Mycofabrication, mechanistic aspect and Multifunctionality of Metal Nanoparticles - Where are we? And where should we go?

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Fungi consists of varied group of heterotrophs, which due to their unique properties show applications in different fields. Nanotechnology covers diverse fields of science and technology, and the fabrication of nanoparticles using the biological route is the need of the day. The introduction of biological agents for the synthesis of nanoparticles has encouraged the researchers to search for the efficiency of different systems to synthesize metallic nanoparticles. Among the different systems harnessed for their potential of synthesizing nanoparticles, the fungal system has emerged as an efficient system synthesizing metal nanoparticles both intra- and extracellularly. The fungus mediated nanoparticles present monodispersity, dimensions and stability. Also, the system is eco-friendly and economically viable for the synthesis of nanoparticles.

The present review focuses on different concepts and mechanism involved in the synthesis of metal nanoparticles, present status, multiple applications, and different areas of research.

Keywords: Fungi, Nanotechnology, nanoparticles, intracellular, extracellular.

1.1 Introduction

Nanotechnology is a multidisciplinary science comprising various aspects of research and technology [1]. Nanoparticles are metal particles in the size range of 1-100nm and form building blocks of nanotechnology [2]. Metal nanoparticles like gold, silver and platinum have gained considerable attention in recent times due to their fundamental and technological interest. These nanoparticles have unique catalytic, electronic and optical properties distinct from the metallic particles [3]. In recent times, many methods have been designed to synthesize nanoparticles such as physical method, chemical method and biological methods [3], [4], [5]. The physical and chemical methods involve the use of strong chemical reducing agents such as sodium borohydride and weak reducing agents like sodium citrate, alcohols, use of gamma rays and UV rays, etc. [6]. Studies have reported that the biological methods depict an inexpensive and eco-friendly route for synthesis of nanoparticles. Till date synthesis of nanoparticles have been demonstrated by the use of biological agents like bacteria, fungi, yeast and plants [3]. A number of bacteria like *Bacillus subtilis* [7], *Pseudomonas stutzeri* [8], *Thermonospora* sp. [9], *Shewanella algae* [10], *Lactobacillus* strains [11], etc. have been studied for the synthesis of metallic nanoparticles. Yeast have also been explored for the biosynthesis of nanoparticles including *Candida glabrata* [12], *Schizosaccaharomyces pombe* [13], MKY3 [14] etc. While, a number of plants like *Medicago sativa* [15], *Pelargonium graveolans* [16], *Azadirachta indica* [17], *Triticum* [18], *Cinnamomum camphora* [19], *Capsicum annum* [20] have been used for the fabrication of metal nanoparticles.

The synthesis of nanoparticles by fungi, and their subsequent application, particularly in medicine are studied under Myconanotechnology. Myconanotechnology is the interface between '*Mycology'* and '*Nanotechnology*' and has considerable potential, partly due to the wide range and diversity of the fungi [21]. When focusing on the synthesis of nanoparticles using fungi, it was observed that nanoparticles of good monodispersity and well dimensions could be synthesized. As fungi are found to secrete high amount of protein they might result in the significant mass productivity of nanoparticles. The fungal proteins are capable of hydrolyzing metal ions. In addition to this, fungi are easy to isolate and culture. Moreover, the downstream processing and the handling of fungal biomass are less complex than the synthetic methods [22].

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 and CdS etc.

Fungi can accumulate metal ions by physico-chemical and biological mechanisms including extracellular binding by metabolites and polymers, binding to specific polypeptides, and metabolism-dependent accumulation [23]. The possible use of fungi has gained much importance, as they are easy to culture in bulk. Also, the extracellular secretion of enzymes has an added advantage in the downstream processing and handling of biomass [24] when compared to the bacterial fermentation process which involves use of sophisticated instruments to obtain clear filtrate from the colloidal broth [25]. Moreover, fungi are excellent secretors of protein compared to bacteria and actinomycetes, resulting into higher yield of nanoparticles [25]. Thus, using these dissimilatory properties of fungi, it could be extensively used for the rapid and eco-friendly biosynthesis of metal nanoparticles. The present review focuses on the synthesis of metal nanoparticles using fungi, various applications, and the present status of research involving its future impressions.

1.2 Where we are in fungus mediated nanoparticle synthesis?

The biological route for the synthesis of nanoparticles implying the use of microorganisms is advantageous over the traditional methods, as biological synthesis is cost-efficient, environment-friendly and simple method. The main advantage of the biological route is its ability to manipulate nanoparticle properties by gaining control over the size and shape of nanoparticles [26], [27]. The feasibility of the fungal system for the synthesis of metal nanoparticles has been successfully demonstrated (Table1). The fungal system shows the capability of both intracellular and extracellular synthesis of nanoparticles [22], [26], [28]. In the recent past, research work using the fungal system has been carried out using both aspects of intracellular and extracellular methods for synthesis of nanoparticles of gold, CdS, silver, silica, titania, zirconia, etc. ([29], [30], [31], [32], [33]). Moreover, a number of fungal species like *Verticillium*, *Phoma* sp., sp. *Fusarium oxysporum*, *Aspergillus fumigatus*, *Trichoderma asperellum*, have been explored ([29], [31], [33], [34], [27]) for the synthesis of metal nanoparticles.

