

Review

## Biogenesis of nanoparticles: A review

Pooja Bansal, Joginder Singh Duhan\* and Suresh Kumar Gahlawat

Department of Biotechnology, Ch. Devi Lal University, Sirsa-125055, India.

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**Advancement in nanotechnology mainly depends upon advancement in nanomaterial. There are many chemical routes known to use toxic chemicals for synthesis of nanoparticles but the need of the hour is to use environmental benign, greener and safer routes. Researchers are looking to use various living organisms as 'nanoparticle factories'. Various biological entities like bacteria, fungi, diatoms, higher plants, actinomycetes and viruses have been used for this purpose. Due to their normal biosynthetic pathways, they can reduce salt into corresponding nanoparticles. This review includes some of the biological sources which have been used by researchers for the synthesis of nanoparticles and their applications.**

**Key words:** Biogenesis, nanofactories, nanoparticles, antimicrobial activity, semiconductor nanoparticles.

### INTRODUCTIONS

The field of nanotechnology has generated great enthusiasm in recent years because of its expected impact on science, industry, economy and our everyday life. Nanomaterial synthesis, characterization and its manipulation is one of the major aspects of nanotechnology (Bansal and Suresh, 2012). Nanoparticles (NPs) have unique properties as compared to their bulk equivalents, thus find application in a number of fields including optics (Kawar, 2011), electronics, sensor technology, clinical biology (Gupta, 2011), catalysis etc. With the advancement in techniques now, we are moving towards 'green synthesis' of nanoparticle production (Bansal et al., 2012).

Besides the traditional, physical and chemical methods of nanoparticles synthesis, biogenesis of nanoparticles is also gaining attention of researchers due to some clear

advantages of using biological entities for NPs production. Physical (Ayyub et al., 2001; Kalishwaralal et al., 2010) and chemical methods (Murray et al., 2002) of production like gel-sol synthesis, chemical reduction method, aerosol technology, lithography, laser ablation method are expensive and involve the use of hazardous chemicals and reagents that pollute environment when we talk about their bulk production. Nature has devised various processes for the synthesis of nano- and micro-length scaled inorganic materials which have contributed to the development of relatively new and unexplored area of research based on the biosynthesis of nanomaterials (Mohanpuria et al., 2007). Microbes may solubilize the metals or can reduce them. Microbial biomass can retain relatively high quantities of metal by biosorption (passive mode) or by bioaccumulation (actively by viable cells)

\*Corresponding author. E-mail: duhanjs68@gmail.com, duhanjs@rediffmail.com. Fax: +91 1666 248123, +91 1666 247143.

(Volesky, 1995). Biogenesis of nanoparticles is one of such environmental friendly approach as it makes use of organisms and their natural biochemical mechanism (Simkiss, 1989; Mann, 1996) for production of nanoparticles thus, provides an economical and safer route. Moreover, the biologically fabricated nanostructures offer substantially different in properties like good adhesion, tribologically good properties, less toxicity and biocompatibility making them more valuable for biological applications. It is interesting to note that a range of organisms including bacteria, fungi and higher plants show remarkable synthesis of nanoparticles thus acting as 'nanofactories'.

## BACTERIA

It has been shown that many bacteria can actively uptake and reduce metal ions. Microbes can either show oxidization, reduction or biosorption of metals. The production of nanoparticle can be extracellular or intracellular depending upon bacterial species. There is a limit of nanoparticle accumulation up to which bacteria can survive and after that, nanoparticle accumulation can be toxic for the microbes (Deepak et al., 2011). First evidence of silver nanoparticle production came from bacteria *Pseudomonis stutzeri* AG 259 which was isolated from silver mine. When placed in silver nitrate solution, it produces NPs in its periplasmic space which are of pyramidal and hexagonal shape and size up to 200 nm (Klaus et al., 1999; Joerger et al., 2000). Synthesis of extracellular silver nanoparticles at room temperature was studied in *Pseudomonas aeruginosa* strain BS-161R and its antimicrobial efficiency was studied against different Gram positive, Gram negative and *Candida* species (Kumar and Mamidyal, 2011).

