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## RESEARCH ARTICLE

### BIOLOGICAL PROSPECTIVE FOR SYNTHESIS OF METAL NANOPARTICLES: A REVIEW

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#### ABSTRACT

Nanoparticle research is currently an area of intense scientific interest, due to a wide variety of potential applications in biomedical, optical, and electronic fields. A *nanoparticle* (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm. Metallic nanoparticles have fascinated scientist for over a century and are now heavily utilized in biomedical sciences and engineering. Today these materials can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands, and drugs of interest and thus opening a wide range of potential applications in biotechnology such as targeted drug delivery and more importantly diagnostic imaging. The greater demand of nanoparticles has therefore led to the development of an eco-friendly method for the synthesis of nanoparticles using biological sources as a potential alternative to numerous chemical and physical methods.

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#### INTRODUCTION

Nano-sized materials, known as *nanoparticles* represents collection of atoms or molecules in the size range of  $10^{-9}$  meters. These particles lie within 1-100 nm. They possess unique and improved properties because of their enhanced surface area to volume ratio as they approaches smaller size. They are therefore considered as *building blocks* of nanotechnology in order to design materials with interesting properties (Bunghez et al., 2012).

##### Types of Metal Nanoparticles

In the last decade, noble metal nanoparticles have attracted much attention of scientific researchers due to their applications in medicine, biology, optoelectronics, material science (Bunghez et al., 2012).

¶ **Gold Nanoparticles:** Colloidal gold, also known as gold nanoparticles, is a suspension (or colloid) of nanometer-sized particles of gold. In ancient Roman times, Gold nanoparticles (AuNPs) were used to stain glasses for decorative purposes. AuNPs were also used for curing various diseases centuries ago. The modern era of AuNPs synthesis began over 150 years ago with the work of Michael Faraday, who firstly observed that colloidal gold solutions have unique fascinating properties that differ from bulk gold (Hayat, 1989).

- ¶ **Silver Nanoparticles:** Like gold nanoparticles, ionic silver has a long history and was initially used to stain the glass for yellow. Currently, there is also an effort to incorporate silver nanoparticles into a wide range of medical devices, including bone cement, surgical instruments, surgical masks, etc. Moreover, it has also been shown that ionic silver, in the right quantities, is suitable in treating wounds (Mody et al., 2010). Silver nanoparticles, like their bulk counterpart also show effective antimicrobial activity against Gram-positive and Gram-negative bacteria, including highly multi resistant strains such as methicillin resistant *Staphylococcus aureus* (Pan'áček et al., 2006).
- ¶ **Magnetic Nanoparticles:** Magnetic nanoparticles are recently developed new materials due to their unique micro configuration and properties like super paramagnetic and high coercive force, and their prospect for broad applications in biological separation and biomedicine fields. Magnetic nanoparticles like  $Fe_3O_4$  (magnetite) and  $Fe_2O_3$  (maghemite) are known to be biocompatible (Li et al., 2011).
- ¶ **Copper Nanoparticles:** Copper has an excellent electrical conductivity. Due to relatively low costs, this metal plays a significant role in modern electronic circuit. Because of its excellent electrical conductivity, catalytic behaviour, good compatibility and surface enhanced Raman scattering activity, Cu nanoparticles have drawn the attention of scientists to be used as essential component in the future nanodevices. Cu nanowires are used in nanoelectronics and have application possibilities for magnetic devices, nanosensors, electron emitters and other electronic applications. Cu nanoparticles have been explored to be

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used as nanoprobe in medicines and bio-analytical areas (Pergolese et al. 2006).

̄ **Metal Oxide Nanoparticles:** Metal oxides play a very important role in many areas of chemistry, physics and materials science (Garcia and Rodriguez, 2007). The metal elements are able to form a large diversity of oxide compounds. Oxide nanoparticles can exhibit unique physical and chemical properties due to their limited size and a high density of corner or edge surface sites. Metal-oxide particles (or pigments), such as TiO<sub>2</sub> and ZnO, serve many functions in the various polymeric materials. Traditionally, they have been used as pigments to enhance the appearance and improve the durability of polymeric products, and usually they have been considered to be inert (Sung et al., 2003).

