**Review Article**

# Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants

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## Abstract

Over the past few decades interest in metallic nanoparticles and their synthesis has greatly increased. This has resulted in the development of numerous ways of producing metallic nanoparticles using chemical and physical methods. However, drawbacks such as the involvement of toxic chemicals and the high-energy requirements of production make it difficult for them to be widely implemented. An alternative way of synthesising metallic nanoparticles is by using living organisms such as bacteria, fungi and plants. This "green" method of biological nanoparticle production is a promising approach that allows synthesis in aqueous conditions, with low energy requirements and low-costs. This review gives an overview of some of these environmentally friendly methods of biological metallic nanoparticle synthesis. It also highlights the potential importance of these methods in assessing nanoparticle risk to both health and the environment.

**Keywords:** Biogenic nanoparticles; Biological nanoparticles; Green nanoparticle synthesis

## Introduction

In recent years the topic of nanoparticles has received particular interest in a wide range of fields. The term "*nano*" comes from the Greek word "*nanos*" meaning dwarf and denotes a measurement on the scale of one-billionth ($10^9$) of a metre in size [1,2]. A strand of DNA is 2.5 nm in diameter [3], a typical virus is around 100 nm wide [4] and a typical bacterium is around 1-3 μm wide [5]. Nanoparticles are defined as particulate dispersions of solid particles with at least one dimension at a size range of 10-1000 nm [2,6]. The most important feature of nanoparticles is their surface area to volume aspect ratio, allowing them to interact with other particles easier [1,2].

In order to survive in environments containing high levels of metals, organisms have adapted by evolving mechanisms to cope with them. These mechanisms may involve altering the chemical nature of the toxic metal so that it no longer causes toxicity, resulting in the formation of nanoparticles of the metal concerned. Thus nanoparticle formation is the "by-product" of a resistance mechanism against a specific metal, and this can be used as an alternative way of producing them. Nanoparticles have unique thermal, optical, physical, chemical, magnetic and electrical properties compared to their bulk material counterparts [7,8]. These features can be exploited for next generation biosensors, electronics, catalysts and antimicrobials [1,8]. Metallic nanoparticles are one important and widely studied group of materials, showing great diversity and many different uses. This review will focus on how material science and biology can work together to create a "green" way of synthesising metal nanoparticles for a wide range of uses.

There are important links between the way nanoparticles are synthesised and their potential uses. Silver nanoparticles (AgNPs) have been shown in numerous studies to display antibacterial properties [8-11]. For instance, nanoparticles such as silver and gold have been shown to be effective in inhibiting growth of both Gram-positive and Gram-negative bacteria [10,12]. With the rise in antibiotic resistance in recent years and the development of fewer new antibiotics, research has begun to focus on these antibacterial nanoparticles as potential new medical tools. Silver nanoparticles have also been used as optical sensors for the formation of small molecule adsorbates [13]. Whereas catalysts based on Pt nanoparticles have been shown to exhibit high activity for the electrooxidation of formic acid [14]. The most common methods for preparing all of these nanoparticles are wet-chemical techniques, which are generally low-cost and high-volume. However, the need for toxic solvents and the contamination from chemicals used in nanoparticle production limit their potential use in biomedical applications [15]. Therefore a "green", non-toxic way of synthesising metallic nanoparticles is needed in order to allow them to be used in a wider range of industries. This could potentially be achieved by using biological methods.

Many bacteria, fungi and plants have shown the ability to synthesise metallic nanoparticles and all have their own advantages and disadvantages (Table 1) [16-18]. Intracellular or extracellular synthesis, growth temperature, synthesis time, ease of extraction and percentage synthesised versus percentage removed from sample ratio, all play an important role in biological nanoparticle production. Finding the right biological method can depend upon a number of variables. Most importantly, the type of metal nanoparticle under investigation is of vital consideration, as in general organisms have developed resistance against a small number of metals, potentially limiting the choice of organism. However synthetic biology; a nascent field of science, is starting to address these issues in order to create more generalised chassis, able to synthesise more than one type of metallic nanoparticle using the same organism [19].

"Natural" biogenic metallic nanoparticle synthesis can be split into two categories. The first is bioreduction, in which metal ions are chemically reduced into more stable forms biologically. Many organisms have the ability to utilise dissimilatory metal reduction, in which the reduction of a metal ion is coupled with the oxidation of an enzyme [20]. This results in stable and inert metallic nanoparticles that can then be safely removed from a contaminated sample. The second category is biosorption. This involves the binding of metal ions from

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| Name of organism | Nanoparticles Produced | Synthesis Location | Method | References |
|---|---|---|---|---|
| **A) Bacteria** | | | | |
| *Thermomonospora sp.* | Au | Extracellular | Reduction | [62] |
| *Rhodococcus* sp. | Au | Intracellular | Reduction | [63] |
| *Escherichia coli* | Pd, Pt | Extracellular | Reduction | [20] |
| *Rhodopseudomonas capsulata* | Au | Extracellular | Reduction | [25] |
| *Pseudomonas aeruginosa* | Au | Extracellular | Reduction | [1] |
| *Delftia acidovorans* | Au | Extracellular | Reduction | [26] |
| *Bacillus licheniformis* | Ag | Intracellular | Reduction | [28] |
| *Shewanella* sp. | AsS | Extracellular | Reduction | [64] |
| | Se | Extracellular | Reduction | [65] |
| *Desulfovibrio desulfuricans* | Pd | Extracellular | Reduction | [66] |
| *Bacillus sphaericus* JG-A12 | U, Cu, Pb, Al, Cd | Extracellular | Biosorption and Reduction | [33] |
| *Bacillus* sp. | Ag | Intracellular | Reduction | [29] |
| *Klebsiella pneumonia* | Ag | Extracellular | Reduction | [30] |
| *Escherichia coli* | Ag | Extracellular | Reduction | [30] |
| *Enterobacter cloacae* | Ag | Extracellular | Reduction | [30] |
| *Lactobacillus* sp. | Ag | Extracellular | Biosorption and Reduction | [32] |
| *Enterococcus faecium* | Ag | Extracellular | Biosorption and Reduction | [32] |
| *Lactococcus garvieae* | Ag | Extracellular | Biosorption and Reduction | [32] |
| **B) Fungi** | | | | |
| *Pediococcus pentosaceus* | Ag | Extracellular | Biosorption and Reduction | [32] |
| *Fusarium oxysporum* | CdS | Extracellular | Enzyme Mediated | [41] |
| | Ag | Extracellular | Reduction | [27] |
| | Pt | | | |
| | Au | Intracellular | Reduction | [67] |
| *Aspergillus fumigatus* | Ag | Extracellular | Reduction | [17] |
| *Neurospora crassa* | Pt | Intracellular & Extracellular | Reduction | [45] |
| *Verticillium sp.* | Au | Intracellular | Reduction | [38] |
| **C) Plants & Extracts** | | | | |
| *Coriolus versicolor* | Ag | Intracellular & Extracellular | Reduction | [43] |
| *Aspergillus flavus* | Ag | Extracellular | Reduction | [68] |
| *Jatropha curcas* latex | Ag | Extracellular | Latex Mediated Reduction | [52] |
| *Acalypha indica* leaf extract | Ag | Extracellular | Reduction | [11] |
| *Medicago sativa* seed exudate | Ag | Extracellular | Reduction | [53] |
| *Cymbopogon flexuosus* extract | Au | Extracellular | Reduction | [57] |
| Live Alfalfa plants | Au | Intracellular | - | [61] |
| *Magnolia kobus* leaf broth | Ag | Extracellular | Reduction | [18] |