Nithya et al. (2011) studied silver hydrosol formation using three bacterial strains; that is, *Streptococcus* sp, *Proteus* sp and *Pseudomonas* sp. Within 30 min, all the three strains started showing change in color. After 24 h, color of bacterial filtrate changed from pale yellow to brown indicating formation of silver nanoparticles. Extracellular production of silver metal nanoparticles by probiotic microbes (*Brevibacterium linens* NCIM 2149) using 1 mM AgNO<sub>3</sub> solution has been reported by Nithya and Raghunathan (2012) and the synthesized NPs showed very good antimicrobial activity against multidrug resistant (MDR) Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Applications of silver nanoparticles and its toxicity have been studied by different researchers (Prabhu and Poulouse, 2012). Silver (Ag) NPs were synthesized by using different root nodule microbes including *Rhizobium* and *Agrobacterium* by Rajkumar and Tamizharasi (2012) and its antimicrobial activity against different pathogens *E. coli*, *Vibrio cholerae*, *Proteus vulgaris* was checked. Silver (Ag) NPs

showed no inhibition on *P. vulgaris* but showed good inhibition on other two bacteria at concentration of 50 µl. Minaeian et al. (2008) investigated Ag NPs synthesis by using a range of bacterial species *Klebsiella pneumoniae*, *E. coli*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus acidophilus* and *Candida albicans* using silver nitrate at concentration of 10<sup>-3</sup> M. Among these *K. pneumoniae*, *E. coli* and *E. cloacae* were found effective for extracellular production of Ag NPs. Potential of nine different *Lactobacillus* strains, extracted from whey, for Ag nanoparticle synthesis was studied. *Lactobacillus* sp VRS-2, showed maximum potential with nanoparticle of size 2 to 20 nm (Rangnathan et al., 2012). Biomass of *Bacillus cereus* isolated from leaves of surface sterilized medicinal plant *Garcinia xanthochymus* synthesized silver nanoparticles by reducing AgNO<sub>3</sub> at room temperature in three to five days (Sunkar and Nachiyar, 2012). *Vibrio alginolyticus* reported to produce intracellular and extracellular spherical Ag NPs depending upon procedure followed by Rajeshkumar (2013). Intracellular reaction started in 4 h and extracellular reaction took 12 h. Extracellular synthesis showed better dispersibility and control over size of synthesized NP.

Gold nanoparticles show very high chemical reactivity as compared to bulk gold which is comparatively inert. Recent study on biosynthesis of gold nanoparticles by *Geobacillus* sp. strain ID17, a thermophilic bacterium isolated from deception Island, Antarctica done by exposing bacterial cells to Au<sup>3+</sup> ions (Correa-Lianten, 2013). Other bacteria for gold nanoparticle synthesis include *E. coli* DH5a and their applications on direct electrochemistry of hemoglobin are reported (Du et al., 2007). *Rhodococcus*, alkalo-tolerant actinomycetes synthesized intracellular gold nanoparticles of uniform size 5 to 15 nm and keep on dividing even after accumulation of gold nanoparticles on its cell wall and cytoplasmic space. Thus, confirming non toxicity of gold nanoparticles against *Rhodococcus* (Ahmad et al., 2003). Effect of pH on shape of gold NPs has been reported. At pH 4, nanoplates were obtained and at pH 7 spherical nanoparticles were accumulated (He et al., 2008).

A well studied example of NPs production is magnetosome. These are either Fe<sub>3</sub>O<sub>4</sub> (magnetite) or Fe<sub>3</sub>S<sub>4</sub> (greigite) nanoparticles in the magneto-tactic bacteria such as *Magnetospirillum magneticum* that can synthesize magnetic nanoparticles (Samuel, 2005; Bazylinski and Frankel, 2004) which have an enormous number of applications in bio and nanotechnology (Lang et al., 2006). Their superior nature allows magnetosomes to be used as magnetic nanoparticles. Semiconductor nanoparticles have exceptional optical and electronic properties and hence, can be of great importance for opto-electronic devices. Under anaerobic conditions, using N-8 and M-8 culture media, Mandal et al. (1998) observed ZnS nanoparticles of 2 to 5 nm produced by the

