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# Biological green synthesis of gold and silver nanoparticles

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**Abstract.** Nanomaterials synthesized by natural bioresources such as microorganisms, animals and plants in nature can also be synthesized in laboratories even on large scale. This is considered as an attractive prospect for eco-friendly or so-called green synthesis. Development of eco-friendly synthesis of biocompatible nanoparticles and their potential biomedical applications introduces the concept of nanobiotechnology. The lower cost and lesser side effects as compare to chemical methods of synthesis are the main advantages of biosynthesis. This review article demonstrates the role of various biological resources e.g. bacteria, fungi, actinomycetes, plant leaves, fruits and honey as well as animal tissues for the synthesis of nanoparticles mainly gold and silver with an overview of their potential applications.

**Keywords:** nanoparticle; nanobiotechnology; microorganism; biosynthesis; biomedical applications; plant extract

## 1. Introduction

Nanotechnology refers to a field where there is control of matter on an atomic or molecular scale. Generally, it deals with structures of dimensions less than 100 nanometers (nm) and involves developing new materials or devices with dimensions on this scale. The word "nano" is Greek, which means dwarf. An atom can be divided into many fundamental particles: electrons, neutrons, meson, protons, quarks etc and it loses its property when it is divided into those particles. In terms of size, atom has the radius in the order of [1Å ]=$10^{-8}$ cm=$10^{-10}$ m. One nanometer is about 8~10 atoms next to one another. Mathematically, 1 nm=$10^{-9}$ m. A new branch of nanotechnology is nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical processes to synthesize nanoparticles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanopaticles formation. The development of eco-friendly protocol for the synthesis of nanomaterials is an important aspect of nanotechnology. The green and cost-effective solution of fabrication of nanomaterials is key for biotechnology development. More specifically, one can define nanobiotechnology involving nanomaterials synthesis using microorganisms, including bacteria, viruses, fungi as well as plant and animal based products. Motivation of understanding biological systems as well as mimicking the synthesis of nanomaterials in nature, scientists have been using the biogenic methods by which a variety of metal, semiconductor (Malarkodi *et al*. 2013) and insulating nanoparticles or their

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assemblies can be synthesized. There are also attempts to synthesize biocompatible, nanomaterials for different biomedical applications such as body implants, drug delivery, cancer therapy etc. Synthesis of nanomaterials using bioresources can be classified roughly into the following types : i)Use of microorganisms like fungi, yeast, or bacteria, ii) use of plant extract, fruit extract and honey iii) use of biological templates like DNA, membranes, viruses and diatoms, iv) use of animal tissues. Currently, there is growing need to develop eco-friendly synthetic protocols of various nanoparticles of different shapes and sizes for avoiding the adverse effects in comparison to traditional chemical methods. There is much scope of further improvement in the synthesis of nanoparticles by biogenic methods using different biological resources as the source of reducing and stabilizing agents and their potential applications. The aim of this paper is to provide an updated overview on the synthesis of gold and silver nanoparticles using various biological methods both intracellularly and extracellularly and their potential applications in optics, optoelectronics, chemical and biosensors, biomedical applications and catalysis. Due to space limitations, a complete review of all recent work on this important subject is not possible. However, we only summarizes a few representative examples, including our own work.

## 2. Synthesis of gold and silver nanoparticles using microorganisms

Microorganisms are the organisms such as bacteria, fungi, yeasts etc. which can be detected under optical microscopes. These microorganisms are capable of interacting with metals when they come into contact with their cells and can form nanoparticles. Many microorganisms can produce inorganic materials either intra or extracellularly. Well-known example is magnetotactic bacteria which able to synthesize magnetic nanoparticles (Bazylinski and Frankel 2004). Magnetotactic bacteria are motile, prokaryotes that move along geometric field lines. They produce magnetosomes, unique intracellular structure contains a magnetic particle, in narrow range of very low oxygen concentration. Magnetotactic bacteria usually mineralize either oxide magnetite $Fe_3O_4$ or iron sulfide $Fe_3S_4$-greigite. Extensive work is in progress using microorganisms for biosynthesis of nanoparticles. Here, some of the organisms exploited as the sources for reducing and stabilizing agents for the synthesis of nanoparticles are discussed along with their brief account of properties**.**

