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# Nanoparticles fabrication using ambient biological resources

-REVIEW ARTICLE-

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## ABSTRACT
Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction at cell level between synthetic and biological materials with the help of nanoparticles. A wide range of nanophasic and nanostructured particles are being fabricated globally with the aim of developing clean, nontoxic and eco-friendly technologies. Use of ambient biological resources in this area of science is rapidly gaining importance owing to its growing success and simplicity. Currently, simple prokaryotes to complex eukaryotic organisms including higher angiospermic plants are used for the fabrication of NPs. Further studies are required on precise and specific analysis of nanoparticles' production, including the nature and activity of diverse sizes of particles to optimize their production. This article presents a review of the ambient biological systems that may support and revolutionize the art of fabrication of nanoparticles and the development of an updated knowledge base.

**Key words:** Nanoscience, nanoparticle fabrication, nanotechnology, biosorption, bioreduction.

**ABBREVIATIONS:** Ag - Silver, Au - Gold, Pd -Palladium, Cd - Cadmium, Pt - Platinum, CdS -Cadmium Sulphide, Pb – Lead, Zr - Zirconium, $Fe_3O_4$ -magnetite, $Fe_3S_4$ - gregite, $AgNO_3$ – Silver nitrate, $HAuCl_4$ - Chloroauric acid, Sphalerite- ZnS, MTB- Magnetotactic bacteria, NADPH -Nicotinamide Adenine Dinucleotide Phosphate (reduced), XAS - X-ray absorption studies, TEM -Transmission electron microscopy, FTIR- Fourier Transform Infrared Spectroscopy, PAGE- Polyacrylamide Gel Electrophoresis, NaCl- Sodium chloride.

## INTRODUCTION
Nanoscience is currently a fast growing niche and nanotechnology is at the cutting edge of this rapidly evolving area (Mandal et al., 2006). Nanotechnology collectively describes technology and science involving nano scale particles (nanoparticles) that increases the scope of investigating and regulating the interplay at cell level between synthetic materials and biological systems (Du et al., 2007). It can be employed as an efficient tool to explore the finest processes in biological processes (Sondi & Salopek-Sondi, 2004) and biomedical sciences (Hütten et al., 2004). Besides this, NPs play an indispensable role in drug delivery, diagnostics, imaging, sensing,

gene delivery, artificial implants and tissue engineering (Morones et al., 2005). In the current context, importance is being given to the fabrication of a wide range of nanomaterials for developing environmentally benign technologies in material synthesis (Bhattacharya et al., 2005).

Nanotechnology, involving nanoparticles (NPs), deals with the synthesis and control of matter at molecular level in a scale smaller than 1 nm (Mude et al., 2009). The size of NPs is analogous to that of most of the biological molecules and structures, i.e. in terms of diameter, fine particles cover a range in between 100 and 2500 nm, while ultra fine particles are between 1 and 100 nm. Despite of their minute structure, they trigger the chemical activity due to their distinctive crystallographic nature that increases surface area, hence the scope of reactivity (Osaka et al., 2006; Singh et al., 2008).

The enormous interest in the biosynthesis of NPs is due to their unusual optical (Krolikowska et al., 2003), chemical (Kumar et al., 2003), photochemical (Chandrasekharan et al., 2000) electronic (Peto et al., 2002) and magnetic (Watson et al., 1999) properties. NPs are either newly created via nanotechnology or are present naturally over the earth's crust or in the environment caused by weathering of Au deposits. The metal formed by evaporation is coupled with minerals and been deposited rapidly from saline groundwater. Sizes of the Au NPs are about 200nm diameter (Hough et al., 2008).

Nanotechnology has been defined as a technology that mainly consists of the process of separation, consolidation and deformation of materials by one atom or molecule (Taniguchi, 1974). It comprises mainly three types (Singh et al., 2008) – (i) Wet nanotechnology, which deals with the study of biological systems that exist primarily in water base system, such as membranes, enzymes and cellular components; (ii) Dry nanotechnology, which is derived from surface science and physical chemistry, and mainly focuses on the fabrication of structures in carbon, silicon as well as other inorganic materials and (iii) Computational nanotechnology, which permits the modeling and stimulation of complex nanometer scale structures. All these technologies are highly reciprocal and interdependent.

Fabrication of NPs, through technology, can be undertaken using either chemical or biological systems (Panacek et al., 2006). The importance of biological synthesis is being emphasized globally at present because chemical methods are capital intensive, toxic, non ecofriendly and have low productivity (Kowshik et al., 2003). Utilizing potential biological systems from higher angiospermic plants or microbes, biosynthesis of NPs is currently under wide exploration (Bansal et al., 2004, 2005; Kumar et al., 2007a). These ambient biological systems provide excellent examples of nanophasic materials with highly optimized characteristics resulting from evolution over a long scale of time (Dickson, 1999) and the synthesis of inorganic materials may occur either extracellularly or intracellularly (Senapati et al., 2004). Exposure to varying temperature, pH and substrate concentration influences, directly or indirectly, the rate of intracellular NPs fabrication (Gericke & Pinches, 2006). Recently, callus extract of *Carica papaya* (Mude et al., 2009), and microbes, e.g. *Klebsiella pneumonia* (Mokhtari et al., 2009) and *Penicillium fellutanum* (Kathiresan et al., 2009) were found to have potential application for Ag NPs fabrication.