### Synthesis of Nanoparticles

There are two approaches for synthesis of nano materials and the fabrication of nano structures. In the bottom-up approach, the structure of nanoparticles is constructed by atoms, molecules or clusters. In top-down approaches, a bulk piece of a required material is reduced to nanosized dimensions using cutting, grinding and etching techniques, i.e., nanomaterials are prepared from larger entities without atomic-level control (Fig. 1).

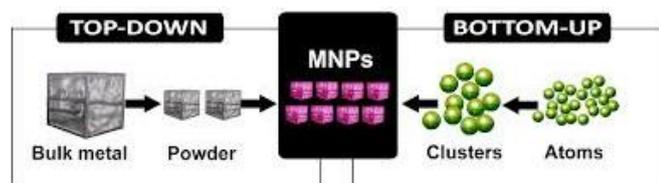


Fig. 1. Approaches for Nanoparticle Synthesis

Chemical reduction, microemulsion (colloidal) techniques, sonochemical reduction, electrochemical, microwave-assisted, and hydrothermal synthesis are the main techniques for the synthesis of nanoparticles through the chemical approach (Umer et al., 2012).

Physical methods for nanoparticles synthesis are laser (pulse) ablation, vacuum vapor deposition, pulsed wire discharge (PWD) and mechanical milling.

A wide range of nanoparticles can be produced using physical methods with little modification for different metals, but the main disadvantages of these methods are the quality of the product, which is less as compared to nanoparticles produced by chemical methods. Usually these methods require costly vacuum systems or equipments to prepare nanoparticles (plasmas) (Umer et al., 2012). Most of the physical or chemical methods of nanoparticles involve the use of hazardous chemicals, low material conversions and high energy requirements. So, there is a growing need to develop an environmental friendly process for nanoparticle synthesis (Logeswari et al., 2013).

### Green Synthesis of Metal Nanoparticles (MNPs)

Green synthesis provides advancement over chemical and physical methods as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Johnson et al., 2014) (Fig. 2). The green synthesis of MNPs should involve three main steps based on green chemistry perspectives, namely (1) the selection of a biocompatible and nontoxic solvent medium, (2) the selection of environmentally benign reducing agents, and (3) the selection of nontoxic substances for stabilization of the nanoparticles (Das and Marsili, 2011). Several microorganisms, such as bacteria, fungi and yeasts, have come up as nanofactories, synthesizing metal nanoparticles of Ag and Au. However, the use of plants for the fabrication of nanoparticles has drawn attention, because of its rapid, economical, eco-friendly protocol, and it provides a single step technique for the biosynthesis process. Biological approaches using microorganisms and plants or plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods (Logeswari et al., 2013).