**Table 1:** Metallic nanoparticles formed by different organisms. The table also illustrates the location of the nanoparticles in relation to the cells and the suggested method of synthesis.

an aqueous or soil sample onto the organism itself, such as on the cell wall, and does not require the input of energy. Certain bacteria, fungi and plants express peptides or have a modified cell wall which binds to metal ions, and these are able to form stable complexes in the form of nanoparticles [21].

Metallic nanoparticles are becoming increasingly important due to their potential application in many fields. The development of an environmentally friendly and inexpensive way of synthesising them is therefore crucial. There are numerous organisms possessing the ability to synthesise nanoparticles and which therefore have the potential to be exploited and modified to optimise them to fulfil this purpose. The following sections will focus on providing an overview and a discussion of metallic nanoparticle synthesis by various biological and non-biological ways.

## Chemical and Physical Methods of Nanoparticle Synthesis

Metallic nanoparticles can be synthesised in many different ways. In order to study the biological methods of synthesising nanoparticles

(NPs), a clear understanding of the current chemical and physical methods is needed to allow comparisons to be made and a basis for improvement to become evident. There is an abundant volume of research on the synthesis of metallic nanoparticles available in the literature. Here a number of studies will be discussed in order to gain insight into the "non-green" methods of NP synthesis.

Wiley et al. were able to produce silver nanoparticles (AgNPs) of cube and tetrahedron shape. They achieved this by heating $AgNO_3$ and Ethylene glycol to 148°C. The resulting nanoparticles were single crystals and had a size range of 20-80 nm in diameter. The AgNPs were relatively monodispersed and the reaction time was only 10 minutes [22]. However the temperature needed for the reaction to occur was relatively high therefore requiring a significant amount of energy if commercial volumes were to be made.

A method using polyamide fabrics developed by Montazer et al. successfully formed AgNPs. The NPs were observed under SEM and were found to be between 20 and 150 nm across with an average size of 90 nm. A small number of aggregates was seen which were attributed to the boiling point temperature during the reaction. The polyamide
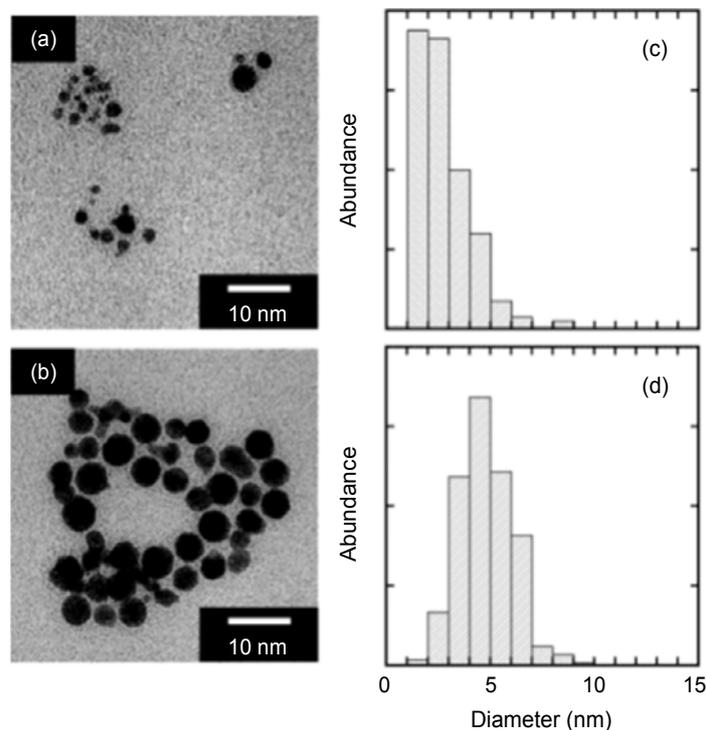
**Figure 1:** TEM micrograph of PtNP synthesis via laser ablation. The histograms show the size distribution of the synthesised nanoparticles. a) Formation of PtNPs in 0.01 M SDS aqueous solution and b) PtNPs produced in pure water. c) and d) display the measured length of PtNPs from a) and b) respectively. Reprinted with permission from Mafuné et al., Copyright 2003 American Chemical Society [24].

fabric itself was used as the reducing agent for the Ag⁺ and was found to stabilise the synthesised NPs. This important result meant that the use of extra stabilising agents was redundant. The fabric was subsequently tested for its antibacterial activity which showed high bactericidal effects even after 30 washing cycles [23].