It is important to understand the biosynthetic mechanism involved in the fabrication of metal nanomaterials mediated by a biological system in order to gain better control of the process and products. So far, little is known about the interaction between biomolecules and Au NPs, though several analyses have been made. It is reported that fabrication of Au nanotriangle crystals is the result of an interaction between *de novo* Au nanoparticles and aldehydes or ketones present in the extract, while in *Cymbopogon* sp. reduction of $HAuCl_4$ is because of the sugar present in the lemongrass extract (Shankar et al., 2004a). At the same time as in *Geranium*, bioreduction of metal ions and stabilization of Au or Ag NPs is primarily caused by various terpenoids or alkaloids present in the extract itself (Shankar et al., 2004b).

One of the major challenges of current nanotechnology is to develop reliable experimental protocols for the synthesis of NPs over a range of chemical composition, size and synchronized monodispersity that must also be non-toxic, clean and eco-friendly, by using ambient biological resources. Though several reviews have been published in the last few years (Bhattacharya et al., 2005; Mandal et al., 2006; Mohanpuria et al., 2008), it is necessary further to elaborate this technology in a consolidated way with an approach that provides an overview of the current trend of research on the biosynthesis of different anisotropic, inorganic metal NPs and their applications. The present review highlights the current knowledge regarding the potential organisms for biosynthesis of NPs and presents a database that future researchers can build upon.

## Fabrication of nanoparticles using bacteria

The most important challenge in nanotechnology today is to cost effectively tailor the optical, electric and electronic property of NPs by controlling the configuration as well as monodispersity. This goal could be achieved using bacterial organisms in an organized manner (Gericke & Pinches, 2006). It has been shown that *Bacillus subtilis* 168 reduces Au ions to produce NPs of 5-25 nm of octahedral nature that are readily precipitated within bacterial cells under incubation with auricchloride solution (Southam & Beveridge, 1994; Fortin & Beveridge, 2000). Extracellular synthesis of Au NPs by *Pseudomonas aeruginosa* has been identified to be of polydispersed nature (Husseiny et al., 2007). A cell free extract of *Rhodopseudomonas capsulata* has been found to have good potential to fabricate Au nano wires with a network structure and the process can be controlled to change the shape of NPs. Exclusively spherical Au NPs with 10-20 nm range have been observed at lower concentrations and nano wires with a network structure at higher concentrations (He et al., 2008). Bioaccumulation of Au NPs has been achieved using $H_2$ as an electron donor in the culture of *Escherichia coli* and *Desulfovibrio desulphuricans*. Here the reduction of the aqueous Au (III) ions is being done by the treatment of $HAuCl_4$ solution (Deplanche & Macaskie, 2008). In spite of this, crystalline Au particles ranging from 20-50 nm have been observed in the periplasmic space, over the cell surface. Introduction of additional cysteine derived thiol residues within the FLiC protein of *Escherichia coli* amplified the Au (III) absorption as well as diminution on the surface of flagellar filament that resulted in fabrication of Au (O) particles of 20-25 nm size (Deplanche et al., 2008).

In the last few years, fabrication of Ag NPs has increased extensively owing to its immense applications (Morones et al., 2005). *Pseudomonas stutzeri* AG259 has been reported to fabricate Ag particles (Joerger et al., 2000), which are accumulated within the periplasmic space of bacterial cell of 200 nm. *Lactobacillus*, a common bacterial strain present in the buttermilk, synthesizes both Au and Ag NPs under standard conditions (Nair & Pradeep, 2002). Rapid synthesis of metallic NPs of Ag using the reduction of aqueous $Ag^+$ has been achieved in the culture supernatants of *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae* (Shahverdi et al., 2007). Recently detailed studies confirmed that synthesis of Ag can be triggered through the liquid mixing process developed in the visible light spectrum by *Klebsiella pneumonia* (Mokhtari et al., 2009). Extracellular biosynthesis of 40 nm Ag NPs by the culture supernatant of *Bacillus licheniformis* has been customized as an easy way to work out the process (Kalishwaralal et al., 2008).

In addition to Ag and Au NPs attention has also been focused on the synthesis of Cd, Zn, Pd, Pt and magnetite (Fe) nanocrystals. It is reported that *Clostridium thermoaceticum* (Cunningham & Lundie, 1993) and *Klebsiella aerogenes* precipitated Cd NPs at the cell surface, whereas intracellular CdS nano crystals are present in *Escherichia coli* (Sweeney et al., 2004). Recently in *Rhodopseudomonas palustris* (a photosynthetic bacterium), change in the coloration of the

biomass within a period of 48 hours implied the fabrication of CdS NPs ranging about 8±0.25 nm, with absorbance maxima at 425 nm (Bai et al., 2009). More precise studies established that although cysteine desulfhydrase producing $S^{2-}$ is located in the cytoplasmic vicinity of bacteria, it is efficiently transferred outside the cell. Through the hydrogen mediated reduction of $[Pd(NH_3)_4]Cl_2$, Pd (0) NPs are obtained in the flagellar filaments of *Desulfovibrio desulphuricans* (Deplanche et al., 2008). Spherical aggregates of 2-5 nm ZnS particles have been extensively retained from the natural biofilms dominated by the members of Desulfobacteriaceae (Labrenz et al., 2000).