Table1: List of Fungi synthesizing metal nanoparticles

Fungi	Mode of Synthesis	Nanoparticles	Reference
Verticillium sp.	Intracellular	Au	Mukherjee *et al*., 2001 [29]
Fusarium oxysporum	*Extracellular*	*CdS*	*Ahmad* et al*., 2002 [30]*
Phoma sp. 3.2883	Intracellular	Ag	Chen *et al*., 2003 [31]
Colletotrichum sp.	Extracellular	Au	Shankar *et al*., 2003 [16]
Usnea longissima	Extracellular	Usnic acid	Shahi & Patra, 2003 [34]
Fusarium oxysporum	Extracellular	Zirconia	Bansal *et al*., 2004 [32]
Trichothecium sp.	Extra/Intra	Au	Ahmad *et al*., 2005
Fusarium oxysporum	Extracellular	Si, Ti	Bansal *et al*., 2005 [33]
Fusarium oxysporum *Verticillium sp*.	Extracellular	Magnetite	Bharde *et al*., 2005 [37]
Fusarium oxysporum	Extracellular	Ag	Duran *et al*., 2005 [38]
Aspergillus fumigates	Extracellular	Ag	Bhainsa & D' Souza, 2006 [34]
Fusarium oxysporum f. *sp. lycopersici*	Intra- & Extracellular	Pt	Riddin *et al*., 2006 [26]
Verticillium luteoalbum	Intracellular	Au	Gericke& Pinches, 2006 [39]
Fusarium semitectum	Extracellular	Ag	Basavaraja *et al*., 2007 [40]

Aspergillus flavus	Intracellular	Ag	Vigeshwaran *et al*., 2007 [41]
Fusarium oxysporum	*Extracellular*	*CdSe quantum dots*	*Kumar* et al*., 2007a [42]*
Fusarium oxysporum	Extracellular	Ag	Kumar *et al*., 2007b [43]
Fusarium oxysporum	Extracellular	Ag	Mohammadian *et al*., 2007 [44]
Aspergillus niger	Extracellular	Ag	Gade *et al*., 2008 [24]
Fusarium acuminatum	Extracellular	Ag	Ingle *et al*., 2008 [28]
Trichoderma asperellum	Extracellular	Ag	Mukherjee *et al*., 2008 [27]
Penicillium sp.	Extracellular	Ag	Sadowski *et al*., 2008 [45]
Fusarium semitactum	Extracellular	Au, Au-Ag alloy	Sawale *et al*., 2008 [46]
Helminthosporum solani	Extracellular	Au	Kumar *et al*., 2008 [69]
Phoma glomerata	Extracellular	Ag	Birla *et al*., 2009 [47]
Fusarium solani	Extracellular	Ag	Ingle *et al*., 2009 [48]
Coriolus versicolor	Extracellular	Ag	Sanghi and Verma, 2009 [70]
Cladosporium cladosporioides	Extracellular	Ag	Balaji *et al*., 2009 [71]
Fusarium oxysporum	Extracellular	Pt	Govender *et al*., 2009 [72]

One of the earliest reports of the synthesis of nanoparticles by fungi was demonstrated by the fungus, *Verticillium* sp. [29]. Gold nanoparticles were synthesized intracellularly by growing the fungal cells in a defined medium and then transferred to aqueous auric chloride solution, the pale yellow color of the fungal cells changed to vivid purple over 24 hours. The UV visible spectra of the fungal cells confirmed the synthesis of gold nanoparticles by depicting an absorption peak at 540nm, characteristic for gold nanoparticles. The TEM (Transmission Electron Microscopy) and Higher magnification TEM studies demonstrated the presence of gold nanoparticles with an average size of 20-28nm on the cell wall as well as in the cytoplasm. The gold nanoparticles were mostly spherical while some were triangular or hexagonal.

The synthesis of CdS quantum dots by the fungus *Fusarium oxysporum* was depicted by extracellular enzymatic reduction of sulphate ions [30]. The CdS quantum dots were supposed to be formed by the reaction of Cd^{2+} ions with sulphate ions and the enzymatic reduction of sulphate ions to sulphide ions thus concluding that the fungus plays the role of a bio-reducing agent (enzyme sulphate reductase). The semiconductor nanoparticles were monodisperse in size from 5 to 20nm. The XRD analysis of particles showed the nanocrystalline nature of nanoparticles with Bragg reflections characteristic for hexagonal CdS particles.

Freeze-dried fungal biomass could also be exploited for the synthesis of silver nanoparticles [31]. Freeze-dried mycelium of *Phoma* sp. 3.2883 was treated with silver nitrate solution for 50 hours. Adsorption assays indicated that the mycelium had absorbed some 13mg of silver and TEM micrographs showed the presence of a large number of silver nanoparticles of around 70nm within the fungal mycelium.

An endophytic fungus *Colletotrichum* sp. isolated from *Pelargonium graveolens* also depicted synthesis of spherical gold nanoparticles [16]. The XRD study showed formation of stable gold nanoparticle aggregates. While, the TEM studies revealed synthesis of predominantly spherical nanoparticles and some aggregated into larger irregular structures with no well-defined morphology. The smaller spherical nanoparticles ranged in size from 8 to 40nm. In the study it was found that the fungal proteins were responsible for the stabilization of gold nanoparticles.