### *2.1 Synthesis of gold and silver nanoparticles using bacteria*

Metal nanoparticles can be extracellularly synthesized generally by the reduction of metal ions in solution. The formation of extracellular and intracellular silver nanoparticles by bacteria (*Pseudomonas stulzeri*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonells typlus*, and *Staphylococcus currens*) had been reported (Lengke *et al*. 2007). Various microbes are known to reduce metal ions to the metals. The formation of extracellular silver nanoparticles can be extracellularly synthesized by photoautotrophic cyanobacterium *Plectonema boryanum* (Lengke *et al*. 2007). The bioreduction of the Ag+ ions could be associated with metabolic processes utilizing nitrate by reducing nitrate to nitrite and ammonium ion (Lengke *et al*. 2007). Cyanobacteria commonly use nitrate as the major source of nitrogen. Nitrate was reduced by cyanobacteria metabolic process.

$$NO_3^- + 2H^+ + 2e^- = NO_2^- + H_2O$$

$$NO_2^- + 8H^+ + 6e^- = NH_4^+ + 2H_2O$$

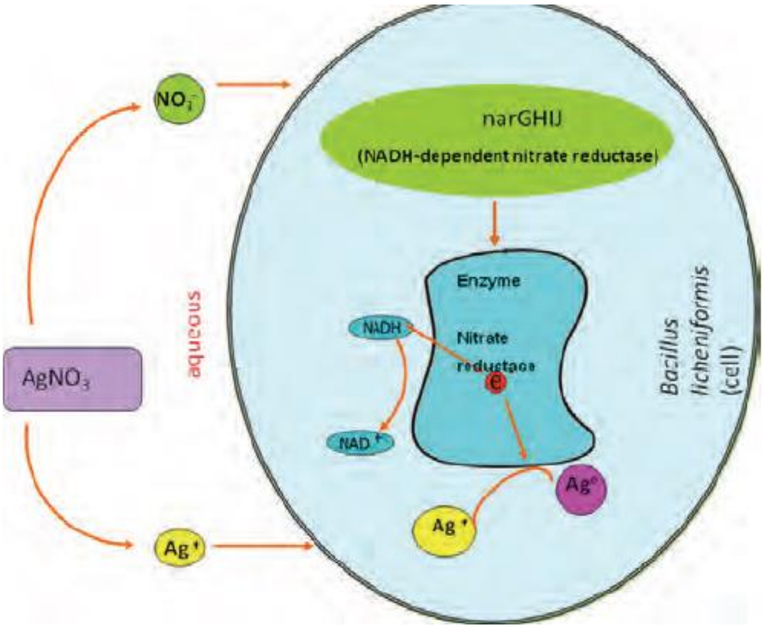


Fig. 1 Schematic representation illustrating the synthesis of silver nanoparticles using *Bacillus licheniformis* (Reprinted with permission from Kalimuthu *et al*. 2008)

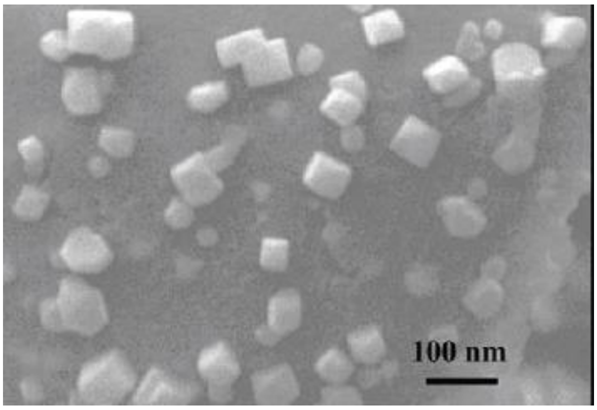


Fig. 2 SEM image of gold nanocubes using *Bacillus licheniformis* (Reprinted with permission from Kalishwaralal *et al*. 2009)