Stable platinum nanoparticles (PtNPs) have been produced using a physical method, which does not require the reducing agents that cause contamination in the nanoparticles produced [24]. Mafuné et al. used laser ablation that creates nanoparticles by heating up bulk material using a laser beam. Mafuné and group performed this method in both SDS and pure water and found in both cases that stable PtNPs were produced (Figure 1). Subsequently the size distribution of the PtNPs was measured under TEM and was found to be 1-7 nm, which proved to be too small for isolating them using centrifugation [24].
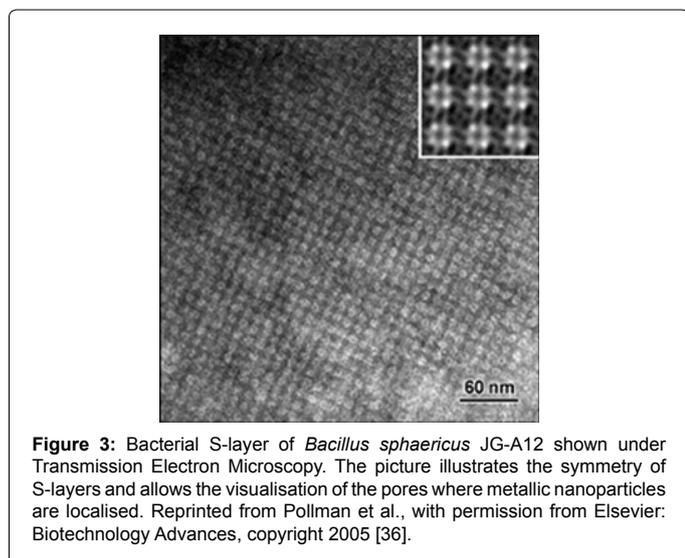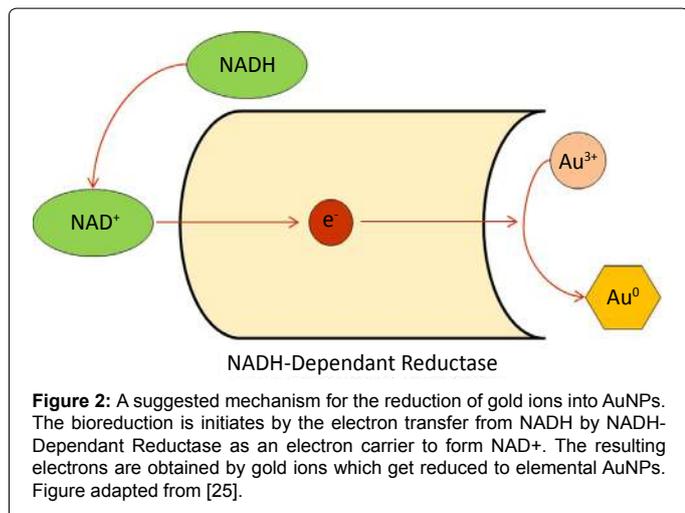
## Nanoparticle Synthesis by Bacteria

Research has focused heavily on prokaryotes as a means of synthesising metallic nanoparticles. Due to their abundance in the environment and their ability to adapt to extreme conditions, bacteria are a good choice for study. 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. it was discovered that changing the pH of the growth medium during incubation results in the production of nanoparticles of differing size and shape [25] Controlling such properties is important, as varying sizes of nanoparticles are required for different applications such as optics, catalysts or anti-microbials.

In a recent study, Johnston et al. illustrated the production of pure gold nanoparticles by the bacterium *Delftia acidovorans*. Reporting

the production of a small non-ribosomal peptide, delftibactin, to be responsible for generating the gold nanoparticles [26]. Johnston et al. believed 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 Johnston group was first to report the mechanism that is responsible for the formation of metallic nanoparticles and how it can vary in different bacteria. Another group suggested an alternative method for gold nanoparticle synthesis by bacteria. He et al. observed the extracellular formation of gold nanoparticles of 10-20 nm by the bacterium *Rhodopseudomonas capsulata* and suggested that these nanoparticles were synthesised via an NADH-Dependant Reductase [25], an enzyme that has been shown in the past to be important in metal biosynthesis (Figure 2) [27].

Pd is a member of the Platinum Group Metals (PGM) which is a collection of highly catalytically active metals and are currently being primarily used as catalysts for dehalogenation and hydrogenation reactions [28]. Recently it has been shown that zero valent palladium (Pd⁰) nanoparticles can be synthesised using bacteria found at Alpine sites heavily contaminated with heavy metals [28]. Of all the variety of heavy metal resistant bacteria that they have found in that environment, they found that *Pseudomonas* cells were involved in producing catalytically active nanoparticles which were successfully used in reductive dehalogenation of tri and tetra-chlorinated dioxin congeners [28]. Macaskie et al. suggested that *Escherichia coli,* can also synthesise Pd⁰ nanoparticles with the help of hydrogenases found in the bacterium [29]. In both studies mentioned, the Pd nanoparticles were found to be formed on the cell envelope of the bacteria which makes them attractive as they are easily accessible.

**Figure 2:** A suggested mechanism for the reduction of gold ions into AuNPs. The bioreduction is initiates by the electron transfer from NADH by NADH-Dependant Reductase as an electron carrier to form NAD+. The resulting electrons are obtained by gold ions which get reduced to elemental AuNPs. Figure adapted from [25].



**Figure 3:** Bacterial S-layer of *Bacillus sphaericus* JG-A12 shown under Transmission Electron Microscopy. The picture illustrates the symmetry of S-layers and allows the visualisation of the pores where metallic nanoparticles are localised. Reprinted from Pollman et al., with permission from Elsevier: Biotechnology Advances, copyright 2005 [36].