Shahi and Patra [35] demonstrated synthesis of bioactive nanoparticles of usnic acid from the lichen-forming fungus *Usnea longissima* in a defined medium. The bioactive nanoparticles were uniform in shape and formulated for the preparation of nanoemulsion by dissolving the usnic acid particles in oleic acid and acetone. The nanoemulsion showed activity against dermatophytes. While performing the *in vitro* investigation minimum inhibitory concentration was found to be 0.1µl/ml for the test fungi, viz., *Epidermophyton floccosum*, *Microsporum audouinii*, *M.canis*, *M.gypseum*, *M.nanum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, and *T. violaceum*. The *in vivo* investigation (for control of fungal infections) was performed on human skin for testing its irritant activity and long term toxicity on human skin. The nanoemulsion did not show any irritation or adverse effect at 5% concentration up to 3 weeks. After topical application of nanoemulsion on human skin improvements were started from first week. Fifty percent showed moderate improvement and 30% mild improvement. In second week, 30% and 25% depicted significant and moderate improvement respectively. While, in the third week 60% patients showed completely diminished fungal infections and 30% significant improvement. Thus, the nanoemulsion prepared by bioactive nanoparticles showed the potential for curing dermatophytic infections in humans.

Zirconia is an oxide of great potential so is titania and silica. *Fusarium oxysporum* has shown the potential for the synthesis of zirconia, titania and silica nanoparticles. Bansal *et al*. [32] demonstrated the synthesis of quasi-spherical zirconia nanoparticles. The fungal protein similar to silicatein was found to be responsible for the synthesis of zirconia nanoparticles. Extracellular synthesis of zirconia nanoparticles was done by exposing the fungal biomass to aqueous solution of zirconium hexafluoride anions at room temperature. After 24 hrs of reaction subsequent bright field and dark field TEM images of the reaction mixture were taken which showed formation of spherical shape nanoparticles with quasi-spherical morphology (3 to 11nm in size). The X-ray Diffraction (XRD) analysis of the thin film of the solution cast nanoparticles showed Bragg reflections characteristic for crystalline zirconia. The Fourier Transform Infrared Spectra (FTIR) of these biogenic nanoparticles depicted Zr-O-Zr vibrational band which was followed by the disappearance of the protein amide I and II bands thus improving the crystallinity of biogenic particles. To find out the proteins secreted by the fungus which were responsible for the hydrolysis of the aqueous anionic metal complex, SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) was carried out which clearly showed the initiation of two extracellular proteins in the presence of zirconium hexafluoride anions. These extracellular proteins were eluted from the gel and on testing showed positive results for hydrolysis of zirconium hexafluoride anions to zirconia nanoparticles. In another study Bansal *et al*. [33] reported the synthesis of titania and silica nanoparticles. *Fusarium oxysporum* was used for the extracellular synthesis of silica and titania nanoparticles. The biosynthesized nanoparticles were quasi-spherical in shape also, the FTIR analysis showed the presence of Si-O-Si and Ti-O-Ti stretching vibration and amide bonds which were considered to be responsible for the synthesis on silica and titania nanoparticles.

Ahmad *et al*. [36] reported that the fungus *Trichothecium* sp. could synthesize gold nanoparticles by both extra and intracellular method. The fungal biomass when kept in stationary condition resulted in rapid extracellular synthesis (Mycelial mat of *Trichothecium* sp. was obtained and separated from culture broth and washed thrice in distilled water and resuspended in 100ml aq. auric chloride solution) of gold nanoparticles whereas; when the biomass was kept in shaking condition on a rotary shaker it resulted in intracellular synthesis of nanoparticles. The TEM photographs of the extracellulary synthesized gold nanoparticles showed a number of individual gold nanoparticles along with few aggregates. The morphological study of these particles depicted presence of polygons (specially triangles and hexagons) as well as some polydisperse spheres and rods. The selected Area Electron Diffraction (SAED) pattern showed the Scherrer ring pattern which is characteristic of face centered cubic (fcc) structure of gold. While, the TEM micrographs of the intracellulary synthesized gold nanoparticles showed formation of small particles with spherical morphology. The SAED pattern of these gold particles showed diffuse rings with lattice spacing which were in agreement with that of gold. The intracellular formation of gold nanoparticles was also studied using the XRD study. The XRD data revealed presence of intense peaks (111, 200, 220, 311) corresponding to Bragg reflections of gold. The mean size of these particles was calculated using the Debye-Scherrer equation which determined that the average size of these gold nanoparticles was 13nm. Thus from the present study, the authors concluded that when the reaction conditions are changed the enzymes and proteins, which are released in the stationary phase do not release in shaking condition and hence result in the intracellular and extracellular synthesis of gold nanoparticles.