sulphate reducing bacteria *C. vulgaris*. Sulphate reducing bacterium *Desulfovibrio desulfuricans* NCIMB 8307 and *Shewanella oneidensis* has been shown to produce palladium nanoparticles, which find application in catalysis (Yong et al., 2002; Windt et al., 2005). A highly cadmium resistant strain *Klebsiella planticola* Cd-1 isolated from reducing salt marsh sediments, precipitated considerable amount of CdS nanoparticles (Sharma et al., 2000). *K. pneumoniae* synthesized stable selenium nanoparticles in elemental form from selenium chloride and were found stable against wet heat sterilization. Thus, wet heat sterilization was done to separate selenium nanoparticles from bacteria (Fesharaki et al., 2010). Precipitation of palladium nanoparticles on bacteria *Citrobacter braakii* to obtain palladium nanoparticles and its application as catalyst for diatrizoate removal with biogenic hydrogen was successfully studied by Hennebel et al. (2011). *Lactobacillus* mediated synthesis of silver oxide nanoparticle of size up to 2 nm has been studied by Dhoondia et al. (2012). CdS NPs synthesized by sulfur reducing bacteria *Serratia nematodiphila*, isolated from chemical effluent of a company has been reported by Malarkodi et al. (2013).

Newman et al. (1997) reported that *Desulfosporo auripigmenti* can precipitate spherical, arsenic trisulfide nanoparticles ( $As_2S_3$ ), both intra and extracellular under sulfate-reducing conditions. The microbial production of extracellular network filamentous arsenic sulphide (As-S) nanotubes by *Shewanella* sp has been reported by Jiang et al. (2009). Aerobically, growing *Duganella* sp. and *Agrobacterium* sp. from selenium contaminated agriculture soils of Punjab (India) has been isolated and found to be effective in converting bio-soluble toxic selenium anion to non toxic elemental selenium NPs (Bajaj et al., 2012), thus, showing its potential in controlling biogeochemical cycle of Se in natural environment. Ion chromatographic analysis released only reduction of Se (IV) to Se (0) and no oxidation. *Duganella* found to be more efficient for producing NPs.

## FUNGI

Fungi have some distinct advantages when used as nanofactories for NP production (Volsky and Holan, 1995)

1. Easy to scale up, large biomass can be obtained as fungi grow easily on large scale by solid state fermentation method.
2. Large scale production of extra cellular enzyme per unit biomass.
3. Ease of biomass handling.
4. Most fungi have a very high wall-binding capacity as well as intracellular metal uptake capacities.

## 5. Good metal accumulation capability.

The biosynthesis of intracellular quantum crystallites in yeast has been reported in *Candida glabrata* and *Schizosaccharomyces pombe* when cultured in the presence of cadmium salt (Dameron et al., 1989). Extracellular synthesis of silver nanoparticles was observed in silver tolerant yeast strains MKY3 when exposed with 1 mM soluble silver in the log phase of growth (Kowshik et al., 2003). Potential of *Aspergillus fumigatus* for extracellular synthesis of mono-dispersed Ag NPs has been explored (Bhainsa and D'Souza, 2006). Crystallized and spherical-shaped Au and Au-Ag alloy nanoparticles have been synthesized and stabilized using a fungus, *Fusarium semitectum* in an aqueous system. Aqueous solutions of chloroaurate ions for Au and chloroaurate and  $Ag^+$  ions (1:1 ratio) for Au-Ag alloy were treated with an extracellular filtrate of *F. semitectum* biomass for the formation of Au nanoparticles (Au NP) and Au-Ag alloy nanoparticles (Au-Ag NP) (Balaji et al., 2008). Extracellular silver nanoparticles of size 5 to 50 nm were obtained by use of *Pleurotus sajorajju* and their antimicrobial activity have been recently reported by Nithya et al. (2009).