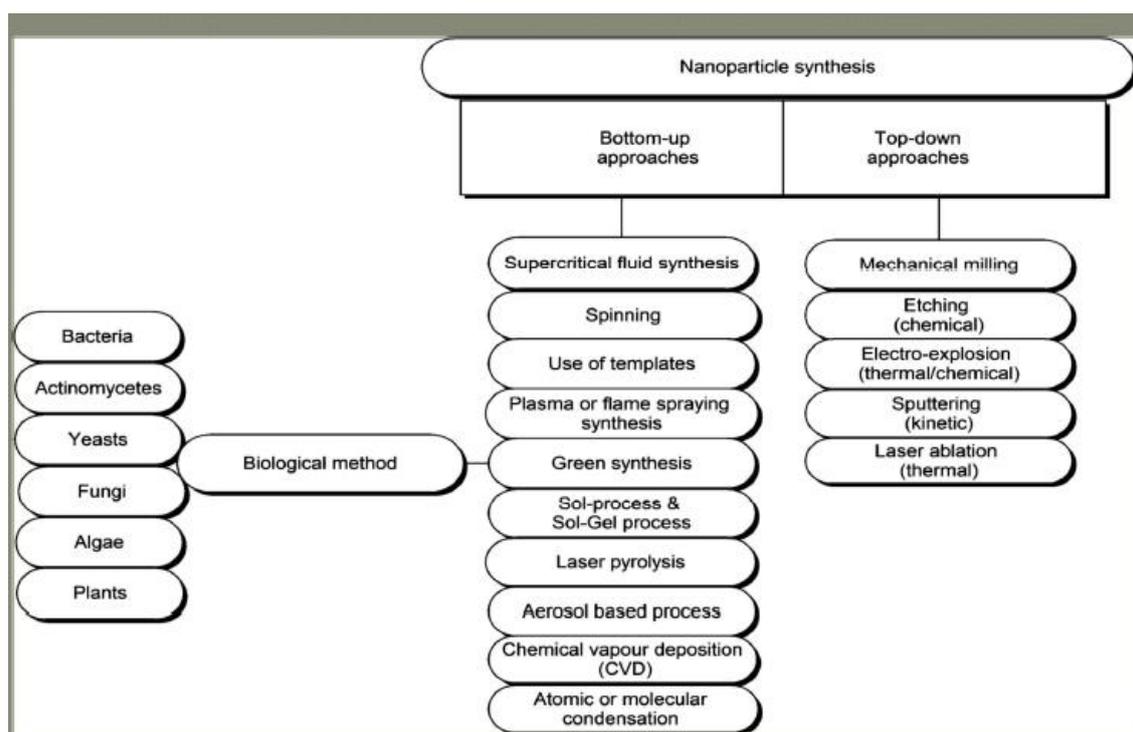


Fig. 2. Methods of Nanoparticle Synthesis

## Bacterial nanoparticle synthesis

Bacteria play a crucial role in metal biogeochemical cycling and mineral formation in surface and subsurface environments. In presence of high concentration and even toxic metal ions, bacteria harbor numerous detoxification mechanisms like dissimilatory oxidation or reduction of metal ions, complication and/or precipitation, impaired transport system, efflux system, etc. Thus microorganisms have been used with a certain success in metal bioremediation of contaminated subsurface environments. However, their role in nanoparticle synthesis has recently been observed. Among the microorganisms, bacteria have received the most attention in the area of metal nanoparticle biosynthesis. The formation of extracellular and intracellular metal nanoparticles by bacteria like *Escherichia coli*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Plectonema boryanum*, *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholerae*, etc., have been reported (Das and Marsili, 2011).

Research has been focused heavily on prokaryotes as a means of synthesizing metallic nanoparticles because of their abundance in the environment and their ability to adapt to extreme conditions. They are also fast growing, inexpensive to cultivate and easy to manipulate. Growth conditions such as temperature, oxygenation and incubation time can be easily controlled. In a study by He *et al.* (2007), it was discovered that changing the pH of the growth medium during incubation results in the production of nanoparticles of differing size and shape. Controlling such properties is important, as varying sizes of nanoparticles are required for different applications such as optics, catalysts or antimicrobials (Pantidos and Horsfall, 2014).

Recently it has been shown that zero valent palladium (Pd<sup>0</sup>) nanoparticles can be synthesized using bacteria found at Alpine sites heavily contaminated with heavy metals. Of all the variety of heavy metal resistant bacteria found in that environment, they found that *Pseudomonas* cells were involved in producing catalytically active nanoparticles (Pantidos and Horsfall, 2014). The bacterium *Bacillus licheniformis* was observed to produce intracellular silver nanoparticles (AgNPs) (Kalimuthu *et al.*, 2008). The colour of the culture after addition of silver ions turned to dark brown indicating the presence of AgNPs.

However, the approach with the greatest potential for the industrial bio production of AgNPs was reported by Shahverdi *et al.* in 2007. Instead of adding metal ions to a live culture, the cultures were centrifuged and the supernatant was tested for the ability to create metallic nanoparticles. Therefore Shahverdi *et al.* were able to create AgNPs in 5 minutes, without the need for a cell lysis step by the use of culture supernatant. This extracellular type of formation is more desirable not only because of the simplicity of purification but also due to the observed increased production rate. An interesting study by Sintubin *et al.*, 2009 focused on the production of AgNPs by lactic acid bacteria. Many bacterial species were tested but only four were found to synthesise AgNPs: *Lactobacillus* species, *Pediococcus pentosaceus*, *Enterococcus faecium* and *Lactococcus garvieae*.

