Nanoparticle synthesis of Fungi

Fungi are a relatively recent addition to the list of microorganisms used for nanoparticle synthesis. Fungi are more beneficial than other microorganisms in many ways. They grow in the form of mycelial mesh which helps them to bear flow pressure and agitation and other conditions to which microbes are subjected to in a bioreactor used for large scale production. Though they require better precision and care to grow but they are easier to handle and manipulate. They secrete enzymes in large amounts. The nanoparticles are generally generated extarcellularly (sometimes intracellular production does occur) they are devoid of various impurities from the cell and can be used directly.

The use of eukaryotic organisms for nanoparticle synthesis was first demonstrated by use of *Verticillium sp*. for the synthesis of gold nanoparticles. In this experiment, gold nanoparticles were reported on the surface and cytoplasmic membrane of the fungal mycelia. Due to the formation of gold nanoparticles the mycelial mass attains a typical purple color demonstrating intracellular generation. TEM analysis shows that particles of well-defined geometry like triangular, hexagonal or spherical shape were formed on the cell wall and quasi-hexagonal morphology were formed of the cytoplasmic membrane. The fungal biomass on exposure to silver nitrate solution was also found generate silver nanoparticles intracellularly. The powder diffraction indicates the crystal nature of both the nanoparticles. The exact mechanism for the synthesis of nanoparticles by *Verticillium* is not yet known. It is thought that the first step is the interaction between positively charged metal ions and negatively charged carboxylates on the enzymes present in fungal cell wall and adhesion of the metal ions to the surface as a result of this interaction. The enzymes reduce these metal ions to elemental metal which serve as nucleation sites and further growth is carried out by subsequent reduction and accumulation. The ability of *Verticillium* to grow and replicate even after exposure to metal ions demonstrate their ability to be used commercially for production of nanoparticles.

A plant pathogenic fungus, *Fusarium oxysporum* has also been studied extensively. It was found that it was able to generate gold and silver nanoparticles extarcellularly rapidly. This was observed from the fact that the supernatant changed its color but the mycelial mass retained its original color. Moreover the fungal extract was also able to generate gold and silver nanoparticles. It is believed that the fungus releases reductases in the solution which are responsible for the reduction of metal ions. This makes in-vitro generation of nanoparticles using an enzyme/cell extract based process possible. It was recently discovered that when *F. oxysporum* is exposed to equimolar solutions of hydrogen tetrachloroaurate (III) and silver nitrate led to the production of gold-silver alloy. The presence of only one plasmon resonance, shifting gradually from gold to silver and back indicates the formation of homogeneous alloy rather than segregated metal or core/shell type structure. The fungus on exposure to cadmium sulfate solution was found to yield cadmium sulfide quantum dots (5-20 nanometers) with hexagonal morphology. Long term incubation of the fungus with cadmium nitrate does not yield cadmium sulfide nanoparticles which indicate the action of sulfate reducing enzyme. Polyacrylamide gel electrophoresis of the extract led to four different protein bands. These proteins were extracted using dialysis and addition of ions to this solution does not yield cadmium sulfide which attests to the presence of some other factor. Addition of ATP and NADH restored the capability to produce quantum dots.

Nanoparticle synthesis by Yeasts

Yeasts are most useful in the synthesis of semiconductor nanoparticles like cadmium sulfide, lead sulfide, antimony oxide, etc. *Candida glubrata* is the yeast which can intracellularly synthesize uniform spherically shaped peptide-bound CdS nano-crystals of size about 20 Å. They tend to form metal–thiolate complex with phytochelatins which neutralize of metal ions *Schizosaccharomyces pombe* can also synthesize CdS nano-crystals with hexagonal crystal structure of particle size 1-1.5 nm. *Torulopsis sp.* was the first yeast in which synthesis of face centered cubic structured PbS nano-crystals, showing semiconductor properties, which was intracellularly produced in the vacuoles having a dimension of 2–5 nm in spherical structure when incubated with Pb2+ reflects λ_{max} of 330 nm in UV–Vis spectrophotometer. Diode junction can be formed using these nanoparticles. *S. cerevisiae* (baker's yeast) can reduce Au^{+3}