Isolates of *Fusarium oxysporum* and *Verticillium* sp. were exploited for the synthesis of magnetite nanoparticles [37]. The fungal filtrates was challenged with 2:1 molar mixture of $K_3[Fe(Cn)_6]$ and $K_4[Fe(Cn)_6]$. The TEM images of nanoparticles synthesized using *F. oxysporum* showed irregular particles with quasi-spherical morphology in size range of 20-50nm. The SAED analysis of individual particles showed crystalline nature of nanoparticles while, the XRD analysis showed well-defined Bragg reflections characteristic of ferric oxide. The FTIR analysis of these biosynthesized nanoparticles showed absorbance peak at 522, 568, 627 cm^{-1} which were attributed to Fe-O-Fe stretching mode vibrations and absorption peaks at 1638 and 1540 cm^{-1} were attributed to the presence of proteins. The TEM images of

the particles synthesized using *Verticillium* sp. depicted number of octahedrally shaped iron oxide particles in size range of 100 to 400nm. The SAED analysis confirmed that the particles were magnetite. The XRD pattern of iron oxide nanoparticles also showed number of Bragg reflections characteristic of ferric oxide. The FTIR analysis showed absorption bands around 522 and 627 cm^{-1} which were characteristic of Fe-O-Fe vibration modes.

Duran *et al*. [38] demonstrated the synthesis of silver nanoparticles using *Fusarium oxysporum*. The authors concluded that the enzyme nitrate reductase might be responsible for the reduction of silver ions and the subsequent synthesis of silver nanoparticles. Extracellular biosynthesis of silver nanoparticles using *Aspergillus fumigatus* was demonstrated by Bhainsa & D'Souza [34]. The synthesis of silver nanoparticles was monitored by UV-visible spectrophotometry, XRD and TEM analysis. TEM micrograph of biosynthesized silver nanoparticles taken after 72 hrs of incubation showed variable shape with majority of them spherical in shape along with some triangular in size range of 5-25nm. The XRD spectrum of the nanoparticles showed intense peaks in consent with the Bragg reflections of crystalline silver.

Gericke and Pinches [39] have demonstrated intracellular synthesis of gold nanoparticles. The fungus *Verticillium luteoalbum* was exploited for the synthesis of gold nanoparticles. TEM micrographs of the biosynthesized nanoparticle showed particle morphologies including spherical, triangular, hexagonal and other shapes. Large variation in the size of particles was observed which varied from a few to approximately 100nm. To obtain better control over size and shape, fungal biomass was grown for 24, 48, 72 hrs and exposed to auric chloride solution for 24 hrs. It was observed from the results that the age of the cells at the time of exposure to $AuCl_4^-$ solution did not have any significant effect on the shape of nanoparticles however, a decrease in number of particles was observed with fungal biomass. The pH of the reaction solution was also found to play an important role in the particle synthesis. TEM images of the particle synthesized at pH 3, 5, 7 and 9 showed particles with shape morphologies including triangles, hexagons, spheres and rods. The EDS spectrum of the nanoparticles indicated that the Au nanoparticles were mainly composed of Au with trace amounts of C, O, Na, Si and Al. Thus, the synthesis of gold nanoparticles was found to be related to the incubation temperature. Increased temperature resulted in faster particle growth rate.

Riddin *et al*. [26] demonstrated Response Surface Methodology (RSM) which consists of a central design to determine the optimal conditions like temperature, pH and concentration of hydrogen hexachloroplatinate (H_2PtCl_6) for the synthesis of platinum nanoparticles. For the intercellular synthesis (RSM_1), fungal biomass (*Fusarium oxysporum* sp. *lycopersici*) was suspended in varying concentrations (1.31, 4.11, 8.22, 12.33, 15.13) of H_2PtCl_6 and placed in water bath at required temperature (24.8, 35, 50, 65, 75.2°C) and the pH was adjusted (3.6, 5.0, 7.0, 9.0, 10.4) with Na_2CO_3. The nanoparticle formation was observed for a period of 72 hours for any color change from yellow to dark brown. Similarly, for extracellular synthesis (RSM_2) the fungal cell free extract was treated with H_2PtCl_6. The results of above experiments were evaluated using the RSM equation. According to the first order model of RSM_1 it was observed that the optimum yield of nanoparticles was found to be 3.23mg/l at pH 3.6, temperature 75.2°Cand H_2PtCl_6 concentration of 15.13mM. While, in case of RSM_2 model predicted that the optimum yield of nanoparticles was found to be 4.85mg/l at pH 10.41, temperature75.2°C and H_2PtCl_6 concentration of 15.13mM.

The TEM analysis of the extracellular solution (0, 2, 16, 24, 48 & 72 hrs) showed different geometrically shaped nanoparticles with varying morphologies after incubation up to 72 hrs. The TEM analysis of particles synthesized at 65°C depicted lower amounts of particles while, particles synthesized at 35°C gave a yield of 0.35mg/l nanoparticles. The wide variation observed in the TEM morphologies of nanoparticles signifies that control over experimental parameters like temperature and pH would help in determination of particle concentration.

Basavaraja *et al*. [40] reported extracellular synthesis of highly stable and crystalline silver nanoparticles using the fungus *F. semitactum*. The fungal cell filtrate was treated with 1mM silver nitrate, the color of the cell filtrate changed after 24 hrs of reaction from colourless to brown. The UV-vis spectral analysis of the samples of the reaction mixture indicated surface plasmon band at 420nm which increased in intensity with time interval of 40 to 120 hrs. For the verification of the UV-vis spectral analysis the XRD analysis of the samples were studied which depicted diffraction signals (111, 200, 220 & 311) corresponding to the face centered cubic structure of silver. The mean diameter of the particles was calculated using Scherrers equation. The average particle size of the silver nanoparticles was found to be 35nm. For further investigation of shape and size of nanoparticles TEM analysis of silver nanoparticles was carried out. The TEM micrographs of silver nanoparticles showed that the nanoparticles were isolated with majority of them spherical in shape with size of 10-60nm. The FTIR analysis of silver nanoparticles illustrated two bands at 1640 and 1540 cm^{-1} and were identified as amide I and amide II which arise due to carbonyl stretch and –N-H stretch vibrations of the amide linkages of proteins. These amide groups and peptide linkages of proteins have found to possess the capability to bind metal hence, in this case the proteins perhaps formed a coat over the silver nanoparticles and helped prevent aggregation of nanoparticles.