They showed that by increasing the pH of the medium, the reduction rate of the nanoparticles increased. The effect of pH on nanoparticle synthesis was also observed by He *et al.* in 2007. By varying the pH levels, nanoparticles of differing size and shape were formed. Although Ag and Au NPs are important due to their antimicrobial abilities, many studies have been done on other metals such as uranium (U). In 2013 a study by Ramanathan *et al.* in 2013, suggested the production of pure elemental Cu nanoparticles by biological means, using *Morganella morganii* was possible. In 2007, Konishi and coworkers reported that platinum nanoparticles were achieved using the metal ion-reducing bacterium *Shewanella algae*. Resting cells of *S. algae* were able to reduce aqueous PtCl<sub>6</sub><sup>2-</sup> ions into elemental platinum at room temperature and neutral pH within 60 min when lactate was provided as the electron donor. Platinum nanoparticles of about 5 nm were located in the periplasm.

## Fungi in nanoparticle synthesis

The use of fungi in producing metallic nanoparticles has received significant interest as they offer certain advantages over the use of bacteria for the synthesis of nanoparticles. The ease of scaling up both at laboratory as well as industrial level and downstream processing, the economic feasibility and the presence of mycelia offering an increased surface area, are important advantages to consider. In 2001, Mukherjee *et al.* also suggested that because fungi secrete significantly higher amounts of proteins than bacteria, this would amplify the nanoparticle synthesis productivity. Different fungal and actinomycete species, i.e. *Fusarium oxysporum*, *Verticillium* sp., *Thermomonospora* sp., *Rhodococcus* sp., and *Rhizopus oryzae* have been reported to synthesize nanoparticles intra- or extracellularly (Ahmad *et al.*, 2003; Mandal *et al.*, 2006).

Growth of a variety of fungi, such as *Penicillium citreonigrum*, *Trametes versicolor*, *Fusarium* sp., *Phanaerochaete cryosporium*, *Trichoderma viride*, *Neurospora crassa*, *Nematolona frowardii*, and *Bjerkandera adusta* was tested in citrate-stabilized colloidal medium containing different noble-metal nanoparticles. The metal nanoparticles bind on the surface of the fungi and forms nano-hybrid system. Even the edible mushroom *Volvariella volvacea* can produce Au and Ag NPs. The mushroom biomass also prevents NPs aggregation after their formation (Philip, 2009). The fungus *Fusarium oxysporum* has been used in a large number of studies attempting to create metallic nanoparticles, especially those made of silver. Pure AgNPs were synthesized at a size range of 5-15 nm and it was suggested that they were capped in order to stable them by proteins secreted by the fungus (Ahmad *et al.*, 2003). Although the most important advance in the fungal synthesis of metal nanoparticles was that *F. oxysporum* produced these nanoparticles extracellularly, in contrast to all previous research which reported only the intracellular production of Ag and AuNPs (Ahmad *et al.*, 2003). *Fusarium oxysporum* has also been shown to produce Cadmium sulphide (CdS), lead sulphide (PbS), zinc sulphide (ZnS) and molybdenum sulphide (MoS) nanoparticles, when the appropriate salt is added to the growth medium (Ahmad *et al.*, 2002).

A later study on the production of Ag NPs was done by **Bhainsa et al., 2006** in which they used *Aspergillus fumigatus* to synthesise extracellular silver nanoparticles in the size range of 5-25 nm. The fungus *Trichoderma reesei* has also been shown to produce extracellular AgNPs. Vahabi et al., 2011 were able to produce AgNPs using this fungus after 72 hours, which was significantly slower than *A. fumigatus* and *Fusarium oxysporum*. However the use of *T. reesei* does have an advantage over the use of other fungi for the production of metallic nanoparticles.

Although extracellular formation has its advantages, such as lower cost, simpler downstream processing, intracellular formation can also be of great importance. In the case of bioremediation, heavy metals such as Cu and Pt need to be removed from contaminated environments. By using fungi that have the ability to produce intracellular nanoparticles, it would be far easier to remove the fungus and its accumulated metal contaminant from the contaminated sample (**Das et al., 2014**). Similar to bacteria, fungi also have an important disadvantage when it comes to safety. Many well studied fungi such as *F. oxysporum* are pathogenic and therefore might pose a safety risk. *Trichoderma asperellum* (**Mukherjee et al., 2001a**) and *Trichoderma reesei* (**Vahabi et al., 2011**) are both fungi that produce AgNPs when exposed to silver salts and have been proven to be non pathogenic which makes them ideal for use commercially. In fact, *T. reesei* has already been used widely in sectors such as food, animal feed, pharmaceuticals, paper and textile industries (**Nevalainen et al., 1994**).