The bacterium *Bacillus licheniformis* was observed to produce intracellular AgNPs [30]. The colour of the culture after addition of silver ions turned a dark brown indicating the presence of AgNPs [30]. Kalimuthu and group illustrated that the nanoparticles were indeed made of Ag and also that they were quite dispersed in solution. However the nanoparticles were synthesised intracellularly, therefore Kalimuthu et al. had to add an additional extraction step. Pugazhenthiran et al. observed a similar result when they attempted to create biological AgNPs. It was found that intracellular AgNPs were created when members of the *Bacillus sp.* were sub-cultured into media containing AgNO$_3$. The reaction was slow and the incubation time was 7 days [31]. This is not suitable for industrial purposes due to the lengthy production time. Whereas, despite the additional extraction step, the method proposed by Kalimuthu et al. was more industrially significant as it took only 24 hours for *Bacillus licheniformis* to create AgNPs. However, the approach with the greatest potential for the industrial bio-production of AgNPs was reported by Shaverdi et al. 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 [32]. Therefore Shaverdi 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 a more desirable not only because

of the simplicity of purification but also due to the observed increased production rate [33].

An interesting study by Sintubin et al. focused on the production of AgNPs by lactic acid bacteria. Many bacterial species were tested but only four were found to synthesise AgNPs: *Lactobacillus spp., Pediococcus pentosaceus, Enterococcus faecium* and *Lactococcus garvieae* [34]. A two-step process of AgNP formation was proposed. First, the Ag ions were accumulated at the cell wall via biosorption and then subsequent reduction of those ions formed the metallic nanoparticles [34]. Sintubin et al. also suggest that the cell wall may act as a capping agent for the nanoparticles, which keeps them stable by preventing aggregation and showed that by increasing the pH of the medium, the reduction rate of the nanoparticles increased [34]. The effect of pH on nanoparticle synthesis was also observed by He et al. By varying the pH levels, nanoparticles of differing size and shape were formed [25]. He et al. illustrated that by increasing the pH, AgNPs of around 10-20 nm were formed and by lowering the pH to 4, nanoplate formation was observed.

Although Ag and AuNPs are important due to their antimicrobial abilities, many studies have been done on other metals such as uranium (U). A significant amount of research has been done on *Bacillus* species due to their metal bioaccumulation abilities [30,31,35]. Pollmann and group studied the ability of *Bacillus sphaericus* JG-A12 to accumulate high concentrations of toxic metals such as Cu, Pb, Al, Cd and U. They found that the S-layer proteins of *B. sphaericus* are responsible for the bioremediation of uranium from aqueous environments. The S-layer is a porous layer consisting of identical proteins which surround the bacterial cell and can contribute up to 15% of the total cell protein content (Figure 3) [36]. Sleytr et al. found that S-layers are approximately 5-15 nm thick and consist of pores with a size range of 2-6 nm [36]. It is this layer that was proposed by Pollman et al. to be responsible for the binding of heavy metals, such as U at up to 20 mg of U per gram of protein, and they suggested that binding was via the carboxyl and phosphate groups of the S-layer resulting in bioaccumulation.

The synthesis of copper nanoparticles (CuNPs) has proven to be a difficult feat in the past, by any available method. Cu is not stable at the nanometre scale and oxidises fairly rapidly to form copper oxide (CuO) [37]. Therefore, if Cu nanoparticles are to be used in an application after their synthesis, they need to be stabilised. In 2013 a study suggested the production of pure elemental Cu nanoparticles by biological means, using *Morganella morganii* was possible [38]. Ramanathan and group state that *M. morganii* 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 Cu$^0$ which then accumulates extracellularly as nanoparticles once effluxed out of the cell [38], however this has not been confirmed. Another study based on *Morganella sp.* showed the extracellular synthesis of AgNPs [39]. Parikh et al. suggested the pathway of Ag resistance is also responsible for the formation of the AgNPs. Bioinformatics analysis of the *silE* gene from *Morganella sp.* showed 99% similarity with previously reported silver-binding proteins. Further results showed the similarity of the *silE* homologue to copper binding proteins from other microorganisms [38]. Using this result, Ramanathan and group suggested that *M. morganii* may use the Ag resistance mechanism to produce elemental Cu nanoparticles.

## Nanoparticle Synthesis by Fungi

The use of fungi in producing metallic nanoparticles has received significant interest as they offer certain advantages over the use of
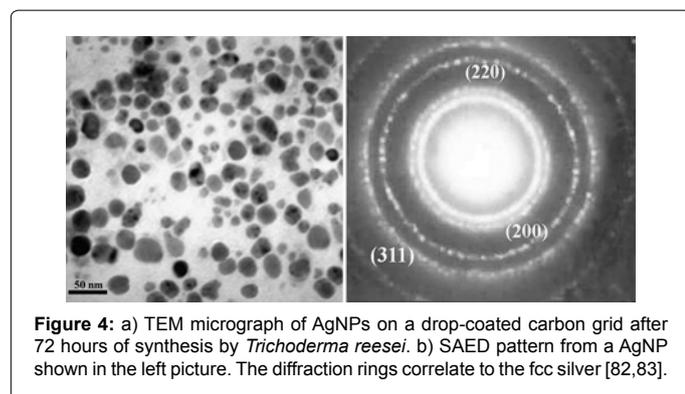
**Figure 4:** a) TEM micrograph of AgNPs on a drop-coated carbon grid after 72 hours of synthesis by *Trichoderma reesei*. b) SAED pattern from a AgNP shown in the left picture. The diffraction rings correlate to the fcc silver [82,83].

bacteria for the synthesis of nanoparticles. The ease of scaling up and downstream processing, the economic feasibility and the presence of mycelia offering an increased surface area, are important advantages to consider [40]. Mukherjee et al. also suggested that because fungi secrete significantly higher amounts of proteins than bacteria, this would amplify the nanoparticle synthesis productivity.

Silver nanoparticle production has been the focus of research by the scientific community, and rightly so. AgNPs have great potential in a number of industries such as antimicrobials and electronics [41,42]. 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 synthesised 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 [27]. 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 [27]. *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 [43].