The fungus *Aspergillus flavus* when treated with aqueous silver ions accumulated silver nanoparticles on its cell wall [41]. The TEM micrographs showed the synthesis of monodisperse silver nanoparticles. The nanoparticles were protein stabilized which were assumed to be fungal proteins.

Synthesis of highly luminescent CdSe quantum dots at room temperature using *F. oxysporum* was demonstrated by Kumar *et al*. [42]. For the experimental purpose, the fungus *F. oxysporum* was challenged with aq. $CdCl_2$, $SeCl_4$ solution. The synthesis of CdSe nanoparticles was depicted after 96 hrs of reaction with a change in coloration to

reddish brown. The UV-vis spectra of the reaction mixture showed strong surface plasmon peak at 370nm. Further, the FTIR analysis showed intense peaks at 100, 111, 220, 311 & 222 corresponding to Bragg reflections of CdSe particles. The TEM analysis of the CdSe particles showed polydisperse particles in size range of 9-15nm with average size 11±2nm with spherical morphology. The X-ray photoelectron spectroscopy of the particles evidently showed presence of Cd, Se, C, O, N and Na as prominent elements. Nitrate reductase mediated method for the synthesis of silver nanoparticles has been employed by Kumar *et al.* [43]. The nitrate reductase enzyme was purified from the fungal cell-filtrate of *Fusarium oxysporum* by column chromatography, and was then used in an anaerobic reaction with silver nitrate, 4-hydroxyquinoline and NADPH. The synthesis yielded individual silver particles of 10-25nm and aggregates. The particles were well dispersed, crystalline and consisted predominantly of Ag, C, O, N and Na.

Photobiological synthesis of silver nanoparticles was investigated using *F. oxysporum* [44]. The UV-vis spectra depicted plasmon peak at 440nm corresponding presence of large metallic silver nanoparticles. The TEM and SEM micrographs of silver nanoparticles illustrated synthesis of spherical nanoparticles with size 10-80nm. The EDS analysis of the samples also confirmed that the nanoparticles were of metallic silver. Plant pathogenic fungus *Aspergillus niger* isolated from soil can serve as an efficient fabricator of silver nanoparticles [24]. The fungus *Aspergillus niger* was isolated from soil and used for the extracellular synthesis of silver nanoparticles. The biosynthesized silver nanoparticles were characterized with the help of UV-visible spectroscopy and TEM analysis. The UV-visible spectra showed an absorption peak around 420nm, while the TEM analysis confirmed the synthesis of spherical nanoparticles with a size of 20nm. Elemental Spectroscopy Imaging (ESI) was done to find out the protein content in the silver nanoparticles. The results of ESI depicted presence of fungal protein around silver nanoparticles thus, increasing the stability of silver nanoparticles. Also, the antibacterial activity of biosynthesized silver nanoparticles (10µg/ml) was evaluated against *Escherichia coli* and *Staphylococcus aureus*. To assess antibacterial activity, silver nanoparticles were treated against *S. aureus* and *E.coli* and characterized by TEM analysis which depicted the presence of elemental silver in the bacterial membrane while, some nanoparticles successfully penetrated the bacterial cells and completely disrupted the bacterial membrane therefore, proving the potency of silver nanoparticles as an efficient antibacterial agent.

Another plant pathogen *Fusarium acuminatum* isolated from infected ginger was also successfully exploited for extracellular synthesis of silver nanoparticles [28]. The plant pathogen when challenged with aqueous silver nitrate solution (1mM) depicted synthesis of silver nanoparticles which was further characterized by UV-visible study and TEM analysis. The optical spectrum showed plasmon resonance at 420nm, while the TEM analysis demonstrated synthesis of spherical nanoparticles in the range of 5-40 nm with average diameter of 13 nm. The synthesis of silver nanoparticles was observed to be due to the enzyme nitrate reductase present in the fungal cell filtrate which was also confirmed by using a specific substrate disc for nitrate reductase (Nitrate Reagent Discs DD 041, Hi-media, Mumbai, India). The color of the disc turned reddish from white when treated with the fungal cell filtrate indicating the presence of nitrate reductase in the fungal filtrate. The mycofabricated silver nanoparticles were also evaluated for antibacterial activity against human pathogenic bacteria like *Escherichia coli*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Staphylococcus aureus* using well diffusion method. Silver nanoparticles proved to be toxic to each of the above species and the effect was found to be 1.4–1.9X stronger than that of pure silver ions. In the present study, *S. aureus* showed the maximum zone of inhibition as compared to other bacteria.

