BioThink, Vol -1, Issue -2
e- ISSN: 3048-7943

Fungi as Natural Nanofactories: Synthesis and Applications

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Introduction

Nanotechnology has gained the spotlight in this decade due to its promising activities in agriculture, medicine, energy, and the environment. Nanoparticles have unique optical, electrical, magnetic and chemical properties that make them significantly practical. However, the biological use of nanomaterials is determined by its process of production and metal characteristics. Nanotechnology enhances the agricultural inputs by enabling targeted distribution, controlled release, increased solubility and longer shelf-life. These properties improve efficiency and lower the danger of environmental pollution. Mycogenesis of nanomaterials enhances the potential of metal and metal oxide nanoparticles in agriculture by decreasing toxicity and improving stability. Nanosensors have enhanced conventional agriculture by detecting pests and soil conditions, leading to smarter systems.

Top-down vs Bottom-up synthesis

Nanoparticle synthesis is the process of generating nanoscale materials with sizes ranging from 1 to 100 nanometers. Nanoparticles can be synthesized using two main approaches *viz.*, top-down and bottom-up methods. Top-down synthesis involves breaking down larger bulk materials into nanoscale particles through techniques like laser ablation, lithography, etching and milling (Arole and Munde, 2014). They can produce very high quality nanoparticles with wider size distribution and requires complex equipments. In contrast, bottom-up synthesis builds nanoparticles from atomic or molecular components using methods such as sol-gel processes, co-precipitation, chemical vapor deposition and biomimetic approaches. It may involve complex chemical reactions they are having better control over size and morphology of nanoparticles (Mazhar *et al*. 2017). The desired characteristics of the nanoparticles and their intended uses will determine which of these approaches is best.

Mycosynthesis of Nanoparticles

The synthesis of nanoparticles using fungi based on green chemistry principles is a feasible method. Biomolecules released by the microbial biomass, such as enzymes and proteins, can act as reducing and capping agents during synthesis. Therefore, these procedures are termed green chemistry since they do not use any harmful compounds (Ahmad *et al*., 2010). Fungal families such as *Aspergillus, Alternaria, Bipolaris, Colletotrichum, Candida, Cladosporium, Fusarium, Helminthosporum, Mucor, Neurospora, Pleurotus, Penicillium, Trichoderma, Verticillium* are fungus species have been extensively studied for synthesis of metallic nanoparticles such as gold, silver, zinc, iron, titanium and platinum etc. Among these

Aspergillus and *Fusarium* are highly used for the mycogenesis of NPs. The synthesis procedure involves preparing a microbiological extract, combining it with a metal salt solution of a certain concentration, and keeping the reaction mixture with the appropriate temperature and pH. The completion of the reaction is signaled by a change in color. The microbe mediated production of nanoparticles occurs through the action of electron shuttle quinones, nitrate reductase or may be both. The nitrate reductase and α-NADPH-dependent reductases are primarily in charge biogenic creation of metallic nanoparticles in most of the microorganism. The enzymes secreted by the fungi influences the green synthesis of NPs by either altering the composition or by affecting the formation of initial particles (Fig. 1).