Ray et al. (2011) showed for the first time the green biosynthesis of silver nanoparticles from the mycorrhizal fungus *Tricholoma crassum* (Berk.) Sacc. The spherical silver nanoparticles were of the size range 5 to 50 nm. These silver nanoparticles showed potent antimicrobial activity against multidrug resistant pathogenic bacteria *E. coli* (DH5 $\alpha$ ), plant pathogenic bacteria *Agrobacterium tumefaciens* (LBA4404) and plant pathogenic fungus *Magnaporthe oryzae*. Room temperature synthesis of Ag NPs from *Penicillium* extracted from field soil has been studied and mechanism of nanoparticle synthesis has also been established. Pure filamentous *Penicillium* is allowed to react with 1 mM  $AgNO_3$  to obtain optimum reduction process (Hemath et al., 2010). Freeze dried *Phoma* sp. 3.2883 mycelia when treated with  $AgNO_3$  solution in shaking conditions synthesized good amount of Ag NPs (Chen et al., 2003). The presence of hydrogenase in the *Fusarium oxysporum* has been demonstrated. This extracellular enzyme shows excellent redox properties and it can act as an electron shuttle in metal reduction. Mukherjee et al. (2001) demonstrated that the fungus *Verticillium* sp. when subjected with an aqueous solution of chloroaurate ( $AuCl_4$ ) resulted in the reduction and intracellular accumulation of gold nanoparticles with average size of around 17 nm. The capability of *F. oxysporum* to synthesize gold nanoparticle by treatment of the fungal biomass with aqueous solution containing  $AuCl_4$  ions was further demonstrated by Mukherjee et al. (2002). The fungus *Verticillium* sp. produces nanoparticles, when exposed to gold and silver ions; metal ions were reduced fairly by biomass and formed respective metallic nanoparticles.

This fungus produces intracellular nanoparticles (Senapati et al., 2004). Live biomass of fungus *Penicillium brevicompactum* successfully used for synthesis of gold nanoparticles and cytotoxic effects of synthesized nanoparticles were studied on mouse mayo blast cancer C2 C12 cells (Mishra et al., 2011). Synthesis of Au NPs on the surface of *Rhizopus oryzae* by *in situ* reduction of H<sub>2</sub>AuCl<sub>4</sub> and its application in water hygiene management has been studied (Das, 2009).

*Aspergillus tubingensis* and *Bionectria ochroleuca* mediated synthesis of extracellular silver nanoparticles, which are spherical in shape with dimensions of  $35 \pm 10$  nm was studied by (Rodreguis et al., 2013). *A. tubingensis* synthesized NPs showed excellent antimicrobial activity and very high surface positive potential as compared to other fungi studied so far. Zirconia nanoparticles have been produced by exposing the fungus *F. oxysporum* with aqueous ZrF<sub>6</sub><sup>2-</sup> anions and extra-cellular protein-mediated hydrolysis of the anionic complexes results in the novel room temperature synthesis of zirconium nanocrystals (Bansal et al., 2004). Kowshik et al. (2002) reported the intracellular synthesis of cadmium sulfide nanoparticles by *S. pombe* strain. In another work, using *Fusarium* sp. biomass as a sustainable synthesis procedure, CdS NPs has been synthesized after its exposition with a CdSO<sub>4</sub> solution (Ahmad, 2002; Reyes et al., 2009).

## ALGAE

The extracellular biosynthesis of silver nanoparticles using marine cyanobacterium *Oscillatoria willei* NTDMO1 which reduce silver ions and stabilizes the silver nanoparticles by a secreted protein was recently reported (Ali et al., 2011). An efficient approach for synthesis of stable gold nanoparticles by the reduction of aqueous AuCl<sub>4</sub> using *Sargassum wightii* was reported (Singaravelu et al., 2007). It was the first report for synthesis of stable metallic nanoparticles by the extract of marine algae. Biological synthesis of gold nanoparticles within 10 min by brown alga, *Stoechospermum marginatum* biomasses through a green route was reported by (Arockia et al., 2012). *Spirulina platensis* biomass directed synthesis of Ag NPs of approximately 12 nm has been successfully achieved and size was confirmed by XRD (Mahdieh et al., 2012). *Nannochloropsis oculata*, *Dunaliella salina* and *Chlorella vulgaris* as three algal species in addition to three *Lactobacilli* including *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus reuteri* were monitored for their potential of silver nanoparticle synthesis. The biosynthesis of silver nanoparticles in all three *Lactobacilli* and two algal species *N. oculata* and *C. vulgaris* was confirmed (Mohseniazar et al., 2012). Recently, copper oxide NPs had been synthesized by brown algae *Bifurcaria bifurcate* and were found to be

effective against two different strains of bacteria *Enterobacter aerogenes* and *Staphylococcus aureus* (Abboud et al., 2013).