In addition, magnetic NPs synthesized biologically have gained scientific interest owing to their magnetic and catalytic properties (Boal, 2004). Several bacteria acquire intracellular magnetosomes that encompass magnetic crystals enveloped in a membrane vesicle and the 'bacterial magnetic particles' (BMP) are of about 50 nm in diameter (Matsunaga &Takeyama, 1998). Uptake, accumulation and deposition of iron particles with a specific dimension is provided by the magnetosome membrane involving the biomineralization mechanism (Schüler, 1999), coupled through *mam* genes (Grunberg et al., 2001) of MTB. Different morphologies of MTB exist that again vary for number, layout and pattern of the BMPs (Schüler, 2002). MTB engineered deposition of $Fe_3O_4$ and $Fe_3S_4$ often occur concurrently, where $Fe_3O_4$ possess a magnetic moment three times that of $Fe_3S_4$. The bacterium *Magnetospirillum magnetotacticum* produce single domain of magnetite crystals that have been subsequently assembled into the folded chain with flux - closure ring morphologies (Philipse & Maas, 2002). The assembly of magnetic NPs into ordered structures has been observed also during the movement of bacteria (Lee et al., 2004). The ambient bacterial resources fabricating NPs have been enlisted in Table 1.

**Fabrication of nanoparticles using actinomycetes**

Besides the eubacteria, actinomycetes also play a key role in fabrication of anisotropic metal NPs. The extremophilic actinomycete, *Thermospora* reduced Au ions in an extracellular mechanism and yielded polydispersed Au NPs (Sastry et al., 2003). The reduction of metal ions as well as stability of the NPs is achieved by an enzymatic process and results in the efficient synthesis of monodispersed Au NPs (Ahmad et al., 2003a). Intracellular synthesis of Au has been observed more accurately and found concentrated on the cytoplasmic membrane rather than on the cell wall of alkalotolerant actinomycete *Rhodococcus* (Ahmad et al., 2003b). Potential actinomycetes fabricating NPs are listed in Table 2.

**Table 1:** List of the ambient bacterial resources fabricating nanoparticles.

| Resources | Type of NPs produced | Size | References |
|---|---|---|---|
| *Bacillus subtilis* | Au | 5-25 nm | Fortin & Beveridge, 2000 |
| *Pseudomonas aeruginosa* | Au | 15-30 nm | Husseiny et al., 2007 |
| *Rhodopseudomonas capsulata* | Au | 10-20 nm | He et al., 2008 |
| *Escherichia coli* | Au | 20-25 nm | Deplanche & Macaskie, 2008 |
| *Desulfovibrio desulfuricans* | Pd | 20-50 nm | Deplanche et al., 2008 |
| *Pseudomonas stutzeri* AG259 | Ag | 200 nm | Joerger et al., 2000 |
| *Klebsiella pneumonia* | Ag | 1-6 nm | Mokhtari et al., 2009 |
| *Bacillus licheniformis* | Ag | 40 nm | Kalishwaralal et al., 2008 |
| *Clostridium thermoaceticum* | CdS | 20-200 nm | Cunningham & Lundie,1993 |
| *Rhodopseudomonas palustris* | CdS | 0±0.25 nm | Bai et al., 2009 |
| *Desulfobacteriaceae* | ZnS | 2-5 nm | Labrenz et al., 2000 |
| *Magnetospirillum magnetotacticum* | $Fe_3O_4$ | 50 nm | Matsunaga & Takeyama, 1998 |

**Table 2**: List of the ambient actinomycetes and yeast resources fabricating nanoparticles.

| Resources | Type of NPs produced | Size | References |
|---|---|---|---|
| ***Actinomycetes*** | Au | 8 nm | Sastry et al., 2003 |
| *Thermospora* sp | | | |
| *Rhodococcus* sp | Au | 5-15 nm | Ahmad et al., 2003b |
| **Yeast** | | | |
| Yeast MKY3 | Ag | 2-5 nm | Kowshik et al., 2003 |
| *Candida glabrata* | CdS | - | Dameron et al., 1989 |
| *Yarrowia lipolytica* NICM 3589 | Au | - | Agnihotri et al., 2009 |
| *Schizosaccharomyces pombe* | CdS | 1-1.5 nm | Kowshik et al., 2002 |

**Fabrication of nanoparticles using yeast**

Among the simple eukaryotic organisms, yeasts are explored mostly for the fabrication of Cd NPs and also known as 'Semiconductor Crystals' or 'Quantum Semiconductor Crystals' (Dameron et al., 1989). Intracellular formation of CdS quantum dots have been reported from *Candida glabrata*, through the degradation of a (-glutamyl-cysteine-) $Cd_{(n)}$ [where n varies from 2-6] complexes (Dameron et al., 1989). *Schizosaccharomyces pombe*, which shows an absorbance maximum at 305 nm, processed a wurtzite ($Cd_{16}S_{20}$) - type hexagonal lattice structure with particles in the range of 1-1.5 nm synthesized intracellularly (Kowshik et al., 2002). Size exclusion chromatography confirmed that Cd is coupled to a protein fraction between 25 and 67 KDa which corresponds to the theoretical molecular weight of CdS nanoparticles of 35 KDa coated with phytochelatins (Krumov et al., 2007). Apart from this, CdS exhibits particle size dependent narrow fluorescence spectrum that enables easy recognition and qualifies them for use as quantum dots or diodes (Kowshik et al., 2002). In addition to this, *Torulopsis* has been found to be proficient in reducing intracellular $Pb^{2+}$ from biomass and a maximum absorption at 330 nm (Kowshik et al., 2002). Ag NPs have been fabricated extracellularly using an Ag tolerant yeast strain MKY3 (Kowshik et al., 2003).