A later study on the production of AgNPs was done by Bhainsa et al., in which they used *Aspergillus fumigatus* to synthesise extracellular silver nanoparticles in the size range of 5-25 nm [17]. This range in size was larger than previously reported by *F. oxysporum* [27], which might cause a disadvantage when application predictability comes into play, as it would be difficult to predict the catalytic activity of the nanoparticles produced if their sizes are different in every batch. However bio-production by *A. fumigatus* is a very attractive prospect as the organism was able to reduce Ag ions into nanoparticles within 10 minutes of exposure [17], in contrastto the research by Ahmad et al. which showed AgNP production by *F. oxysporum* within an hour [27]. The results of the Bhainsa group present the biological synthesis of metallic nanoparticles as comparable to the chemical and physical processes that exist to create them [22]. The fungus *Trichoderma reesei* has also been shown to produce extracellular AgNPs (Figure 4) [44]. Bahabi et al. were able to produce AgNPs using this fungus after 72 hours, which was significantly slower than *A. fumigatus* and *Fusarium oxysporum* [17,27]. However the use of *T. reesei* does have an advantage over the use of other fungi for the production of metallic nanoparticles. As a comparatively well studied organism it can be manipulated to produce high quantities of enzymes, up to 100 g/L [44], which may help increase the nanoparticle production rate in the future. The size range of the AgNPs reportedly produced by *T. reesei* was 5-50 nm [44], which

were not as homogenous as those produced by either *A. fumigatus* [17] or *F. oxysporum* [27].

Although extracellular formation has its advantages, such as lower-cost, simpler downstream processing [33], 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. A noteworthy study was done on the white rot fungus *Coriolus versicolor* by Sanghi et al. into the production and accumulation of intracellular AgNPs [45]. The group manipulated the reaction conditions and observed that *C. versicolor* had the ability to produce AgNPs intracellularly and extracellularly. Therefore the production process is not fixed and can be adapted according to specific requirements.

Gold nanoparticles have been increasing in their importance over recent years but, despite this, there are fewer examples of their synthesis by fungi than those composed of silver. Their small size makes them reactive, unlike the bulk form of gold, which makes AuNPs ideal for use as catalysts and as precursors for electronics applications [40,46]. Mukherjee et al. reported the synthesis of AuNPs using the fungus *Verticillium sp.* whereby intracellular AuNPs were observed localised on the surface of the mycelia via the biological reduction of $AuCl_4^-$ [47].

Unlike the most commonly researched metallic nanoparticles, such as AgNPs and AuNPs, very little is known about the biological synthesis of Platinum nanoparticles (PtNPs). An enlightening study by Castro-Longoria et al. showed the synthesis of PtNPs using the fungus *Neurospora crassa*. This fungus was reported to produce intracellular single PtNPs of 4-35 nm in diameter and spherical nano-agglomerates of 20-110 nm in diameter [48]. What is interesting about this study is that they attempted to create PtNPs using *N. crassa* extract and then compared the results with the PtNPs produced from the *N. crassa* biomass. They noticed that after the reaction, the sample from the extract contained almost exclusively single-crystal nanoagglomerates [48]. An additional study by Riddin et al. on the previously mentioned fungus *F. oxysporum*, also confirmed the production of PtNPs. In this case the PtNPs were formed both intracellularly and extracellularly, however the amount synthesised intracellularly was deemed to be statistically insignificant. The amount of extracellularly produced PtNPs was reported to be 5.66 mg $l^{-1}$ [49], with temperature variation effecting production rates of the PtNPs and even slight pH variation away from the standard inhibiting the formation of PtNPs [49]. Knowledge of such results is crucial in order to understand the effects of environmental factors on NP synthesis as they can help us optimise the biological synthesis of metallic NPs.

Not all metallic nanoparticles are made of elemental metals in their zero-valent form. Magnetite ($Fe_3O_4$) is a common iron oxide that possesses magnetic properties and magnetite NPs (MaNPs) have been shown to be produced by the pathogenic fungus *F. oxysporum* and the endophytic fungus *Verticillium* sp [50]. The use of magnetite nanoparticles has been talked about extensively for use in biomedical applications such as magnetic resonance imaging [51] and for oscillation damping and position sensing [52], in addition to non-medical applications, such as in magnetic recording devices. The synthesised MaNPs described by Bharde and group were formed intracellularly, which again means an extra step in the purification of MaNPs if they are intended to be of commercially such use.

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 [53]. *Trichoderma asperellum* and *Trichoderma reesei* are both fungi that produce AgNPs when exposed to silver salts [44,54] and have been proven to be non-pathogenic which makes them ideal for use commercially [54,55]. In fact, *T. reesei* has already been used widely in sectors such as food, animal feed, pharmaceuticals, paper and textile industries [55].