## VIRUSES

Viruses do not synthesize nanoparticles as such, but there are several reports on use of viruses for template dependent synthesis of nanoparticles. Viroid capsules (Douglas et al., 1998) are used in template mediated production of inorganic nanomaterials and microstructured materials. Tobacco mosaic virus (TMV) has been successfully used as template for the synthesis of iron oxides by oxidative hydrolysis, co-crystallization and mineralization of CdS and lead sulphide (PbS) crystalline nanowire. A hybrid nanowire (ZnS-CdS) is obtained with a dual peptide virus engineered to express A7 and J140 within the same viral capsid (Shenton et al., 1999; Mao et al., 2003).

## PLANT AND PLANT EXTRACTS

The plant mediated synthesis of nanoparticles is more advantageous than the other biological process as there is no troublesome of preserving and maintaining the cell culture. Plant mediated nanoparticle synthesis is an easy, one step synthesis method with no chances of mutation as in microorganisms. Extraction and separation can be easily scaled up for the large-scale synthesis of NPs (Veerasamy et al., 2011). By using a natural plant reducing constituent geraniol, extracted from different plants, silver NPs up to 10 nm size range were synthesized with average particle size of 6 nm and cytotoxicity studies of synthesized NPs were done by Safaepour et al. (2009). It was the first time when only the active component from plant was used for biosynthesis of NPs.

Synthesis of anisotropic gold and spherical-quasi-spherical silver nanoparticles by reducing aqueous chloroauric acid and silver nitrate solution at room temperature was studied and it was found that size and shape of the nanoparticles can be controlled by varying the concentration of phyllanthin extract (Kasthuri et al., 2009). *Cinnamon zeylanicum* bark extract, upon evaluation for synthesis resulted in cubic and hexagonal silver nanocrystals with size ranging between 31 to 40 nm (Sathishkumar et al., 2009). A report synthesis of AuNPs using *Gnidia glauca* flower extract (GGFE) and its evaluation of chemocatalytic potential under different environmental conditions were used to study optimal conditions and it was found that 0.7 mM H<sub>2</sub>AuCl<sub>4</sub> at 50 °C is optimum for maximum production (Ghosh et al., 2012). By using extract of *Rosmarinus officinalis*, silver NPs of size 60 nm and its antimicrobial activity against pathogens

**Table 1.** List of some higher plants reported for production of NPs.

Plant	Synthesized nanoparticle	Reference
<i>Azadirachta indica</i> leaf extract	Gold, silver and gold core silver shell	Shankar et al., 2004
<i>Aloe vera</i> extract	Gold nanotriangles and silver NPs	Chandran et al., 2005
Coriander leaf extract	Gold NPs	Narayanan et al., 2008
Honey	Gold NPs	Philip et al., 2009a,b
<i>Syzygium aromaticum</i>	Gold NPs	Deshpande et al., 2010
<i>Moringa oleifera</i>	Silver	Prasad et al., 2011
	Silver	Mubayi et al., 2012
<i>Ocimum basilicum</i>	Gold NPs	Singhal et al., 2012
<i>Momordica charantia</i>	Gold NPs	Pandey et al., 2012
<i>Ficus benghalensis</i>	Silver NPs	Saxena et al., 2012
<i>Justicia gendarussa burm F</i> leaf extract	Gold NPs	Fazaludeen et al., 2012

including *S. aureus* and *S. pneumonia* was reported by Sulaimana et al. (2013).

