodopseudomonas capsulata*. Consequently, Au ions accept electrons and get reduced (Au^{3+} to Au^0), leading to the formation of gold nanoparticles (He et al. 2007).

7.4 Factors Affecting Enzyme-Mediated NP Synthesis

Several factors including the quantity of enzyme, pH, temperature, enzyme to substrate concentration ratio and incubation time of reaction are limiting factors in synthesis and controlling the size of metallic NPs. The following section discusses various factors affecting the enzyme-mediated nanoparticle synthesis.

Phanerochaete chrysosporium derived enzymes like Laccase and ligninase have been reported for the formation AuNPs of 10–100 nm in particle size. Several factors like incubation age of the fungal culture, concentration of $AuCl^{4-}$ solution and temperature affect the shape of AuNPs (Sanghi et al. 2011). He et al. (2007) reported the synthesis of AuNPs by *Rhodopseudomonas capsulata*-mediated via NADH and NADH-reliant enzymes. Several factors like concentration of the predecessor, pH, temperature and duration of reaction are limiting factors in controlling the size of MtNPs. pH value of the reaction mixture was found to be an important factor for controlling the size and shape of AuNPs. In another study, Riddin et al. (2010) demonstrated the effect of platinum salt concentration (H_2PtCl_6) on Pt NP synthesis. The ratio of H_2PtCl_6 to bacterial hydrogenase enzyme varied from 0.7:1 to 4:1. Amongst them, H_2PtCl_6 to bacterial hydrogenase enzyme at 1.5:1 was reported to be the optimized condition for Pt NP synthesis. One of the important parameter affecting NP synthesis in more economical and efficient way is concentration of substrate. Gradual increase in the concentration of $AgNO_3$ to 5 mM, AgNP production was increased using *Fusarium oxysporum*-mediated enzymatic AgNPs synthesis. However, further increasing to 10 mM, the production of AgNPs decreased (Korbekandi et al. 2013).

7.5 Limitations of Enzyme-Mediated NP Synthesis

In spite of a wide range of benefits obtained from enzyme-mediated synthesis of metal NPs, there exists a number of challenges to overcome. One of the major limitations in enzyme-mediated synthesis is lack of complete and thorough understanding of mechanical aspects of biofabrication of nanoparticles. Detailed analysis of metabolic pathways is required to obtain tailor-made nanoparticles (Ovais et al. 2018). Considering the requirement in biomedical purposes, it remains an indispensable agent owing to biocompatibility of NPs. It is important that nanoparticles remain stable without any significant change of morphology, shape, size and structure (Dauthal and Mukhopadhyay 2016). Surged studies are required to ensure the efficacy and long-term stability of enzyme-fabricated nanoparticles. Large-scale production is yet a major bottleneck for commercialization of enzyme-mediated nanoparticle fabrication along with controlled sizes and shapes. Bulk processing methods for enzyme-mediated nanomaterials and downstream processing techniques also need substantial improvement.

7.6 Conclusion and Future Prospective

There is immense potential for enzyme-mediated metal nanoparticle synthesis as the process is eco-friendly, low in toxicity, less expensive, high biodegradability and are applicable for therapeutic purposes. However, enzyme-mediated green metallic nanoparticle synthesis requires in depth knowledge of the biochemical and molecular mechanisms of the reactions involved during synthesis for a better understanding of chemical composition, shape, size and mono dispersity of nanoparticles. Therefore, detailed studies are required to find out the exact role of enzymes and their optimised reaction conditions required for synthesis, stabilization or pharmaceutical activities of NPs. With improvement of our knowledge, enzyme-mediated nanoparticle synthesis could be the leading large-scale production method for nanoparticles in coming days. Detailed characterization of enzymes used for the NP formation and biogenic activities could open up a new pool of proficient enzymes which could be utilized for various biomedical applications in future.

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