## Nanoparticles synthesis assisted by plants

Among the use of living organisms for nanoparticle synthesis, plants have found application particularly in metal nanoparticle synthesis. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell cultures. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts and in a controlled manner according to their size, dispersity and shape. Plant use can also be suitably scaled up for large-scale synthesis of nanoparticles (Fig. 3). Noble metals, especially Au and Ag, have been extensively tested for the biosynthetic process assisted by plants, in order to obtain metallic nanoparticles with control over shape and size. A number of researchers have listed the metallic nanoparticles obtained by biosynthesis employing plants biomass or extracts in their reports (**Kumar and Yadav, 2009; Bali et al., 2006**).

The first successfully report of synthesis of nanoparticles assisted by living plants appeared in 2002 when it was shown that gold nanoparticles, ranging in size from 2 to 20 nm, could form inside alfalfa seedlings (**Torresday et al., 2002**). An interesting study by **Bar et al. in 2009** illustrated a simple green synthesis route for AgNPs from AgNO<sub>3</sub> salts using the extract from *Jatropha curcas*. The result was the production of fairly homogenous (10-20 nm) AgNPs in 4 hours.

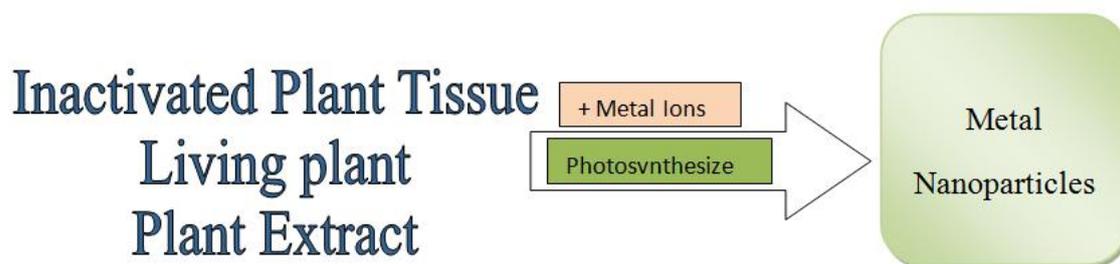


Fig. 3. Metal Nanoparticle Synthesis by Plants

## Nanoparticle synthesis by yeast

The extracellular synthesis of nanoparticles in huge quantities, with straightforward downstream processing, has been reported by **Kowshik et al. in 2003**. This group has been involved in isolation of silver tolerant yeast strain MKY3, by inoculating with aqueous silver nitrate. The formation of 2-5 nm silver nanoparticles takes place in the forced ecological conditions. The synthesis of cadmium nanoparticles by using *Candida glabrata* and *Schizosaccharomyce pombe* has been reported by **Dameron et al., 1989**. The silver and gold nanoparticles biosynthesis was also investigated by Mourato et al. in 2011, using an extremophilic yeast strain isolated from acid mine drainage.

Most bacteria and fungi produce nanoparticles of a much wider size range as described earlier. However, for the synthesis process to occur the sample needs to be heated to a temperature of 85°C, which would be fairly costly if high volumes are required.

Another study, using *Acalypha indica* leaf extracts showed that AgNP synthesis using plants is possible (**Krishnaraj et al., 2010**). Lukman et al. reported the production of AgNPs using *Medicago sativa* seed exudates. Ag<sup>+</sup> reduction occurred almost instantly as AgNPs were observed within 1 minute of exposure to the silver salt. In less than 50 minutes 90% Ag<sup>+</sup> reduction occurred when the reaction was carried out at 30° C which is significantly lower than the temperature used for *J.*

*curcas*. The nanoparticles produced were spherical, flower-like and/ or triangular in shape with a size range of 5-108 nm (Lukman *et al.*, 2011). In contrast to Krishnaraj *et al.*'s study, not only were the purified AgNPs from *M. sativa* heterogenous in size, they did not show significant inhibition of bacterial growth. However, it was also suggested by Lukman *et al.* that they could act synergistically with the seed exudate in order to eliminate bacteria.