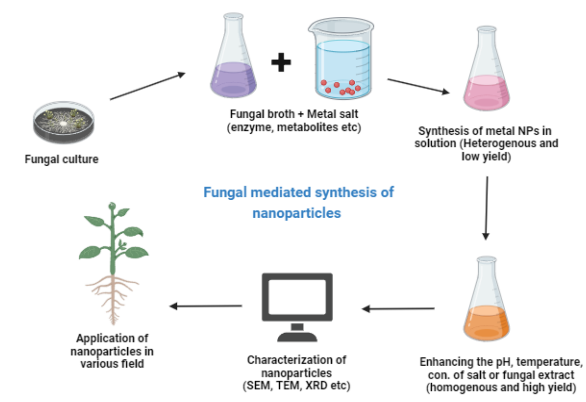


Fig. 1: Steps involved in mycogenesis of nanoparticles

Exploration of fungi in nanoparticle synthesis

Gold and silver NPs are the widely studied nanoparticles owing to their natural compatability with living beings. *Verticillium* sp. was utilized to reduce aqueous $AuCl^{-4}$ ions, producing gold nanoparticles with their preferred structure and monodispersity by the process of intracellular synthesis. A comprehensive investigation found that metal ions $AuCl^{-4}$ were bound on the surface of fungal biomass through electrostatic interaction with charged residues of amino acids in enzymes in the fungal cell wall (Alghuthaymi *et al*., 2015). *Aspergillus niger* produces very stable silver nanoparticles via an extracellular pathway. *A. niger* excretes quinone and nitrate-dependent reductase, which act as capping and chelating agents in the production of silver nanoparticles, as demonstrated by elemental spectroscopy. *Fusarium oxysporum* produces silver nanoparticles by hydrolyzing $AgNO_3$ solution. When the fungal mycelia were exposed to aqueous silver nitrate at a concentration of 1 mmol/L. The filtrate color shifted from light yellow to brown and gets intensified after 2-3 hours. The NPs were detected and characterized TEM and UV-Vis spectrophotometer which generated spherical NPs in the range of 5 - 40 nm (Ingle et al., 2009). The Magnetotactic bacteria *Magnetospirillium magneticum* produces two different types of iron NPs *viz.*, Fe_3O_4 and Fe_3S_4. Similarly a sulfate-reducing bacterium, *Desulfovibrio desulfuricans* was reported to synthesize palladium nanoparticles in the presence of an exogenous electron source (Omajali *et al*., 2015) (Table 1).

Intracellular synthesis extracellular synthesis of nanoparticles

Fungi secrete a large quantity of various enzymes especially reductase which are highly involved in the bioreduction and stabilization of nanoparticles. So fungi synthesize large quantity of nanoparticles when compared to bacteria. Since the cell wall of the fungal biomass is negatively charged with sticky materials, the metal ions adhere to it through electrostatic contact with these adhesive materials. NPs can be synthesized either intracellular or extracellular synthetic methods. In case of intracellular synthesis, the metal ion binds with the negatively charged suface of fungal culture. The metal ions will undergo reduction process because of the enzyme nitrate reductase and produces nanoparticles in the fungal cell surface. Whereas, in extracellular synthesis the reduction of metal ions and the formation of nanoparticles will be occur in the the fungal cytoplasm (Fig. 2).

Table 1: Exploration of fungi in nanoparticle synthesis

Fungi	Metal ions	Mechanism	Size	Shape	Application	Reference
Aspergillus niger	Ag	Extracellular	3-30	Spherical	Antibacterial, antifungal	Jaidev and Narasimha, 2010
Aspergillus oryzae	Ag	Extracellular	5-50	Spherical	Bactericidal	Binupriya *et al*., 2010
Aspergillus fumigatus	ZnO	Extracellular	1.2-6.8	Spherical, hexagonal	Industrial, medical & agricultural	Raliya and Tarafdar, 2013
Aspergillus flavus	TiO_2	Extracellular	12-15	Spherical	Plant nutrient	Raliya *et al*., 2015
Aspergillus flavus	TiO_2	Extracellular	62-74	Spherical	Antimicrobial	Raliya *et al*., 2015
Aspergillus tubingensis	Ca_3P	Extracellular	28.2	Spherical	Agricultural, biomedical & engineering	Tarafdar *et al*., 2012
Alternaria alternata	Ag	Extracellular	5-20	_	Antimicrobial	Gajbhiye *et al*., 2009
Coriolis versicolor	Au	Extra- and	20-100	Spherical,	_	Sanghi and