*Pseudomonas aeruginosa* were used by Husseiny *et al*. for the extracellular biosynthesis of gold nanoparticles (Husseiny *et al*. 2007) with dimension in the range of 15-30 nm. As shown in this study, the gold nanoparticles were synthesized in the extracellular cell supernatant of the bacteria. Silver nanoparticles were synthesized using *Bacillus licheniformis* (Kalimuthu *et al*. 2008). Here, the plausible mechanism for the formation of silver nanoparticles involves the enzyme nitrate reductase as shown in Fig. 1. Saifuddin and coworkers reported the eco-friendly rapid biosynthesis of silver nanoparticles using a culture supernatant of bacteria *Bacillus subtilis* and microwave irradiation at the frequency of 2.45 GHz (Saifuddin *et al*. 2009). Kalishwaralal reported the biosynthesized of gold nanocubes using *Bacillus licheniformis* (Kalishwaralal *et al*. 2009). Fig. 2

shows the SEM image of gold nanocubes synthesized by using *Bacillus licheniformis*. Nair and Pradeep (Nair and Pradeep 2002) have synthesized nanocrystals of gold, silver and their alloys by reaction of the corresponding metal ions within cells of lactic acid bacteria present in buttermilk.

### *2.2 Synthesis of gold and silver nanoparticles using the fungal systems*

The fungal systems are extremely good resources in the synthesis of metal nanoparticles. A novel biological method for the synthesis of silver nanoparticles using the fungus *Verticillium* has been reported (Mukherjee *et al*. 2001). Exposure of the fungal biomass to aqueous Ag+ ions resulted in the intracellular reduction of the metal ions and formation of silver nanoparticles of dimensions ~25 nm. Electron microscopy analysis of thin sections of the fungal cells indicated that the silver particles were formed below the cell wall surface, possibly due to reduction of the metal ions by enzymes present in the cell wall membrane. The metal ions were not toxic to the fungal cells and the cells continued to multiply after biosynthesis of the silver nanoparticles. Duran *et al*. reported the extracellular synthesis of Ag nanoparticles using *Fusarium oxysporum* (Duran *et al*. 2005). Sadowski *et al*. (2008) synthesized silver nanoparticles using two *Aspergillus niger* strains. Bhainsa and D'Souza reported the extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Aspergillus fumigatus* (Bhainsa and D'Souza 2006). They studied the kinetics of synthesis including the spectroscopic and microscopic characterization of the synthesized silver nanoparticles. The extracellular synthesis of silver nanoparticles by a marine fungus *Penicillium fellutanum* has been reported by Kathiresan and coworkers (Kathiresan *et al*. 2009). The fungus *P. fellutanum* was isolated from a coastal mangrove sediments. The obtained silver nanoparticles were spherical in shape with size ranging from 5 to 25 nm as shown by the TEM image in Fig. 3.