Another plant that has the potential to reduce  $\text{Ag}^+$  is *Ocimum sanctum*. Its leaf extract was mixed with 1 mM  $\text{AgNO}_3$  and the production of AgNPs of 3-20 nm in size was observed. The particles were spherical in shape and it was suggested by Mallikarjuna *et al.* in 2011, that they may be stabilised by a component of the leaf broth. In a study by Kasthuri *et al.* in 2008, phyllanthin was extracted from the plant *Phyllanthus amarus* and subsequently used for the production of Ag and AuNPs. Phyllanthin concentrations played a key role in the size and shape of the nanoparticle produced. Low concentrations resulted in the slow formation of triangular and hexagonal AuNPs and higher concentrations of phyllanthin gave rise to greater levels of spherical NPs which was confirmed by UV-Visible and TEM analysis (Kasthuri *et al.* 2008). Park *et al.* talked about the use of plant derived polysaccharides and phytochemicals which can be used for the synthesis of gold and silver nanoparticles. The use of such compounds for nanoparticle synthesis offers advantages such as decreased use of toxic chemicals and the ability of creating nanocomposites with different metals. Soluble starch, chitosan, cellulose, dextran, alginic acid and hyaluronic acid have been used for the production of silver and gold nanoparticles successfully (Pantidos and Horsfall 2014).

An extensive study was done by Song *et al.* in 2009 on the production of AgNPs from a number of different plant leaf extracts. They examined the use of Pine, Persimmon, Ginkgo, Magnolia and Platanus extracts and compared their ability to produce AgNPs. The Magnolia leaf broth was found to be the best  $\text{Ag}^+$  reducer as it took only 11 minutes to reduce 90% of the  $\text{Ag}^+$  present in the sample. In this example, once again, reaction conditions such as temperature needed to be controlled closely during the synthesis stage as they affected the rate, size and shape of the NPs.

Song *et al.* in 2009 tested the effect of different reaction temperatures and found that at 95°C,  $\text{Ag}^+$  conversion was much higher than at 25°C. However the size of the nanoparticles decreased at higher temperatures. It was hypothesized this was caused by the increased turnover of the nanoparticles by the reducing agent, as it has less time to build upon the pre synthesized nanoparticles before starting to synthesize new ones.

Extracellular synthesis of AuNPs was illustrated using extracts from a lemongrass plant, *Cymbopogon flexuosus*. Liquid-like nano triangles formed by aggregates of spherical AuNPs were obtained when the extract was incubated with gold tetrachloride ( $\text{AuCl}_4^-$ ). This fluidity was attributed to the nanoparticle surface complexation of aldehydes and/or ketones that were present in the plant extract (Shankar *et al.*, 2004). The leaf broth of a Neem plant, *Azadirachta indica* was mixed

with the salts of Au, Ag and then both metal ions simultaneously. The production was rapid with the synthesis rate starting to plateau at approximately 2 hours (Shankar *et al.* 2004). The production of PtNPs by bacteria and fungi has been researched in the past but not to a great extent and research on PtNP production by plants is even scarcer (Pantidos and Horsfall 2014).

All the aforementioned studies on the synthesis of metallic NPs by plants involved using part or whole plant extracts, though it is also possible to synthesize metallic nanoparticles inside live growing plants. However there were no reports where plants have been used as the biological producers of Pd NPs until Parker *et al.* in 2014 presented a method of creating such nanoparticles using the plant, *Arabidopsis thaliana*. After growing *Arabidopsis*, its medium was then replaced with potassium tetra chloro palladate ( $\text{K}_2\text{PdCl}_4$ ) and incubated for 24 hours in the salt solution. Subsequent TEM results visualised the PdNPs produced were at 2-4 nm size range. Alfalfa plant seeds were prepared and grown for two weeks with various concentrations of K ( $\text{AuCl}_4^-$ ). The nanoparticles were then extracted and analyzed by various methods which confirmed the production of elemental AuNPs (Torresday *et al.*, 2002).