Yeast strains are now under specialized exploration to engineer Au NPs. Standardization of the growth and other cellular activities for controlled synthesis of NPs has been achieved (Gericke & Pinches, 2006). Recently, synthesis of Au NPs using a non-conventional yeast *Yarrowia lipolytica* NCIM3589 has been observed (Agnihotri et al., 2009). Dispersive spectrum analysis revealed the presence of Au crystals at a diverse range of pH (2, 7 and 9), with an absorbance peak at 540 nm and well associated with the cell wall. Studies are still being carried on to search further for diverse groups of beneficial yeasts. Useful and potential yeast sources fabricating NPs are listed in Table 2.

**Fabrication of nanoparticles using algae**

Alga is a diverse group in plant kingdom that is being explored for application in nanotechnology. Besides the production of NPs, algae are also being explored for determining its nutritional value, efficacy in bio-diesel improvement as well as its vast potential for therapeutic application. Accumulation of elemental Au in the suspension of dried cells of a green alga, *Chlorella vulgaris* has been reported (Hosea et al., 1986). The morphological control over the shape of Au NPs has been well established using *Plectonema boryanum* UTEX485, blue-green algae, while treated with aqueous Au $(S_2O_3)_2^{3-}$ and $AuCl_4$ solutions (Lengke et al., 2006a). The mechanism involves interaction with aqueous Au (III) chloride solution

that promotes the precipitation of NPs of amorphous Au(I) sulphide at the cell walls and finally deposits metallic Au in the form of octahedral platelets near the cell surfaces and in solutions (Lengke et al., 2006b). Rapid formation of Au NPs through extracellular biosynthesis has been made feasible in a marine alga *Sargassum wightii* Greville (Singaravelu et al., 2007).

Studies are ongoing on the biosorption and bioreduction of Au (III) ions and *Fucus vesiculosus*, a brown alga is important in this respect (Mata et al., 2009). Bioreduction with *Fucus vesiculosus* could be used as an alternative eco-friendly process for recovering Au from dilute hydrometallurgical solutions and leachates of electronic scraps. Diatoms have been found to be a resource for fabrication of siliceous materials (Kroger et al., 1999). Phytoplanktonic alga, *Phaeodactulum tricornatum* possess phytochelatin coated CdS nano crystals fabricated in response to Cd (Scarano & Morelli, 2003). *Shewanella algae* has been reported to reduce aqueous $PtCl_6^{2-}$ to elemental Pt at neutral pH under room temperature within 60 min using lactate as the electron donor. Biogenic Pt NPs of 5 nm are observed in the periplasm, which is a preferable position for simple and quick recovery (Konishi et al., 2007). UV-Vis spectrum exhibits an absorbance peak at 527 nm in respect to the plasmon resonance of the Au NPs with a range of 8-12 nm and reveals Bragg's Reflections. Further potential algal resources fabricating NPs are listed in Table 3.

**Table 3:** List of the ambient algal resources fabricating nanoparticles.

| Resources | Type of NPs produced | Size | References |
|---|---|---|---|
| *Chlorella vulgaris* | Au | - | Hosea et al.,1986 |
| *Phaeodactylum tricornatum* | CdS | - | Scarano & Morelli, 2003 |
| *Sargassum wightii* | Au | 8-12nm | Singaravelu et al., 2007 |
| Diatoms | $SiO_2$ | - | Kroger et al., ,1999 |
| *Shewanella* algae | Au | - | Konishi et al., 2004 |
| *Shewanella* algae | Pt | 8-12nm | Konishi et al., 2007 |
| *Fucus vesiculosus* | Au | ~20nm | Mata et al., 2009 |
| *Plectonema boryanum* UTEX 485 | Au | 6-10nm | Lengke et al., 2006a,b |

## Fabrication of nanoparticles using fungi

The fungal mediated green chemistry approach towards the fabrication of NPs has many advantages. This includes easy and simple scale up method, economic viability, easy downstream processing and biomass handling, and recovery of large surface area with optimum growth of mycelia (Sastry et al., 2003). Exposure of the biomass of *Verticillium* to $HAuCl_4$ solution ($10^{-4}$ M) expresses the formation of intracellular Au NPs with a distinct purple coloration (Mukherjee et al., 2001). An endophytic fungus, *Colletotrichum* growing in *Geranium* leaves, exposed to aqueous chlorate ions, leads to the fabrication of prismatic and rod like Au NPs (Shankar et al., 2003). Production of NPs is enhanced by different growth conditions of fungal cultures. An excellent work carried with biomass of *Trichothecium* manifest the production of extracellular Au NPs under static conditions and of intracellular Au NPs under shaking conditions (Ahmad et al., 2005). When *Fusarium oxysporum* is reacted with equimolar solutions of $HAuCl_4$ and $AgNO_3$, highly stable Au-Ag alloy NPs of different mole fractions have been recovered (Senapati et al., 2005).