to give gold nanoparticles. Reduction happens in the peptidoglycan layer of the cell wall by the aldehyde group present in reducing sugars. *Pichia jadinii* is another yeast which can intracellularly synthesize the gold nanoparticles of different morphologies like spherical, triangular, hexagonal etc. of the size less than 100 nm in the cytoplasm of the cell within a day. Gold nanoparticles are also synthesized by the tropical marine yeast *Yarrowia lipolytica* by reducing the gold ions using pH control regulations. It synthesizes nanoparticles of various morphologies when subjected to different pH cultures. At pH 2.0 it produce hexagonal and triangular gold crystals because of the nucleation of the nanoparticles on the cell surfaces giving rise to golden color which falls in the visible range spectrum having wavelength 540 nm and at pH 7.0 and pH 9.0 gold nanoparticles gives pink and purple colors with an average size of ~ 15 nm. *S. cerevisiae* can also synthesize face-centered cubic unit cell antimony oxide (Sb2O3) nanoparticles with spherical morphology of size 2-10 nm at room temperature conditions. This was possibly due to the radial tautomerization of membrane-bound quinines or by membrane-bound or cytosolic pH-dependent oxidoreductases. Antimony oxides are an important ingredient for semiconductor industry. *MKY3* is the only yeast so far discovered which is capable of extracellular silver nanoparticles synthesis of hexagonal crystal structure of size 2-5 nm in log phase growth of the yeast. These silver nanoparticles are used to make silver tolerant strain.

Nanoparticle	**Bacteria**	**Fungi**
Au	*Rhodococcus sp.*	*Candida albican*
	Shewanella oneidensis	*Yarrowia lipolytica*
	Plectonema boryanum UTEX 485	*Neurospora crassa*
	Escherichia coli	*Phanerochaete chrysosporium*
	Pseudomonas aeruginosa	*Candida utilis*
	Rhodopseudomonas capsulate	*Neurospora crassa*

	Brevibacterium casei	
	Ureibacillus thermosphaericus	
Ag	*Bacillus licheniformis*	*Trichoderma viride*
	Escherichia coli	*Phaenerochaete chrysosporium*
	Corynebacterium glutamicum	*Aspergillus flavus*
	Bacillus cereus	*Aspergillus fumigatus*
	Pseudomonas stutzeri	*Verticillium sp.*
		Fusarium oxysporum
		A. tubingensis
		Cladosporium cladosporioides
		Chrysosporium tropicum
		Phoma glomerata
Hg	*Enterobacter sp*	
Se	*Shewanella sp*	
CdTe	*Escherichia coli*	
Zn	*Streptomyces sps*	*A. flavus*
		A. terreus
		A. fumigatus
		A. tubingensis
Mg		*A.tubingensis*
		A. fumigatus
		Aspergillus brasiliensis

Conclusion

Ever-growing awareness about the necessity to turn towards environment-friendly approaches for materials synthesis to protect earth's environment has led to the development of eco-friendly, safe and green biological methods for nanoparticle production. Unlike other physical and chemical processes which involve the use expensive and sometimes hazardous chemicals and equipments, biological processes are cost-effective and eco-friendly. Hence, microbial synthesis of nanoparticles has emerged as an important part of nanotechnology. It is an interdisciplinary field which requires collaborations of physicists, chemists, biologists and engineers. Due to their vast diversity, bacteria, fungi and yeasts are able to produce a wide variety of nanoparticles and can act as biofactories for nanoparticles. However, a number of issues still need to be addressed both from nanotechnology and microbiology point of view before biosynthesis procedures can replace the traditional methods. The rate of production and monodispersity of the particles need to be improved and to achieve this microbial cultivation method and downstream processing have to be improved. The biochemical pathways involved in the synthesis of nanoparticles must be studied thoroughly and the specific genes and enzymes involved must be characterized. This will help us to have better control over the parameters that define the properties of a nanoparticle such as size, shape and monodispersity. Genetic engineering can also be used to make possible the use of organism whose growth process is known in detail and can be manipulated to our best interests. Future research on microbial synthesis of nanoparticle with unique properties is of great importance for applications in the field of medicine, agriculture and electronics.

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