#### Mechanism of nanoparticle synthesis by Micro organisms

Synthesis of metal nanoparticles in different types of microbial species are reported, however, the exact mechanism of nanoparticle biosynthesis has not been established. Recent works by Xie *et al.* (2007) demonstrated that proteins are the principal biomolecules involved in the algal synthesis of gold NPs. Enzymatic reduction (Ahmad *et al.*, 2003; He *et al.*, 2007) of metal ions and subsequent growth of metal nanoparticles have postulated for fungal mediated synthetic mechanism. Ahmad *et al.* (2003) postulated that in *Fusarium oxysporum*, a NADH-dependent reductase is involved in AgNPs synthesis. Daniel *et al.* (2008) demonstrated that the FAD-dependent enzyme glutathione reductase (GR) catalyzes the NADPH-dependent reduction of  $\text{AuCl}_4^-$ . The synthesized AuNPs at the active site tightly bound through the catalytic cysteines group. Involvement of nitrate reductase in *B. licheniformis* in the fabrication of nanoparticles has also been reported (Das and Marsili, 2011).

Johnston *et al.* in 2013 believed that the production of delftibactin was associated with the resistance mechanism of *D. acidovorans* to toxic gold ions. By producing inert gold nanoparticles bound to delftibactin, the gold no longer posed any toxicity problem for the cells. The extracellular formation of gold nanoparticles of 10-20 nm by the bacterium *Rhodospseudomonas capsulata* is possible and these nanoparticles were synthesized via an NADH-Dependant Reductase (Pantidos and Horsfall, 2014).

*Escherichia coli*, can also synthesise  $\text{Pd}^0$  nanoparticles with the help of hydrogenases found in the bacterium (Lloyd *et al.* 1998). Ramanathan and group in 2013 stated that *M. morgani* synthesises the Cu nanoparticles intracellularly by uptake of the Cu ions and subsequent binding of the ions to either a metal ion reductase or similar protein.

This results in the reduction of the ion to metallic  $\text{Cu}^0$  which then accumulates extracellularly as nanoparticles once effluxed out of the cell; however this has not been confirmed. Another study based on *Morganella* sp. showed the extracellular synthesis of AgNPs (Parikh et al., 2008).

Different microorganisms have different mechanisms of forming nanoparticles. However, nanoparticles are usually formed following this way: metal ions are first trapped on the surface or inside of the microbial cells. The trapped metal ions are then reduced to nanoparticles in the presence of enzymes. In general, microorganisms impact the mineral formation in two distinct ways: They can modify the composition of the solution so that it becomes supersaturated or more supersaturated than it previously was with respect to a specific phase. A second means by which microorganisms can impact mineral formation is through the production of organic polymers, which can impact nucleation by favoring (or inhibiting) the stabilization of the very first mineral seeds (Li et al., 2011).

### Mechanism of nanoparticle synthesis in plants

On the whole, the mechanism of metal nanoparticles synthesis in plants and plant extracts includes three main phases (Fig. 4): 1) the activation phase during which the reduction of metal ions and nucleation of the reduced metal atoms occur; 2) the growth phase during which the small adjacent nanoparticles spontaneously coalesce into particles of a larger size (direct formation of nanoparticles by means of heterogeneous nucleation and growth, and further metal ion reduction; a process referred to as Ostwald ripening), which is accompanied by an increase in the thermodynamic stability of nanoparticles; and 3) the process termination phase determining the final shape of the nanoparticles (Makarov et al., 2014).

As the duration of the growth phase increases, nanoparticles aggregate to form nanotubes, nanoprisms, nanohexahedrons, and a variety of other irregularly shaped nanoparticles, as well. In the termination phase, nanoparticles acquire the most energetically favorable conformation, with this process being strongly influenced by the ability of a plant extract to stabilize metal nanoparticles. For example, nanotriangles have a very high surface energy, which makes them less stable, and if the stability of nanoparticles is not supported in given extracts, then the nanotriangles will acquire a more stable morphology, such as a truncated triangle, in order to minimize the Gibbs free energy (Makarov et al., 2014).

Therefore, the reduction process of metal ions with the formation of nanoparticles is affected by a large number of factors; besides the nature of a plant extract containing active biomolecules in different combinations and concentrations, these include the reaction mixture pH, incubation temperature, reaction time, concentration, and electrochemical potential of a metal ion. The pH value of a plant extract exerts great influence on the formation of nanoparticles. A change in pH results in a charge change in the natural phytochemicals contained in an extract, which affects their ability to bind and reduce metal cations and anions in the course of nanoparticle synthesis, and this in turn may affect the shape, size, and yield of nanoparticles (Makarov et al., 2014).