It has been observed that most of the fungal genera are coupled with the synthesis of Ag NPs either intracellularly or extracellularly showing the onset of deep brown coloration (Sastry et al., 2003). Aqueous Ag ions exposed

to *Fusarium oxysporum* leads to the fabrication of extremely stable Ag hydrosol. The particles are in the 5-15 nm range and are stabilized in solution by the proteins excreted through the fungus (Ahmad et al., 2003a). Extracellular biosynthesis of Ag NPs in the 5-25 nm range using *Aspergillus fumigatus* is found to be quite fast and manifested the production of dense fungal biomass (Bhainsa & D'Souza, 2006). White rot fungus, scientifically known as *Phaenerochaete chrysosporium* has also been used for biomimetics of Ag NPs (Vigneshwaran et al., 2006). When *Aspergillus flavus* has been challenged with $AgNO_3$ solution it accumulated Ag NPs (Gade et al., 2008) on the surface of its cell wall within 72 h and also showed an absorbance peak at 420 nm in UV-Vis spectrum corresponding to the plasmon resonance of Ag NPs. The transmission electron micrographs of dislodged NPs in aqueous solution confirmed the fabrication of convincingly monodispersed NPs of size 8.92 ± 1.61 nm. FTIR confirmed the existence of proteins adjoining the Ag NPs with a distinctive emission peak at 553 nm excited at 420 nm in photoluminescence spectrum (Vigneshwaran et al, 2007). Fabrication of NPs, phytochelatin and NADPH dependent nitrate reductases for *in vitro* production of Ag NPs have been isolated from *Fusarium oxysporum* and been elucidated (Kumar et al., 2007a).

Since 2008, several fungal strains are being dynamically used as a promising resource for Ag NPs fabrication. *Fusarium acuminatum* has been studied intensely for the formation of Ag NPs, where the fungal cell filtrate, treated with $AgNO_3$ (1 mM) showed a sharp peak at 420 nm with high absorbance (Ingle et al., 2008a). *Fusarium semitectum* fabricated 10-60 nm spherical NPs and the colloidal suspensions are stable for several weeks but after that stability. Possible medicinal applications of these Ag NPs are envisaged (Basavaraja et al., 2008). Extracellular biosynthesis of Ag NPs by *Fusarium solani* (USM-3799), a phytopathogen, when treated with AgNO3 (1mM) fabricates spherical, polydispersed NPs with an average diameter of 16.23 nm. FTIR analyses provide evidence for the presence of proteins as a capping agent that enhanced the stability of synthesized Ag NPs (Ingle et al., 2008b). Further, it has been reported that functional groups, e.g. ≡C-O-C≡, =C=O,≡C-O-R and =C=C= are being derived from heterocyclic compounds like proteins that are present in the fungal extract and act as capping ligands of NPs (Sanghi & Verma, 2009).

The genus *Penicillium* seems to have extremely good candidates for the fabrication of Ag NPs. Production proceeds via extracellular mechanism with high negative zeta potential and stable at pH above 8.0 because of electrostatic repulsion (Sadowski et al., 2008). *Penicillium fellutanum,* isolated from coastal mangrove sediment, is found to be highly efficient in synthesis of Ag NPs and confirmed by 430 nm absorption peak. Production is optimized at 0.35% NaCl, pH 6.0, incubated at 5ºC and treated with $AgNO_3$ (1mM) for 24 hours. A protein band with a molecular weight of 70 KDa is detected when analyzed by PAGE (Kathiresan et al., 2009). As *Penicillium* is a very common biomass waste from pharmaceutical industry that possesses a potential to fabricate particles, it would enhance the opportunity for cost-effective preparation of various Ag based nanostructures.

Controlling the scale-up process for biosynthesis of Ag NPs with fungal proteins of *Coriolus versicolor* has been carried out (Sanghi & Verma, 2009). Fungal biomass accumulated Ag NPs in 72 h, which is reduced to 1 h in customized reaction conditions. FTIR studies revealed that amino groups bound to particles account for the stability of NPs and established the existence of protein as the stabilizing and capping agent. Extracellular synthesis took place whereby other than the fungal proteins, glucose is found to be responsible for the reduction. While in fungal mycelium, intracellular development of Ag NPs could be modified to give both intracellular and extracellular Ag NPs under alkaline conditions, wherein the surface -S-H groups of the fungus play a major role. Extracellular synthesis of Ag-NPs from *Phoma glomerata* (MTCC-2210) has been traced and its



efficacy against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* has been evaluated (Birla et al., 2009).

Apart from the Au and Ag NPs production, tetragonal barium titanate ($BaTiO_3$) NPs of 10 nm size has been fabricated by *Fusarium oxysporum* under optimized parameters (Bansal et al., 2006). The fungus is found to be highly potent for the synthesis of luminescent CdSe quantum dots, when incubated with a mixture of $CdCl_2$ and $SeCl_4$ (Kumar et al., 2007b). Besides this *Fusarium oxysporum* lead to the production of $TiO_2$ and $SiO_2$ NPs from aqueous anionic complexes $SiF_6^{2-}$ and $TiF_6^{2-}$, respectively (Bansal et al., 2005). Experimental evidence suggests that the extracellular production of NPs is stabilized by proteins as well as reducing agents secreted by the fungus itself. NPs have been found to be coupled with at least four high molecular weight proteins released from the biomass of fungus. FTIR indicates the native forms of these proteins (Duran et al., 2005). Reduction of metal ions and surface binding of proteins to the NPs has been elucidated little far (Kumar et al., 2007a). Studies further indicate that genera *Fusarium*, *Aspergillus* and *Penicillium* have large potential for the fabrication of different metal NPs. The list of functional ambient fungal resources that aid in fabricating different NPs has been provided in Table 4.