Trichoderma asperellum a non-pathogenic fungi also possess capability to synthesize silver nanoparticles was depicted by Mukherjee *et al*. [27]. The biosynthesis of silver nanoparticles on treatment of fungal cell filtrate with 1mM silver nitrate was easily identified by the color change from yellow to dark brown within 24 hours. The UV-vis spectra exhibited intense peak at 410nm corresponding to the surface plasmon frequency of nanocrystalline silver. The XRD patterns of the reaction mixture showed diffraction pattern at 111 & 220 planes which were in agreement with the face centered cubic structure of metallic silver. Typical high resolution TEM micrographs of silver nanoparticles showed synthesis of highly stable nanoparticles with size range of 13-18nm.

A hypothetical mechanism for the synthesis of silver nanoparticles was corroborated according to the FTIR study of silver nanoparticles. The FTIR spectra of silver nanoparticles depicted intense peak at 1240cm^{-1} corresponding to stretching vibrations of Ag-N bonds and two broad bands at 1350 and 1565 cm^{-1} attributed to symmetric and asymmetric C=O stretching vibration of CO_2. Selective enhancement of these Raman bands indicated that C=O bonds and Ag-N bonds lie perpendicular to the nanosilver surface and gets associated with the formation of a cap over nanoparticles. Also, the symmetric and asymmetric stretching bonds of CO_2 significantly broaden due to distortion of the respective bond angles and bond lengths which further support in the encapsulation of silver nanoparticles. The band at 240 cm^{-1} confirmed the formation of a chemical bond between silver nanoparticles and the nitrogen of amino groups.

Sadowski *et al*. [45] exploited the fungus, *Penicillium* sp. For the extracellular synthesis of silver nanoparticles the fungal cell filtrate was treated in the dark with Ag^+ ions for the biosynthesis process. The reaction mixture showed color change from colorless to brown which intensified with the increase in incubation period. The UV-vis spectral analysis showed absorption peak around 440nm corresponding to larger silver nanoparticles. Further analysis of the particles was done by laser diffraction study, SEM and measurement of zeta potential. The laser diffraction study showed that the reaction mixture contained polydisperse nanoparticles ranging from hundreds of nanometers to micrometers. The SEM

micrographs of the nanoparticles were in accordance with the laser diffraction study and also depicted that the nanoparticles were partially aggregated. The effect of pH on the zeta potential was investigated in natural condition with pH close to 8. The zeta potential of the nanoparticles was found to be equal to -26.3±0.2mv thus concluding that silver nanoparticles possess negative zeta potential.

Gold (AuNP) and Gold-Silver (Au-AgNP alloy) nanoparticles were synthesized by extracellularly treating the fungus *F. semitactum* with Au and Ag^+ ions [46]. The AuNP and Au-AgNP nanoparticles were characterized by XRD, TEM and FTIR studies. The XRD study of the AuNP nanoparticles showed Bragg reflections 911, 200, 220, 3110 corresponding to the face centered cubic structure of silver. The XRD data of Au-AgNP also showed Bragg reflections corresponding to both Au and Ag patterns. The mean particle size of AuNP and Au-AgNP nanoparticles was calculated using Scherrers equation which revealed the average particle size of particles, 25 and 18nm respectively. The bright field TEM images of AuNP showed polydisperse spherical shaped particles in the size range of 18-80nm. While, the bright field TEM images of Au-AgNP showed variable sizes of polydisperse spherical nanoparticles in the size range of 10-35nm with an average size of 20nm. The FTIR analysis of AuNP and Au-AgNP particles showed peaks at 1643, 1543, 1405 and 1075cm^{-1} corresponding to the presence of amide I and amide II bonds responsible for the bioreduction of metal ions. N-H vibrations were found to be responsible for the formation of a cap over nanoparticles and prevent agglomeration.

Combined effects of biosynthesized silver nanoparticles from *Phoma glomerata* [47], was evaluated in combination with commercially available antibiotics using disc diffusion method. Further, the data was statistically analyzed by evaluation of increase in fold area. The silver nanoparticles were synthesized by treating the fungal filtrate with 1mM silver nitrate solution and analysis of samples using UV-visible, FTIR, and SEM analysis. The UV-visible spectra depicted absorption spectra around 440nm. SEM study showed spherical nanoparticles in size range of 60-80nm with some aggregates. The FTIR analysis confirmed capping of silver nanoparticles by biomolecules. The combined effect of silver nanoparticles was appraised against three human pathogenic bacteria (*S. aureus, E. coli* and *P. aeruginosa*). The antibacterial activities of ampicillin, gentamycin, streptomycin and vancomycin were comprehensively increased in combination with silver nanoparticles against *E. coli*, *P. aeruginosa* and *S. aureus*. In contrast, the synergistic activity observed was better in *E. coli* and *P. aeruginosa* than *S. aureus*.

A phytoapthogen *F. solani* (USM-3799) was harnessed for the extracellular biosynthesis of silver nanoparticles by Ingle *et al*. [48]. The biosynthesized nanoparticles were characterized using FTIR and TEM study. The FTIR spectra of the silver nanoparticles depicted presence of functional groups like C-N, C-O-C, amide linkages and –COO-. These functional were found to play an important role in the capping of nanoparticles and their further stability in aqueous solution. The TEM images of the nanoparticles further revealed the synthesis of Polydisperse spherical nanoparticles in the size range of 5-35nm with average size of 16.23nm.