## Nanoparticle Synthesis by Plants

Bacteria and fungi have been studied extensively in the past few decades for their ability to synthesise metallic nanoparticles, however there has been less of a focus on plants in this matter. In the past decade an increasing amount of research is being performed on the green synthesis of metallic nanoparticles using plants or plant extracts [56-58]. This area is relatively underexplored and offers promising results
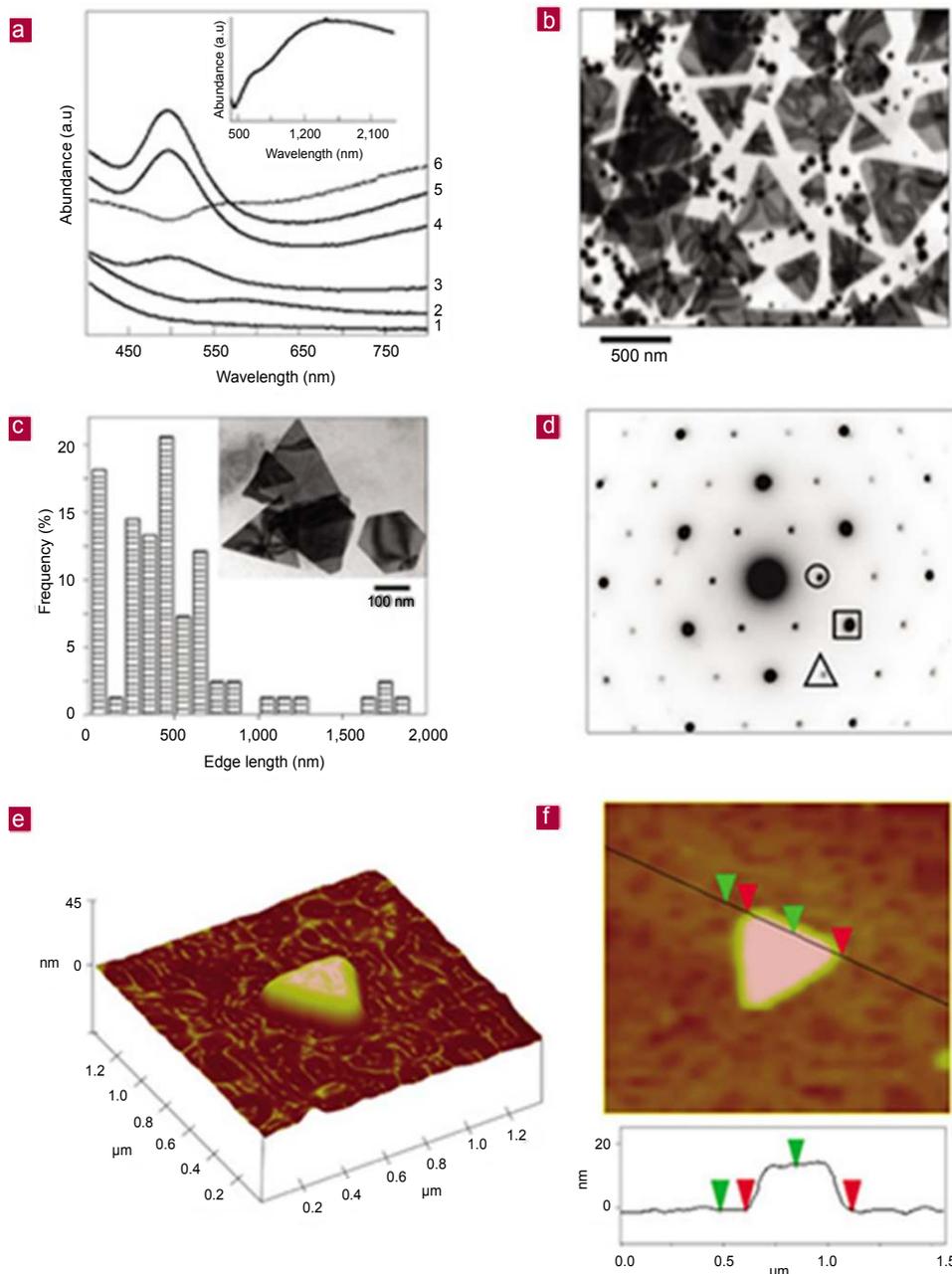


**Figure 5:** UV-vis-NIR kinetics, TEM and Atomic Force Microscopy (AFM) images of AuNPs produced by lemongrass. a) UV-vis spectra illustrating the time of reaction assay with gold ions over time. Curve 6 shows the spectrum obtained from a purified AuNP solution; inset shows the UV-Vis-NIR spectrum of a drop-cast film of purified gold nanotriangles attained from the reaction of AuCl$_4^-$ and lemongrass extract. b) TEM micrograph of synthesised circular and nanotriangular AuNPs by lemongrass extract. c) Histogram representing the size of triangular AuNPs; inset demonstrates purified gold nanotriangles. d) Selected Area Electron Diffraction (SAED) pattern of a gold nanotriangle. The highlighted spots correspond to the 1/3{422},{220} and {311} Bragg reflections with lattice spacings found to be 2.5, 1.44 and 1.23 Å respectively. e) AFM image of a gold nanotriangle and f) its dimensional profile. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials [71], copyright 2004.

for the field. A very important aspect of using plants instead of bacteria or fungi for NP production is the lack of pathogenicity.

An interesting study by Bar et al. illustrated a simple green synthesis route for AgNPs from $AgNO_3$ salts using the extract from *Jatropha curcas*. The result was the production of fairly homogenous (10-20 nm) AgNPs in 4 hours [59]. 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 [11]. The size of the AgNPs was 20-30 nm, which is again significantly homogenous. Krishnaraj and group went on to test the synthesised AgNPs' antimicrobial capacity on *Escherichia coli* and *Vibrio cholera*. They observed that the AgNPs inhibited bacterial growth at a concentration as low as 10 μg/ml [11]. Lukman et al. reported the production of AgNPs using *Medicago sativa* seed exudates. $Ag^+$ reduction occurred almost instantly as AgNPs were observed within 1 minute of exposure to the silver salt. In less than 50 minutes 90% $Ag^+$ reduction occurred when the reaction was carried out at 30°C [60] 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 [60]. 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 suggested that they could act synergistically with the seed exudate in order to eliminate bacteria [60]. Another plant that has the potential to reduce $Ag^+$ is *Ocimum sanctum* [61]. Its leaf extract was mixed with 1 mM $AgNO_3$ and the production of AgNPs of 3-20 nm in size was observed. The particles were spherical in shape and it was suggested that they may be stabilised by a component of the leaf broth [61].

In a study by Kasthuri et al., phyllanthin was extracted from the plant *Phyllanthus amarus* and subsequently used for the production of Ag and AuNPs. This is a unique study as only a single constituent of a plant extract was used for the synthesis of metallic nanoparticles, contrasting other studies mentioned earlier in which whole plants or extracts were used. 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 [62].

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 [63]. Soluble starch [64], chitosan [65], cellulose [66], dextran [67], alginic acid [68] and hyaluronic acid [69] have been used for the production of silver and gold nanoparticles successfully [63].

An extensive study was done by Song et al. 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 $Ag^+$ reducer as it took only 11 minutes to reduce 90% of the $Ag^+$ present in the sample [70]. 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. tested the effect of different reaction temperatures

and found that at 95°C $Ag^+$ conversion was much higher than at 25°C. However the size of the nanoparticles decreased at higher temperatures. It was hypothesised this was caused by the increased turnover of the nanoparticles by the reducing agent, as it has less time to build upon the presynthesised nanoparticles before starting to synthesise new ones [70].