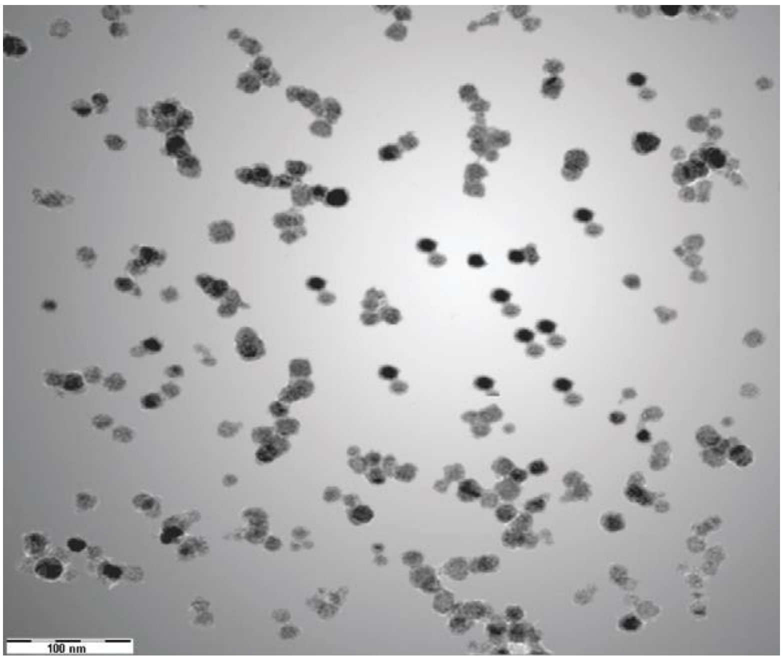


Fig. 3 TEM image of silver nanoparticles synthesized using fungus *Penicillium fellutanum* (Reprinted with permission from Kathiresan *et al*. 2009)

*Cladosporium cladosporioides* is a commonly available fungus found in marshland regions. This fungus was employed to biosynthesis of silver nanoparticles (Balaji *et al*. 2009). D. Philip had synthesized gold and silver nanoparticles using the extract of a naturally occuring edible mushroom *Volvariella volvacea* (Daizy 2009).

### *2.3 Synthesis of gold and silver nanoparticles using the actinomycete*

Actinomycetes are microorganisms which can exhibit important characteristics of both fungi and prokaryotes such as bacteria. Focus on actinomycetes has primarily centered on their phenomenal ability to produce secondary metabolites such as antibiotics. Murali Sastry and his group were pioneer on the synthesis of metal nanoparticles using actinomycete (Sastry *et al*. 2003). Extremophilic Actinomycete *Thermomonospora* sp. (Ahmad *et al*. 2003) were exploited by Ahmad *et al*. for the extracellular biosynthesis of monodisperse gold nanoparticles of dimension ~ 8 nm. Alkalotolerant Actinomycete, *Rhodococcus* species were used for the intracellular synthesis of gold nanoparticles (Ahmad *et al*. 2003).

## 3. Synthesis of gold and silver nanoparticles using fruit extract

Fruit and plant extracts can be used to synthesize metal nanoparticles. The use of these extracts can eliminate the elaborate process of maintaining cell cultures using microorganisms for the nanoparticle synthesis. In addition to this, large-scale synthesis of nanocrystals can be carried out at lower cost. Ankamwar *et al*. had synthesized gold and silver nanoparticles using *Emblica officinalis* fruit (Ankamwar *et al*. 2005) extracts. Fig. 4 shows the photograph of *Emblica officinalis* fruit.

Fig. 4 Photograph of *Emblica officinalis* fruit (photo courtesy : Dr. Balaprasad Ankamwar)

Fig. 5 Photograph of some plant which can be used for extract preparation in metal nanoparticles synthesis (photo courtesy : Dr. Balaprasad Ankamwar)

Fig. 6 Photograph of Lemon grass plant

## 4. Synthesis of gold and silver nanoparticles using plant extract

An important protocol for the eco-friendly biosynthesis of nanoparticles is the application of plant extract. Use of different plants in the synthesis is quite novel leading to true green chemistry. Fig. 5 shows some popular plants which can be used for the extract preparation.

Murali Sastry and his group had used the leaves of geranium plant (*Pelarogonium graveolens)* to synthesize gold nanoparticles (Shiv Shankar *et al*. 2003). It should be noted that there is also a plant associated fungus which can produce compounds such as taxol and gibbarellins. There is an exchange of intergenic genetics between fungus and plants. Nanoparticles obtained using *Colletotrichum sp* fungus related to geranium plant have a wide distribution of sizes with mostly spherical shapes. However, rod and disk shaped nanopaticles can be synthesized using geranium plant extract.

Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts had been reported (Song *et al*. 2009). There are reports about the synthesis of gold nanoparticles using *Murraya koenigi* leaf (Ankamwar 2010), Lemon grass (Shiv Shankar *et al*. 2004), Aloe vera leaf (Chandran *et al*. 2006), Neem leaf (Shiv Shankar *et al*. 2004), *Tamarindus indica* leaf (Ankamwar *et al*. 2005) and silver nanoparticles using *Murraya koenigi* leaf

(Ankamwar *et al*. 2012). Similarly, we find the synthesis of gold and silver nanoparticles using Krishna tulsi leaf extract (Daizy and Unni 2011) and *Hibiscus rosa sinensis* (Daizy 2010).

Triangular gold nanoprisms can be synthesized biologically (Shiv Shankar *et al*. 2004) in high yield at room temperature by the reduction of aqueous chloroaurate ions ($AuCl_4^-$) by using the plant lemongrass (*Cymbopogon flexuosus*) extract. Fig. 6 shows the photograph of Lemon grass plant.

Dr. Murali Sastry of Physical & Materials Chemistry Division, Dr. Absar Ahmed of Biochemical Sciences Division of National chemical laboratory (NCl), Pune and the team had demonstrated biological synthesis of large amounts of triangular gold nanoprisms by a single-step, room-temperature reduction of gold salt solution by the extract of the plant, lemongrass (*Cymbopogon flexuosus*). The lemongrass extract on mixing with gold salt solution exhibits a change of colour from pale yellow to a vivid ruby red. The reaction mixture is allowed to stand for six hours to yield a large number of triangular gold nanoparticles of 8-18 nm thickness with an edge length of 200-500 nm. The scientists also enhanced the percentage of gold nanotriangles in the reaction medium up to ninety-five per cent of the nanoparticle population by repeated centrifugation.The reducing sugars (aldoses) present inside the lemongrass extract were found to reduce the $Au^{3+}$ into nanoprisms (Shiv Shankar *et al*. 2005). By simple variation in the concentration of the lemongrass extract in the reaction medium, it is possible to vary the size of the nanoprisms, thereby, the longitudinal SPR band in the NIR region can be easily tuned. It is reported that tamarind leaf extract can also be used as the reducing agent for making gold nanotriangles (Ankamwar *et al*. 2005). On treating aqueous $Au^{3+}$ solution with tamarind leaf extract, rapid formation of flat and thin single crystalline gold nanotriangles was observed. The effect of different organic solvent vapours such as methanol, benzene, and acetone on the conductivity of these gold nanotriangles was investigated by measuring the I-V characteristics. The results suggest that these nanotriangles can be used as vapor sensors. *Cinnamomum zeylanicum* leaf broth can be used as reducing agent to reduce $Au^{3+}$ into gold nanoprisms (Smitha *et al* 2009). Nanoparticles of diverse shapes such as hexagon, truncated triangle, and triangle can also be synthesized by reducing aqueous chloroauric acid solution with the extract of seaweed, Sargassum *sp*., at room temperature (Liu *et al*. 2005).

## 5. Synthesis of gold nanoparticles using honey

Daizy (Daizy 2009) and her group carried out honey-mediated green synthesis gold nanoparticles. 10 mL of aqueous solution of honey was added to 30 mL of $HAuCl_4$. The reduction of $AuCl_4^-$ was completed in 3 hours as verified by observing the light purple colour of the solution. The speed of reduction is found to increase with increase in the addition of honey and colloid is obtained within 30 min. The final pH of the colloids was 3. A slight increase of pH could also speed up the reduction and was complete within a few minutes. The primary ingredient of honey is fructose, a monosaccharide and a strong reducing agent. Also, it contains vitamin C (ascorbic acid), a weak reducing agent. Further, when honey is diluted with water, chemical reaction between glucose, water and oxygen produces small amounts of $H_2O_2$ and gluconic acid. This slow release of $H_2O_2$ makes honey a mild antiseptic. The acidity of honey reduces the number of organisms that can live in it. As glucose is changed to gluconic acid due to dilution with water, the presence of the reducing agent fructose in honey may be responsible for reduction. It is also possible that sucrose and proteins/enzymes play a role in the reduction. When an excess of honey was used to reduce the aqueous $HAuCl_4$, the biomolecules acting as capping agents strongly shaped spherical nanoparticles rather than nanotriangles though the reductive biomolecules were enhanced. The presence of large quantity of honey, causes strong interaction between protective biomolecules and surfaces of nanoparticles preventing nascent gold nanocrystals from sintering. With larger quantities of honey, the interaction is intensified, leading to size reduction of spherical nanoparticles. FTIR measurement was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of Au nanoparticles synthesized using honey. Similarly, silver nanoparticles can be synthesized using honey (Daizy 2010).