		intracellular		ellipsoidal		Verma, 2010
Candida albicans	Au	_	20–40, 60–80	Spherical, nonspherical	Detection of liver cancer	
Fusarium oxyporum	Ag	Extracellular	20-50	Quasi-spherical	Antibacterial	Durán *et al.*, 2010
Fusarium oxysporum	Fe_3O_4, Bt	Extracellular	20–50 4-5	Quasi-spherical	Antibacterial	Bharde *et al.*, 2006
Fusarium semitectum	Au	_	25	Spherical	Optoelectronics	Sawle *et al.*, 2008
Pleurotus sajor caju	Ag	Extracellular	30.5	Spherical	Antibacterial activity	Vigneshwaran and Kathe, 2007
Penicillium brevicompactum	Au	_	10−50	Spherical	To target cancer cells	Mishra *et al.*, 2011
Rhizopus oryzae	Au	Cell surface	10	Nanocrystalli ne	Agricultural pesticides	Das *et al.* 2009
Trichoderma viride	Ag	Extracellular	5-40	Spherical, rod-like	Synergistic effect with antibiotics	Fayas *et al.*, 2010
Trichoderma viride -	Ag	Extracellular	2–4	Mostly spherical	Biosensor and bio imaging	Fayas *et al.*, 2010
Volvariella volvacea	Au	_	20-150	Spherical	Therapeutic	Philip, 2009
Verticillium sp.	Fe3O4	Extracellular	100–400, 20–50	Octahedral, quasi-spherical	_	Bharde *et al.*, 2006

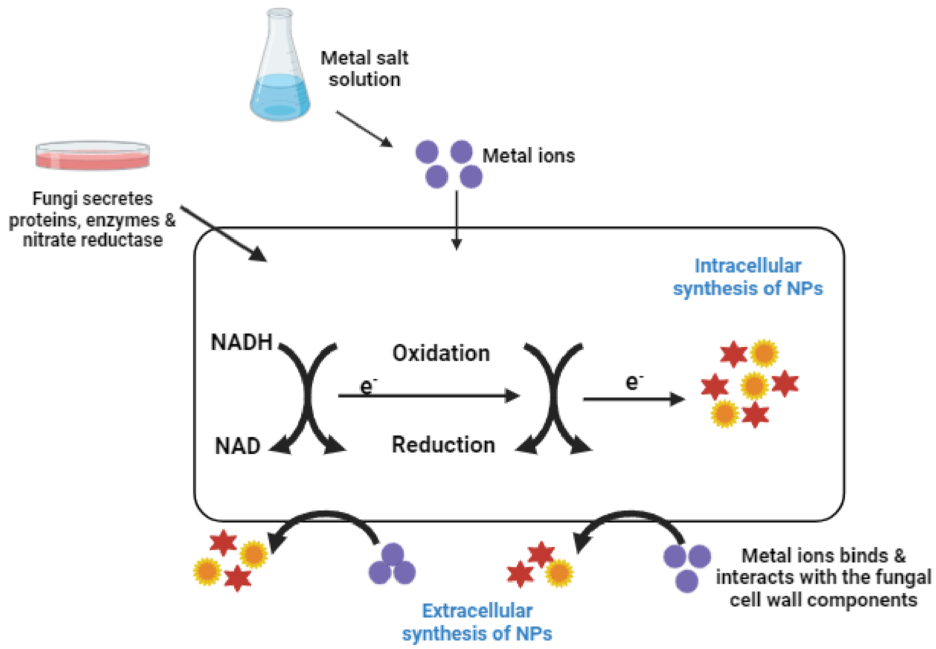


Fig. 2: Intracellular and extracellular synthesis of nanoparticles

Advantages of NPs synthesized from fungal organisms

1. Fungi have a high potential for metal absorption and tolerance.
2. It minimizes the dependency on hazardous chemicals, increasing the sustainability of nanoparticle manufacturing and lowering environmental toxicity.
3. It improve the effectiveness of fungicides and biopesticides, offering a more focused method of controlling pests while using less chemicals.
4. Microbial nanoparticles can improve soil fertility by increasing nutrient intake and soil microbial activity, resulting in greater plant development.
5. Extracellular enzyme synthesis requires fewer stages in the downstream pathway.
6. The fungal culture can be easily grown and are easy to handle
7. Fungal biomass can easily produces the metal nanoparticles of varied forms, size and shapes.