Extracellular synthesis of AuNPs was illustrated using extracts from a lemongrass plant, *Cymbopogon flexuosus* [71]. Liquid-like nanotriangles formed by aggregates of spherical AuNPs were obtained when the extract was incubated with gold tetrachloride ($AuCl_4^-$) (Figure 5). This fluidity was attributed to the nanoparticle surface complexation of aldehydes and/or ketones that were present in the plant extract [71]. An additional study showed the synthesis of Au, Ag and bimetallic Au-AgNPs. 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. It is believed that the terpenoid and flavanone components of the leaf broth may keep the NPs stable [72].

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. Song et al. 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 [73]. 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°C and no protein peaks were found in their FTIR analysis [73].

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 synthesise metallic nanoparticles inside live growing plants. However there were no reports where plants have been used as the biological producers of PdNPs until Parker et al. presented a method of creating such nanoparticles using the plant, *Arabidopsis thaliana*. After growing Arabidopsis, its medium was then replaced with potassium tetrachloropalladate ($K_2PdCl_4$) and incubated for 24 hours in the salt solution. Subsequent TEM results visualised the PdNPs produced were at 2-4 nm size range. The produced PdNPs were subsequently used in Suzuki-Miyaura coupling reactions where the biogenic PdNPs were shown to convey a higher catalytic activity that commercially available PdNPs [74].

Moreover, Gardea-Torresdey et al. reported the synthesis of AuNPs, also by live plants. Alfalfa plant seeds were prepared and grown for two weeks with various concentrations of $K(AuCl_4)$. The nanoparticles were then extracted and analysed by various methods which confirmed the production of elemental AuNPs [75]. Although an interesting study, the NP synthesis time was long, over two weeks including the NP extraction. In order for a biological process for NP synthesis to be commercially feasible, the production time would need to be shortened significantly. On the other hand this would be a cheap and green method for synthesising such nanoparticles.

## Applications of Metallic Nanoparticles

This review focuses on a number of metallic nanoparticles including gold (Au), silver (Ag), copper (Cu), Platinum (Pt), Palladium (Pd) and magnetite ($Fe_3O_4$). There are many reports on metallic nanoparticles used for industrial and medical applications and all of them show promising

results. Catalysts, cancer treatment, cosmetics and electronics are just a few examples of all the applications that metallic nanoparticles can be used for.

Au nanoparticles have found use in modern electronics where they can be used to print low resistance Au conductors. Conductors made of such nanoparticles have certain advantages over their bulk metal equivalent as they can be more flexible and also have a lower sintering temperature. This makes them suitable for electronics in plastic as they can be hardened at lower temperature and hence form low resistance conductors [76]. Au has also been studied on its effects on breast cancer cells. Stuchinskaya et al., conjugated the Au nanoparticles with a phthalocyanine-antibody complex which proved to be a promising way of targeting and killing breast cancer cells by the Au nanoparticles and cytotoxic singlet oxygen producer phthalocyanine [77].

Ag nanoparticles have been extensively studied in the recent years due to their antibacterial and therapeutic potential. Ag has been used in much a similar way to Au nanoparticles where an antibody-photosensitizer-nanoparticle complex was fused and targeted to cancer cells in order to create free radicals which would then kill the affected cells [78]. Ag nanoparticles have also sparked interest in other biomedical uses as well due to their high versatility in different areas of research. Their powerful antibacterial effects have been studied extensively and offer promising results for future antimicrobials [79]. They have the ability to create reactive oxygen species which cause irreversible damage to bacteria and also have a strong affinity in binding to DNA or RNA which interferes with the microbial replication process [78].

A study by Zain et al. suggested that Cu nanoparticles can also be used as antibacterial agents. It was also found that when Cu and Ag nanoparticles were fused together to create bi-metallic nanoparticles, their antibacterial effects increased and that nanoparticle size played a key role in the strength of the bactericidal effect [79].

Pt nanoparticles have also been extensively studied as they possess superior catalytic activities. They are primarily used for catalysis and fuel cell technology as they have can control redox reactions effectively [80]. For fuel cell applications Pt is used in cathodes and acts as an oxygen reducer. However Pt can also be used as the anode in which case it oxidises different types of fuels such as methanol in the methanol oxidation reaction (MOR) [80]. Pt has also been applied in catalysis where it acts as the catalyst for chemical reactions such as hydrogenation processes [81]. Similarly to Pt nanoparticles, Pd nanoparticles have also been used for catalytic purposes [79]. Bunge et al. was able to isolate bacterial strains from Alpine sites and use them to synthesise biogenic Pd nanoparticles. These nanoparticles when tested; were found to be highly catalytically active during dehalogenation reactions of polychlorinated dioxins [79].

## Conclusions

It is clear that metallic nanoparticles have great potential in many different industries. The need for a process to synthesise such nanoparticles in a reliable and green way is becoming more pressing. Current chemical and physical methods involve toxic chemicals and high temperatures that are not only dangerous to the environment but costly too. Numerous groups have focused on alternative ways of synthesising nanoparticles as described here. Biological systems have been investigated in an effort to provide a sustainable, resource efficient and cheap method of synthesis. Many different biological chassis have been studied for their ability to resist the toxic effects of metal ions whilst producing metallic nanoparticles.

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 the near-term bio-production of nanoparticles that require optimised 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 nanoparticle producers. The plants physiology has allowed for many different types of studies on them. Single components of plant extracts to whole plants have been utilised for the synthesis of nanoparticles. However before any industrial relevance can be attributed to the synthesis of nanoparticles by plants many more examples must be identified and, especially in the case of whole plant synthesis, the risks must be thoroughly assessed.

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 risks of such nanoparticles must also be assessed, but here biological synthesis may offer yet another advantage. The rapidly developing field of synthetic biology aims to create predictable, standardised systems and with such new technologies directed towards the production of metallic nanoparticles, biogenic nanoparticle samples are likely to become more homogenous and more reproducible, therefore the environmental and health risks posed will be more easily and more reliably assessed.

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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### References

1. Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. Adv Colloid Interface Sci 156: 1-13.

2. Thakkar KN, Mhatre SS, Parikh RY (2010) Biological synthesis of metallic nanoparticles. Nanomedicine 6: 257-262.

3. Lyubchenko YL, Shlyakhtenko LS (1997) Visualization of supercoiled DNA with atomic force microscopy in situ. Proc Natl Acad Sci U S A 94: 496-501.