## 6. Synthesis of gold and silver nanoparticles using animal tissue

Animal tissue can be a potential source of reducing and capping agents for the biosynthesis of nanomaterials such as gold and silver nanoparticles. Recently, Jha and Prasad (Jha and Prasad 2013) employed the use of cockroach (*Periplaneta Americana*) broth to synthesize gold nanoparticles. The synthesized nanoparticles were characterized by X-ray diffraction and transmission electron microscopic analysis to ascertain the formation of nanoparticles. The synthesis of nanoparticles might have resulted due to the activity of chitin, metallothioneine and tropomyosin to explain the possible mechanism of biosynthesis of nanoparticles. This work provides a new step for utilizing animal wastes in synthesizing different nanomaterials and subsequently addressing environmental issues.

## 7. Applications of gold and silver nanoparticles

The major intention of biological synthesis of nanoparticles is to develop eco-friendly and synthetic protocols with minimum cost of production and applications, specially in the biomedical field. Obviously, large number of researchers diverted their attention towards the use of biological systems for the synthesis of biocompatible metal nanostructures. From the nanotechnology point of view, identification of prokaryotic microorganisms such as actinomycetes in the extracellular synthesis of metal nanoparticles should offer greater scope for development via genetic manipulation through modern methods of recombinant DNA technology and protein engineering (Ahmad *et al*. 2003). The nanoparticles bound to the surface of the fungal cells may be used for the catalysis and as precursors for synthesis of coatings for electronic application. Extra-cellular synthesis offers a great advantage over an intra-cellular process of synthesis from the application point of view as the nanoparticles formed inside the bacteria would have required additional step of processing for the release of the nanoparticles from the bacteria by ultrasound or chemical treatment with suitable reagents. The silver nanoparticles obtained by biosynthesis can be used in catalysis, data storage, energy storage, microelectronics etc., (Ganesh Babu *et al*. 2009). Duran *et al*. (2007) reported silver nanoparticles incorporated cloths are sterile and can be useful in hospitals to prevent or to minimize infection with pathogenic bacteria. Ankamwar *et al*. used (Ankamwar *et al*. 2005) biogenic gold nanotriangles films as vapour sensors. The high absorption coefficient of these gold triangles in the NIR region make them useful in fabricating photonic devices such as optical sensors and NIR absorbers. Another application based on the large NIR absorption of the gold nanoparticles could be in hyperthermia of tumors (Hirsch *et al*. 2003). The extremely flat nature of the nanoparticles would facilitate excellent thermal contact between the nanotriangles and tumor cells. The gold nanoparticles synthesized in aqueous medium using *Murraya koenigi* leaf extract can be used for cancer therapy due to anti-carcinogenic properties of biomolecules in the extract.

## 8. Conclusions

Various bioresources such as bacteria, fungi, actinomycete, plant leaves, fruits, honey as well as animal tissues can be used as a possible source of reducing and capping agents for the synthesis of nanoparticles. This review article demonstrates the role of various biological resources e.g., bacteria, fungi, actinomycete, plant leaves, fruits and honey as well as animal tissues for the synthesis of nanoparticles mainly gold and silver with an overview of their potential applications. It is very difficult to find out the possible mechanism of these biosynthetic protocols. However, one can identify the reducing and capping agents in the bioresources e.g., nitrate reductase in bacteria; cytochrome c in fungi; hydrolysable tannins, antioxidants in fruits; reducing sugars, aldehydes/ketons in plant leaves; fructose sugars and proteins in honey; chitin, metallothioneine and tropomyosin in animal tissues.

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