**Table 4:** List of fungal isolates fabricating nanoparticles.

| Resources | Type of NPs produced | Size | References |
|---|---|---|---|
| *Verticillium* | Au | 20 nm | Mukherjee et al., 2001 |
| *Colletotrichum* | Au | 20-40 nm | Shankar et al., 2003 |
| *Trichothecium* | Au | - | Ahmad et al.,2005 |
| *Fusarium oxysporum* | Au-Ag alloy | - | Senapati et al., 2005 |
| *Fusarium oxysporum* | Ag | 5-15 nm | Ahmad et al., 2003a |
| *Aspergillus fumigatus* | Ag | 5-25 nm | Bhaisa & D'Souza, 2006 |
| *Aspergillus flavus* | Ag | 8.92±1.61 nm | Vigneshwaran et al., 2007 |
| *Fusarium acuminatum* | Ag | 10-30 nm | Ingle et al., 2008a |
| *Fusarium semitectum* | Ag | 10-60 nm | Basavaraja et al., 2008 |
| *Fusarium solani* | Ag | 16.23 nm | Ingle et al., 2008b |
| *Penicillium fellutanum* | Ag | - | Kathiresan et al., 2009 |
| *Fusarium oxysporum* | Zr | 3-11 nm | Bansal et al., 2004 |
| *Verticillium* | Ag | 25±12 nm | Senapati et al., 2004 |
| *Fusarium oxysporum* | $TiO_2$ & $SiO_2$ | 5-15 nm | Bansal et al., 2005 |

**Fabrication of nanoparticles using higher angiospermic plants**

Several microorganisms such as bacteria, fungi and yeasts have come up as nanofactories synthesizing metal NPs of Ag and Au. However, use of plants for the fabrication of NPs has drawn attention of workers because of its rapid, economical, eco-friendly protocol and it provides a single step technique for the biosynthesis process (Huang et al., 2007). The first report of phytofabrication of Au NPs has been reported from *Medicago sativa* (alfalfa), grown in $AuCl_4$ enriched environment and absorption of metal is confirmed by XAS and TEM (Gardea-Torresdey et al., 2002). Atomic resolution analyses revealed the nucleation and growth of Au NPs inside plant are of crystalline nature. Another dimension has been added to this approach with

the use of *Cymbopogon* sp. commonly known as lemon grass (Shankar et al 2004a) when challenged with aqueous $HauCl_4$. It yields a high percentage of thin, flat, and single-crystalline Au nanotriangles which seem to grow by a process involving rapid reduction, assembly and room-temperature sintering of 'liquid-like' spherical Au NPs. Similarly, in the extract of *Geranium* sp. fabrication of Ag as well as Au NPs have been elucidated (Shankar et al., 2004b). pH dependent binding trend of rod shaped Au (III) ions have been well elucidated from *Avena sativa* biomass. At pH 2.0, large NPs of 25-85 nm range are developed in less numbers, while at pH 3.0 or 4.0, smaller NPs of 5-20 nm range developed in good amount. As Au (III) occurs as an anion, the biomass might carry more functional groups that allow them to get closer to the binding sites (Armendariz et al., 2004a). Rapid synthesis of stable Au nano triangles on higher concentration of leaf extract of *Tamarindus indica* has been made possible (Ankamwar et al, 2005b). Along with stability, the control over the shape of NP production is accounted by the concentration range and plant part. *Sesbania drummondii*, a leguminous shrub with subsequent reduction of Au (III) ions to Au (O) inside plant cell, resulted into intracellular arrangement of fabrication of Au NPs of 6-20 nm range. *In situ* catalytic role of the NP rich biomass bearing biomatrix authenticated the initial report of reducing aqueous 4-Nitrophenol (4-NP), a toxic pollutant (Sharma et al., 2007). Recent trends in extensive use of phytochemicals of *Camellia sinensis* as dietary supplement and pharmaceuticals found significant agents that reduce Au salts to the corresponding Au NPs and also act as stabilizers, providing strong coating in a single step (Nune et al., 2009). The *Camellia sinensis* (tea) generated Au NPs (T-AuNPs) have established significant *in vitro* stability in various buffers.

The leaf broth of *Azadirachta indica* extracellularly fabricates pure metallic Ag-Au-NPs and bimetallic Au/Ag NPs. Competitive reduction of $Au^{3+}$ and $Ag^{+}$ ions present simultaneously in solution during exposure to plant leaf extract generates bimetallic Au core and Ag shell NPs. TEM revealed Ag particles have been adsorbed on Au NPs and constructed the former structure (Shankar et al., 2004c). Flavonone and terpenoid components of leaf broth are being predicted to stabilize the formation of NPs in contrast to high molecular weight proteins of fungal biomass (Shankar et al., 2004c). The fruit extract of *Emblica officinalis* enhances the extracellular synthesis of highly stable Ag and Au NPs (Ankamwar et al., 2005a). Utilizing leaf extract of *Aloe vera* as a reducing agent, fabrication of Au nano triangles and Ag NPs in single triangular form has been achieved (Chandran et al., 2006). The percentage of Au nano triangles to the spherical particles has been altered as the amount of extract varied. *Cinnamomum camphora* has also been revealed to fabricate Au and Ag NPs (Huang et al., 2007).