Characterization of Nanoparticles

UV–visible spectroscopy

Noble metal nanoparticles may be characterized via UV-visible spectroscopy because of their brilliant color and high extinction coefficient. Their surface plasmon resonance varies by size and shape. As a result, this approach can provide qualitative information on nanoparticles. For example in case of gold NPs the colour change occurs from ruby red to purplish blue.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR can be used to detect certain types of chemical bonds or functional groups based on their distinct absorption fingerprints by measuring chemical bond stretching and bending via energy absorption using infrared spectroscopy. This energy is in the infrared (IR) range of the electromagnetic spectrum.

X-ray diffraction technique (XRD)

This non-destructive approach examines the crystalline phase of nanoparticles. The technique involves placing a crystalline or powdered sample on a sample holder, exposing it to X-rays of a certain wavelength, and measuring the strength of the reflected radiation with a goniometer. The data is then evaluated using Bragg's equation.

Atomic force microscopy (AFM)

AFM is an analytical instrument which provides provides data pertaining to the surface shape and phase by producing a 3D surface map of the sample. It uses the Vander Waal’s force on the sample and the received signal are processed to obtain the topographical information of the surface.

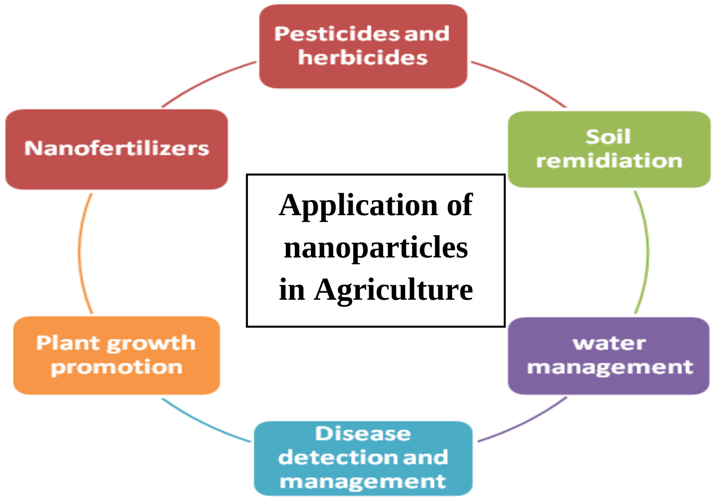
Transmission electron microscopy (TEM)

It is employed to determine the metal nanoparticles size, shape and dispersion. Diffraction effects prevent optical microscopy from seeing molecules smaller than 1 µm. The resolution of a monograph relies on the wavelength of the radiation beam used for imaging. A short wavelength beam provides high resolution. The transmitted beam is directed on a phosphorous screen to generate the final picture.

Scanning electron microscopy (SEM)

SEM is used to determine the shape and topology of metal nanoparticles. The picture of the specimen's surface is obtained by scanning it with an electron beam of elevated voltage.

The detector collects and analyzes backscattered and secondary electrons to produce images. It can detect the NPs even below 10 nm.



Application of nanoparticles

Conclusion

The fungal mediated synthesis of nanoparticles is a potential green synthesis technique for synthesis of nanoparticles as it takes advantage of fungi metabolic processes to create materials with unique features. This approach has several advantages, including eco-friendliness, sustainability and the capacity to produce nanoparticles with desirable properties like as size, shape, and surface functioning. The nanoparticles created by this technology have showed promise in agriculture, including improved nutrient delivery, insect management, plant growth and maintaining soil health. As the agriculture industry pursues more sustainable methods, fungal-mediated synthesis offers an innovative way to satisfy these demands while reducing environmental effect.

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