Iron and silver nanoparticles were synthesized at room temperature using a rapid, single step biosynthetic method employing aqueous *Sorghum* extracts as both the reducing and capping agent. Highly crystalline silver nanoparticles with an average diameter of 10 nm were obtained (Njagi et al., 2011). Geranium leaves (*Pelargonium graveolens*) and its endophytic fungus *Colletotrichum* sp used in the extra-cellular synthesis of gold nanoparticles. Leaves and fungus growing in the leaves were separately exposed to aqueous chloroaurate ions and in both cases, rapid reduction of the metal ions was observed resulting in the formation of stable gold nanoparticles of variable size (Shankar et al., 2003). Torresdey et al. (2002) reported the formation of gold nanoparticles in *Alfalfa* (*Medicago sativa*) plants. *Alfalfa* roots have ability of absorbing reduced silver ions Ag (0) from production medium and transferring it to shoot of the plant in the same state of oxidation. Inside the plant tissue, reactions occur and the accumulation of Ag NPs takes place. Another quick method of Ag NPs biogenesis was reported where *R. officinalis* extract was heated with 1 mM AgNO<sub>3</sub> at 7°C for 3 min. The obtained NPs were tested for its antimicrobial potential against six different pathogens (Ghassan et al., 2013).

Copper NPs were synthesized using the latex of *Calotropis procera* by using copper acetate. Cysteine proteases in the latex are responsible for the reduction of copper ions. Cytotoxicity studies were performed on latex stabilized copper nanoparticle on HeLa, A549 and BHK21 cell lines by MTT dye conversion assay and Cu NPs, thus synthesized were excellent in terms of biocompatibility (Harne et al., 2012). Biosynthesis of copper (Cu), zero valent iron (ZVI), silver (Ag) nanoparticles using leaf extract of *Dodonaea viscosa* has been investigated by Daniel et al. (2013). The synthesized spherical nanoparticles showed the average size of 29, 27 and 16 nm for Cu, ZVI, and Ag nanoparticles, respectively. Biosynthesized Cu, ZVI, and

Ag nanoparticles were tested against various human pathogens viz. Gram-negative *E. coli*, *K. pneumonia*, *Pseudomonas fluorescens* and Gram-positive *S. aureus* and *B. subtilis* and showed good antimicrobial activity. Some other higher plants which produced NPs are shown in the Table 1.

Diatoms are single celled photosynthesizing algae. It has been shown that the freshwater diatom, *Stauroneis* sp. can be used to fabricate silicon - germanium oxide nanoparticles by a two step process (Ali et al., 2011). Mono-dispersed platinum and palladium nanoparticles of size up to 6 nm have been synthesized at room temperature by using root extract of *Asparagus racemosus* (Linn.) (Raut et al., 2013). Natural hydrocolloids isolated from trees are new class of potentially economical and environmental friendly biomaterial that exhibits a high specificity for the production of nanomaterials. Gums extracted from plants may act as both reducing and capping agents in nanoparticle synthesis. Vellora et al. (2013) synthesized CuO nanoparticles using gum karaya, a natural nontoxic hydrocolloid that explored its antimicrobial properties.

## CONCLUSION

Biologically synthesized nanoparticles are gaining attention day by day in various fields including electronics, optics, medicines and chemical industries. Due to simplicity, cost effectiveness and eco friendly nature of this method, biogenesis of NPs is of much interest for future prospective. Range of biological entities including bacteria, fungus, viruses, algae, viruses including higher plants has been used so far. Production can be classified as intracellular and extracellular on the basis of site of NP accumulation in biological entity. By controlling different physical parameters like temperature, pH, media, reaction time etc. shape, size and quantity of NPs can be controlled. Different metallic, non metallic and semiconductor NPs have been produced and their

antimicrobial activity has been studied over different pathogens. As compared to physical and chemical methods, biogenesis of NP is time consuming process but it is much cheaper and environmental friendly. Moreover when we talk about biological applications biogenetically synthesized NPs are supposed to be free of toxic chemicals and hence, supposed to be more compatible with biological entities and safer to use. Still much refinement is needed for using this method for large scale synthesis of NPs in terms of efficiency and control over shape and size.

### Conflict of interests

The author(s) have not declared any conflict of interests.

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