Fungus mediated synthesis of silver nanoparticles was reported using the fungus *Penicillium*(J3 strain) [49]. Ten different strains of *Penicillum* (J1, J2, J3, F4, F16, MEA F16, MEA F5, MEA P22, MEA W4 and MEA W18) were screened for the synthesis of silver nanoparticles. The fungal cell filtrate of each of the ten strains of fungus was treated with Ag^+ ions and observed for change in coloration. The J3 strain of *Penicillum* showed change in coloration from colorless to brown while the other test strains did not. Hence, the J3 strain of the fungus was further analyzed. The UV-vis spectra of the samples showed absorbance peak at 425nm implying the bioreduction of Ag^+ ions. The SEM micrographs of the fungi also depicted synthesis of silver nanoparticles. The synthesis of silver nanoparticles was further confirmed by TEM analysis which depicted presence of spherical nanoparticles in the size range of 10-100nm with average size of 60nm.

1.3 Mechanistic Aspects

Fungal cell wall and cell wall sugars are likely to play an important role in the reduction of metal ions [29]. The fungal cell wall is a dynamic structure, which changes and modifies at different stages in the life cycle of a fungus. It is composed of a microfibrillar component located to the inner side of the wall and usually embedded in an amorphous matrix material. The prime components of the fungal cell wall include β-linked glucans and chitin, while the matrix consists mainly of polysaccharides that are mostly water-soluble [50].

The fungal cell wall also plays a pivotal role in the absorption of heavy metals. The intracellular synthesis of nanoparticles can be explained using a step wise mechanism. In the preliminary step of bioreduction, trapping of metal ions takes place at the fungal cell surface. This is probably due to the electrostatic interaction of the positively charged groups in enzymes present on the cell wall mycelia. In the next step, the metal ions are probably reduced by the enzymes within the cell wall, which leads to the aggregation of metal ions and formation of nanoparticles [29] (Figure1). The TEM analysis of the fungus also, depicts presence of nanoparticles on the cytoplasmic membrane as well as within cytoplasm. This shows the possibility that some Au^+ ions diffuse through cell wall and are reduced by the enzymes present on the cytoplasmic membrane and cytoplasm while, some of the smaller nanoparticles diffuse through the fungal cell wall and get trapped within the cytoplasm [25].

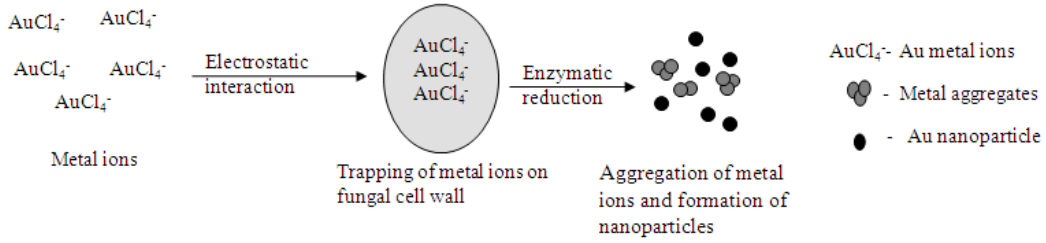

Figure 1: Hypothetical mechanism for intracellular synthesis of silver nanoparticles

To explore the mechanism of extracellular synthesis of nanoparticles using fungi whether it is possibly due to reductase action or by electron shuttle quinones or both [38] conducted the nitrate reductase assay test through the reaction of nitrite with 2, 3-diaminophthalene. The emission spectrum demonstrated two major peaks of fluorescence intensity at 405 and 490 nm relating to the emission maximum of nitrite and 2, 3-diaminonapthotriazole (DAN) respectively. The intensity of these two bands was found to be increased with the addition of a 0.1% KNO_3 solution, confirming the presence of nitrate reductase. Thus, it was concluded that the enzyme reductase is responsible for the reduction of Ag^+ ions and the subsequent formation of silver nanoparticles. The role of nitrate reductase in the synthesis of nanoparticles was also studied by Kumar *et al*. [43]. The enzyme a-NADPH-dependent nitrate reductase was isolated from *Fusarium oxysporum* and used for the *in vitro* synthesis of silver nanoparticles. The UV-visible spectra of the control samples depicted absorption bands at 260-270nm corresponding to proteins, a-NADPH and hydroxyquinoline. The spectra of reaction mixture showed strong surface plasmon resonance at 413nm which intensified with time while, the absence of absorption band at 413 nm for the reaction mixture in the absence of enzyme clearly depicted that the reduction of silver involves enzymatic reduction of nitrate to nitrite. Thus, indicating that the synthesis of silver requires the reduction of a-NADPH to a-$NADP^+$ and the hydroxyquinoline probably acts as an electron shuttle transferring the electron generated during the reduction of nitrate to Ag^+ ions converting them to Ag^0 (Figure 2).

In accordance with the above studies Ingle *et al*. [28] conducted the nitrate reductase test using commercially available nitrate reductase discs to clarify the above assumptions. The color of the disc turned reddish from white when challenged with fungal filtrate signifying the presence of nitrate reductase. Thus, it can be concluded that the enzyme a-NADH dependent reductase is associated with reduction of Ag^+ to Ag^0 in the case of fungi.