Ag NPs have also gained significance due to their broad-spectrum activity against bacterial infections. The roots of *Medicago sativa* accumulate Ag ions inside the plant tissue from the enriched medium and undergo nucleation and fabricate Ag NPs (Gardea - Torresdey et al., 2003). Using *Pelargonium graueoleus* leaf extract, $Ag^{+}$ is reduced to Ag NPs that are crystalline as well as highly stabilized (Shankar et al., 2003). Bioreduction activity of leaf extracts of *Helianthus annus*, *Basella alba*, and *Saccharum officinarum* resulted in the fabrication of Ag NPs in which *Helianthus annus* is found to exhibit strong potential for quick reduction of $Ag^{+}$ (Leela & Vivekanandan, 2008). The polyol components and the water soluble heterocyclic components are mainly responsible for reduction of $Ag^{+}$ as well as stabilization of NPs. Information regarding the activity of reductases in NP fabrication are well illustrated (Kumar et al., 2007a). No correlation is observed between the color development and increase in abundance exhibited by the synthesized nano metal. Differences in morphology of nanoparticles synthesized, is one possible reason for variation in optical properties (Xu & Kali, 2002). Further studies reflect that several

parameters together determine the NP synthesis including plant source, the organic compounds in the crude leaf extract, the concentration of $AgNO_3$, the temperature and the pigments of the corresponding leaf extract (Leela & Vivekanandan, 2008). Recently, fabrication of Ag NPs using the callus extract of *Carica papaya* has been reported (Mude et al., 2009). Brown coloration of the MS medium and analyses of FTIR with peaks ranging 1000-2000 per centimeter confirmed the presence of proteins and other ligands that are mandatory for the synthesis and stabilization of derived spherical NPs within a range of 60-80 nm. The use of latex of *Jatropha curcas* as reducing as well as capping agent in the fabrication of Ag NPs has recently been reported (Bar et al., 2009). The NPs have been characterized using high-resolution transmission electron microscopy (HRTEM), X-ray diffraction and UV–Vis absorption spectroscopy and confirmed as face centered cubic structure. Comparison of the NPs radius obtained from HRTEM image with the optimized cavity radius of the cyclic peptides present within the latex indicated that particles of size 10–20 nm are typically stabilized by the cyclic peptides. The list of the ambient plant resources that aid in phytofabrication of various NPs has been provided in Table 5.

**Table 5:** List of the ambient plant resources fabricating nanoparticles.

| Resources | Type of NPs produced | Size | References |
|---|---|---|---|
| *Avena sativa* | Au | 5-85 nm | Armendariz et al., 2004a |
| *Azadirachta indica* | Ag, Au-Ag, Au | 5-100 nm | Shankar et al., 2004c |
| *Berkheya chicory* | Au | - | Lamb et al., 2001 |
| *Sesbania drummondii* | Au | 6-20 nm | Sharma et al., 2007 |
| *Berkheya coddii* | Au | - | Lamb et al., 2001 |
| *Aloe vera* | Ag | 15.2±4.2 nm | Chandran et al., 2006 |
| *Emblica officinalis* | Ag | 10-20 nm | Ankamwar et al., 2005a |
| *Emblica officinalis* | Au | 15-25 nm | Ankamwar et al., 2005a |
| *Brassica juncea* | Au , Ag | - | Lamb et al., 2001 |
| *Cinnamomum camphora* | Au & Ag | 55-80 nm | Huang et al., 2007 |
| *Triticum aestivum* | Au | - | Armendariz et al., 2004b |
| *Carica papaya* | Ag | 60-80 nm | Mude et al., 2009 |
| *Jatropha Curcas* | Ag | 10-20 nm | Bar et al., 2009 |
| *Capsicum annum* | Ag | - | Li et al., 2007 |
| *Tamarindus indicus* | Au | 20-40 nm | Ankamwar et al., 2005b |
| *Chilopsis linearsis* | Au | - | Rodrıguez et al., 2007 |
| *Helianthus annus* | Ag | - | Leela & Vivekanandan 2008 |
| *Camellia sinensis* | Au | 15-42 nm | Nune et al., 2009 |
| *Pelargonium graveolans* | Au | - | Shankar et al., 2003 |

## Future perspectives

Improvement in nanoscale technologies has brought about new vistas towards revolutionizing the fundamentals of disease diagnosis, treatment, and prevention by innovating nanomedicines. They have the potential to alter molecular discoveries arising from genomics and proteomics into wide spread benefits for patients. Emergence of antibiotic resistance is also of alarming concern presently and by combating it with the aid of nanotechnology it is expected that new avenues could open which prevent diseases using tailored materials of atomic scale (Singh et al., 2008). Increasingly, agents with 'antimicrobial' effects are being coated on materials and medical aids as a

prophylaxis to check bacterial growth. Since the role of bioresources in nature is so diverse, the process of mining their genetic variation for new applications will continue long into the future.

Additionally, the advantage of biological production systems is in the controlled production at a molecular level. NPs are formed in highly defined structures, complex morphologies and narrow particle size distribution (Sharma et al., 2007). Recent technologies of impregnation of Au or Ag NPs enable to solve the burning problem of resistance against antibiotics. Microbes are unlikely to develop resistance against Ag, as they commonly do against conventional and narrow-target antibiotics. Metallic Ag in the form of Ag NPs has made a remarkable comeback as a potential antimicrobial agent and has developed into diverse medical applications ranging from Ag based dressings, Ag coated medicinal devices, e.g. nanogels and nanolotions among others (Rai et al., 2009).