Song et al. in 2010, attempted to create PtNPs using the leaf extract of *Diopyros kaki*. A greater than 90% reduction of Pt ions into nanoparticles was illustrated in approximately 2.5 hours. Song and group suggested that the reduction of the Pt ions was due to the presence of functional groups within the leaf extract such as amines, alcohols, ketones and carboxylic acids; as opposed to an enzyme mediated process. This was based on the fact that the reaction temperature was  $95^\circ\text{C}$  and no protein peaks were found in their FTIR analysis.

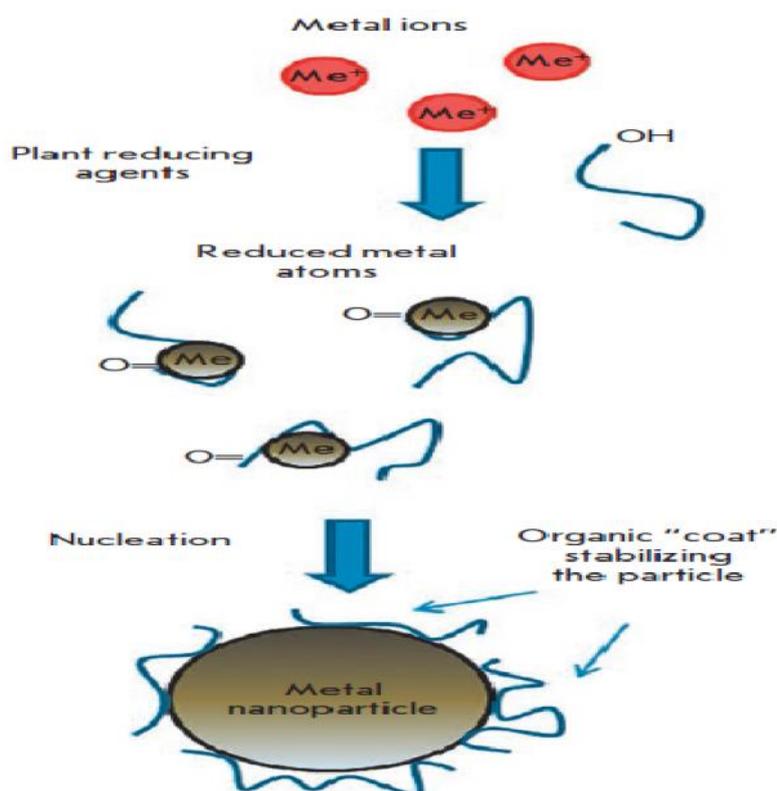


Fig. 4. Mechanism of Nanoparticle Synthesis by Plants

## Conclusion

Green synthesis usually involves processes that reduce or eliminate the use and/or generation of hazardous substances and thus emerging as an alternative synthesis method in comparison to already existing physical and chemical methods. It is usually a microbe (Bacteria, Fungi, Algae, Yeast) or plant mediated synthesis. Bacteria are relatively cheap to cultivate and have a high growth rate compared to other biological systems such as fungi or plants. Their ease of manipulation gives them the advantage over plants and fungi as the chassis of choice for bio-production of nanoparticles that require optimized synthesis through genetic engineering. Alternatively, fungi have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate. Many fungi have mycelia that provide a much higher surface area than bacteria and this area could be used to support the interaction of metal ions and fungal reducing agent thus enhancing the conversion of ions to metallic nanoparticles.

Fungi also have the advantage of ease of downstream processing when extracellular nanoparticles are produced, allowing for a more efficient way of extracting nanoparticles from them. Scalability, another factor for consideration in the case of commercial production of nanoparticles, gives fungi the edge as the chassis of choice for long term development as they can be used more easily in large-scale reactors than bacteria. Finally plants have also been found to be nanoparticles producers. Whatever the choice of biological chassis may be, whether it is a bacterium, fungus or plant, they all need to be studied comprehensively in order to gain a clearer understanding of mechanism and to close the knowledge gap in biological nanoparticle synthesis methods by different organisms. The field of biological production of metallic nanoparticles is relatively new and underexplored, however it shows great potential in the biotechnology sector. There are many aspects of these biological methods to be discovered, and later manipulated, as the technology emerges.

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