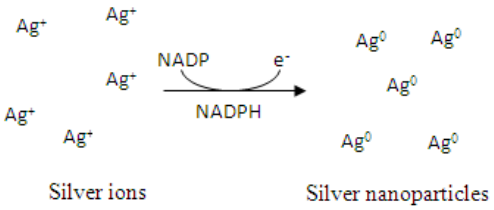

Figure 2: Hypothetical mechanism for extracellular synthesis of silver nanoparticles

1.4 Multifunctionality of metal nanoparticles

The study of biosynthesis of nanomaterials using the fungal system offers valuable contribution into material synthesis. Advancement of nanoscale devices using biosynthesized nanomaterials and their use in a wide range of applications has recently fascinated researchers towards bionanotechnology [51], [3].

Silver nanoparticles (SNPs) have been recognized to exhibit antibacterial properties [52]. The use of silver in its metallic state has been conceded since centuries but with the appraisal of silver in the form of nanoparticles extensive research has been done to improve the efficacy of silver based antibacterial agents in the form of surgical instruments [53], topical drugs [54], dressings [55], [56] etc. Silver nanoparticles have also depicted use in preparation of material for electrical batteries, polarizing filters, staining pigments for glasses and ceramics [49].

Silver nanoparticles have also demonstrated antiviral properties by undergoing size-dependent interaction with HIV-1. Silver nanoparticles preferentially bind with the gp120 subunit of the viral envelope glycoprotein and inhibit the HIV-1 virus infectivity [57].

Gold nanoparticles have been efficiently used in cancer therapy as they show strong absorption of light which is efficiently converted into thermal energy and destructs malignant cells [58]. Binding gold nanoparticle surfaces with drug molecules is also practical in biomedical applications. Plate-like gold nanoparticles have been reported such as to

slice through the cells after surface functionalization with drugs or labelling the particles with various desired carrier biomolecules. Additionally, the infrared absorption of the anisotropic particles could also be utilized for hyperthermia in various therapeutic treatments like for cancer and in architectural applications like infrared-absorbing optical coatings [59]. But recently gold nanoparticles have come up as alternative materials to assist in the purification of water as selective photothermal agents using visible light to control microorganisms in water [60].

Optical properties of non-spherical gold nanoparticles are easy to tune along with particle size and shape hence, show a wide range of applications like optical labelling for biosensing events and biomedical labelling. Thus, it is speculated that these non-spherical gold nanoparticles could play a major role in future cancer diagnosis and therapy [61]. Gold nanoparticles are also found to play important role in areas like pollution control (e.g. water & air filter), chemical processing, fuel cell technology (e.g. solar & fuel cells) [62].

Zirconia nanoparticles due to their intrinsic physico-chemical properties are used as resistant coating tool, in high temperature engine components and as catalyst [32]. Titanium nanoparticles have been used in sunscreen, cosmetics [33]. Samarium nanoparticles have paved a new application of nanotechnology in nuclear medicine for detection and treatment of cancerous tumours [63]

Alginate nanoparticles have been used to develop a natural polymer based nanoparticle drug delivery system for four key Anti-Tuberculosis drugs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol). The efficiency of drug encapsulated alginate nanoparticles was tested against TB-infected mice, which depicted complete bacterial clearance from lungs, liver and spleen [64]. Platinum nanoparticles are noteworthy in the industrial production of fuel cells [26].

Magnetite nanomaterials synthesized using the biosynthetic approach can be used as precursors instead of cyanide complexes to avoid cyanide toxicity to the environment [65]. Magnetic nanoparticles are also useful in the removal of environmental contaminants from water [66]. Semiconductor CdSe quantum dots due to their optical and electronic properties can be used as luminescent probes [42].

Antimony oxide (Sb_2O_3) nanoparticles are applicable as conductive materials, effective catalyst, functional filler and optical material [67].

Recent application of nanoparticles includes areas like biological sensing and labelling and imaging of live cells and tissues [68]. Future applications of nanoparticles involve development, transport and detection of digital information for security of home land, high speed data communication and computing components [61].

1.5 Future Perspectives

Nanotechnology is one of the most significant areas of research and its impact is felt in our present day life. With the advancement of the technology nanoparticles are expected to solve large scale problems using nanoscale solutions.

The biosynthetic route for the synthesis of metal nanoparticles using fungi is a simple process involving the reaction of fungal culture with aqueous solutions of metal ions. But there are a number of questions, which need to be addressed. The synthesis process points out that there are a number of reducing agents involved in the reduction of metal ions and corresponding formation of nanoparticles. These reducing agents also affect the size and shape of nanoparticles hence, there is a need to investigate the exact mechanism involved in the biosynthesis of nanoparticles. Studies on the synthesis of nanoparticles of specific size and shape depend on different factors like temperature and light intensity. Biosynthetic approach for nanoparticle synthesis also needs to focus on the shape selectivity and size monodispersity of nanoparticles. Studying the novel shape and size dependent physical and chemical properties of nanoparticles and their subsequent interaction could help in development of a new range of photonic and electronic devices that can control and manipulate light at nanoscale. Establishment of low-cost recovery techniques to make the synthesis process commercially feasible also needs to be undertaken.

1.6 Conclusion

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.

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