The major mechanism through which Ag NPs manifest high antibacterial properties is by anchoring and penetrating the bacterial cell wall and modulating the cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues (Singh et al., 2008). Earlier, it has been established that Ag ions strongly intermingles with thiol groups of essential enzymes present in the target microorganisms, inactivates them and plays an inhibitory role (Matsumara et al., 2003). Activity is principally dose-dependent and more prominent against Gram-negative bacteria (Morones et al., 2005). Comparative analysis with a Gram negative bacterium *Escherichia coli*, both in liquid systems and on agar plates reveals that triangular nanoplates displayed the strongest biocidal action, than the spherical and rod-shaped NPs of different shapes (Pal et al., 2007). Consequently, Ag NPs have emerged as potential sources of bactericidal agents that can maintain their efficacy in colloidal system.

The Au NPs with unique and anisotropic planar shapes might find application in photonics; optoelectronics and the reduction of Au NPs through different solvents reveal them as future chemical sensors (Ankamwar et al., 2005b). The current interest in Au NPs is based on their extensive potential applications in catalysis, microelectronics and biomedicine (Shankar et al., 2004a). Quantum dots having vast application in nanobiotechnology could be fabricated from yeasts and fungal genera (Kumar et al., 2007b). Nevertheless, quantum dots also particularly play significant roles in the preparation of biosensors, organic dyes and light-emitting diodes (Mohanpuria et al., 2008; Rai et al., 2008). Quantum wires have many magnetic and electrical properties. Metallic wires are generally used in the fabrication of nanodevices, electronic and electro-optical devices and semi-conductors (Mandal et al., 2006). In future, nanoscale devices with novel properties could be used to identify plant health issues before these become noticeable to the farmer and make one capable of responding to different situations with appropriate remedial actions. Additionally, nanotechnologies such as encapsulation and controlled release methods will revolutionalize the use of pesticides and herbicides (Rai et al., 2008). Different ambient bioresources supporting fabrication of NPs and their applications are presented in Table 6.

## CONCLUSION

This paper has reviewed recent knowledge and built a data base of bioreductive approaches to Ag, Au, Cd, Pt, Pd, $SiO_2$ and $TiO_2$ NPs using different biological systems. The exact mechanism for the fabrication of NPs in biological resources is still being investigated and several possible ways have been proposed (Shankar et al., 2004a; Huang et al., 2007; Rai et al., 2008). Microorganisms where proteins (Deplanche et al., 2008; Sanghi & Verma, 2009) and angiosperms where carboxylic groups, amino groups, proteins and carbohydrates (Huang et al., 2007, Rai et al., 2008) are present in the source extract have been proposed to play a key role in the biosorption and bioreduction process for the fabrication of NPs.

However, the technological applications of these NPs have been restricted as the existing physical, chemical and biosynthetic methods lack sufficient size and shape selectivity. Several studies still need to be executed to understand the effect of time, temperature, light and other parameters regarding the phytofabrication of NPs. As nanotechnology has gained incredible thrust in the last few years, and is expected to develop more in the future, the foremost challenge is to expand consistent experimental protocols in microbial synthesis, mycosynthesis, phycosynthesis or phytosynthesis of NPs. In addition, an enhanced understanding of the exact mechanism of the fabrication of NPs and the bioreduction phenomenon of metal ions is needed.

**Table 6:** List of organisms and applications of NPs.

| Sources | NPs produced | Applications | References |
|---|---|---|---|
| *E.coli* DH5 | Au | Electro chemistry of hemoglobin is detected directly | Du et al., 2007 |
| *Phoma glomerata* | Ag | Comprehensive bactericidal activity against most gram negative bacteria. | Birla et al.,2009 |
| *Fusarium oxysporum* | $TiO_2$ & $SiO_2$ | Can be used as inorganic building blocks for multilayer formation on polystyrene (PS) sphere templates and have applications in cosmetic industry. | Bansal et al., 2005 |
| *Camellia sinensis* | Au | Affinity towards prostrate (PC- 3) & breast (MCF – 7) cancer cells | Nune et al., 2009 |
| *Magnetospirillum magnetotacticum* | $Fe_3O_4$ | Used in ultra violet density, magnetic recording, magnetic fluids, biomedical materials | Boal, 2004 |
| | | Detection of biomolecular interaction between biotin and streptavidin in the field of DNA, Protein micro array chip assay | Osaka et al., 2006 |
| *Sesbania drummondii* | Au | Reduce the toxic pollutant 4 Nitro Phenol (4-NP) | Sharma et al., 2007 |
| *Rhodopseudomonas palustris* | CdS | Development of quantum dots | Bai et al., 2009 |
| *Tamarindus indica* | Au | High absorption coefficient of Au NPs in near infra red (NIR) region makes them useful in fabricating photonic devices as optical sensors & NIR absorbers. | Ankamwar et al., 2005b |
| *Saccharomyces pombe* | CdS | Fabrication of an ideal diodes (exhibit ~75 mA/cm$^2$ current at 10V when forward diased & breakdown occurred at ~15V in reverse biased mode) | Kowshik et al., 2002 |
| *Desulfovibrio desulfuricans* & *Escherichia coli* | Pd & Au | Catalytic applications in molecular techniques | Deplanche et al., 2008 |
| *Fusarium oxysporum* | Zr | can be useful as quantum dots and possess space applications | Bansal et al., 2004 |
| *Fucus vesiculosus* | Au | Recover gold from dilute hydrometallurgical solution & leachates of electronic scraps | Mata et al., 2009 |

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