4. Wagner E, Plank C, Zatloukal K, Cotten M, Birnstiel ML (1992) Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin-polylysine-DNA complexes: toward a synthetic virus-like gene-transfer vehicle. Proceedings of the National Academy of Sciences 89: 7934-7938.

5. Katz A, Alimova A, Min X, Rudolph E, Shah MK, et al. (2003) Bacteria size determination by elastic light scattering. Selected Topics in Quantum Electronics, IEEE Journal of 9(2): 277-287.

6. Mohanraj VJ, Chen Y (2006) Nanoparticles – A Review. Tropical Journal of Pharmaceutical Research 5: 561-573.

7. Husseiny MI, El-Aziz MA, Badr Y, Mahmoud MA (2007) Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa. Spectrochim Acta A Mol Biomol Spectrosc 67: 1003-1006.

8. Durán N, Marcato PD, De Souza DIH, Alves OL, Esposito E (2007) Antibacterial Effect of Silver Nanoparticles Produced by Fungal Process on Textile Fabrics and Their Effluent Treatment. Journal of Biomedical Nanotechnology 3: 203-208.

9. Guzman M, Dille J, Godet S (2012) Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine 8: 37-45.

10. Guzmán M, Jean GD, Stephan G (2009) Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. International Journal of Chemical and Biomolecular Engineering 2: 104-111.

11. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, et al. (2010) Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 76: 50-56.

12. Lima E, Guerra R, Lara V, Guzmán A (2013) Gold nanoparticles as efficient antimicrobial agents for Escherichia coli and Salmonella typhi. Chem Cent J 7: 11.

13. McFarland AD, Van Duyne RP (2003) Single Silver Nanoparticles as Real-Time Optical Sensors with Zeptomole Sensitivity. Nano Letters 3: 1057-1062.

14. Waszczuk P, Barnard T, Rice MC, Masel RI, Wieckowsky A (2002) A nanoparticle catalyst with superior activity for electrooxidation of formic acid. Electrochemistry Communications 4: 599-603.

15. Li X, Xu H, Chen ZS, Chen G (2011) Biosynthesis of Nanoparticles by Microorganisms and Their Applications. Journal of Nanomaterials 2011: 1-16.

16. Suresh K, Prabagaran SR, Sengupta S, Shivaji S (2004) Bacillus indicus sp. nov., an arsenic-resistant bacterium isolated from an aquifer in West Bengal, India. Int J Syst Evol Microbiol 54: 1369-1375.

17. Bhainsa KC, D'Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids Surf B Biointerfaces 47: 160-164.

18. Song JY, Kim BS (2009) Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng 32: 79-84.

19. Edmundson MC, Capeness M, Horsfall L (2014) Exploring the potential of metallic nanoparticles within synthetic biology. N Biotechnol 31: 572-578.

20. Deplanche K, Caldelari I, Mikheenko IP, Sargent F, Macaskie LE (2010) Involvement of hydrogenases in the formation of highly catalytic Pd(0) nanoparticles by bioreduction of Pd(II) using Escherichia coli mutant strains. Microbiology 156: 2630-2640.

21. Yong P, Rowson AN, Farr JPG, Harris IR, Mcaskie LE (2002) Bioaccumulation of palladium by Desulfovibrio desulfuricans. Journal of Chemical Technology and Biotechnology 55: 593-601.

22. Wiley B, Herricks T, Sun Y, Xia Y (2004) Polyol Synthesis of Silver Nanoparticles - Use of Chloride and Oxygen to Promote the Formation of Single-Crystal, Truncated Cubes and Tetrahedrons. Nano Letters 4: 1733-1739.

23. Montazer M, Shamei A, Alimohammadi F (2014) Synthesis of nanosilver on polyamide fabric using silver/ammonia complex. Mater Sci Eng C Mater Biol Appl 38: 170-176.

24. Mafuné F, Kohno JY, Takeda Y, Kondow T (2003) Formation of Stable Platinum Nanoparticles by Laser Ablation in Water. The Journal of Physical Chemistry B 107: 4218-4223.

25. He S, Guo Z, Zhang Y, Zhang S, Wang J, et al. (2007) Biosynthesis of gold nanoparticles using the bacteria Rhodopseudomonas capsulata. Materials Letters 61: 3984-3987.

26. Johnston CW, Wyatt MA, Li X, Ibrahim A, Shuster J, et al. (2013) Gold biomineralization by a metallophore from a gold-associated microbe. Nat Chem Biol 9: 241-243.

27. Ahmad A, Mukherjee P, Senapat S, Mandal D, Khan MI, et al. (2003) Extracellular biosynthesis of silver nanoparticles using the fungus Fsarium oxysporum. Colloids and Surfaces B: Biointerfaces 28: 313-318.

28. Schlüter M, Hentzel T, Suarez C, Koch M, Lorenz WG, et al. (2014) Synthesis of novel palladium(0) nanocatalysts by microorganisms from heavy-metal-influenced high-alpine sites for dehalogenation of polychlorinated dioxins. Chemosphere 117C: 462-470.

29. Lloyd JR, Yong P, Macaskie LE (1998) Enzymatic recovery of elemental palladium by using sulfate-reducing bacteria Appl Environ Microbiol 64: 4607-4609.

30. Kalimuthu K, Suresh Babu R, Venkataraman D, Bilal M, Gurunathan S (2008) Biosynthesis of silver nanocrystals by Bacillus licheniformis. Colloids Surf B Biointerfaces 65: 150-153.

31. Pugazhenthiran N, Anandan S, Kathiravan G, Udaya Prakash NK, Crawford S, et al. (2009) Microbial synthesis of silver nanoparticles by Bacillus sp. Journal of Nanoparticle Research 11: 1811-1815.

32. Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi AA (2007) Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. Process Biochemistry 42: 919-923.

33. Das V, Thomas R, Varghese R, Soniya EV, Mathew J, et al. (2014) Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area. 3 Biotech 4: 121-126.

34. Sintubin L, De Windt W, Dick J, Mast J, van der Ha D, et al. (2009) Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl Microbiol Biotechnol 84: 741-749.

35. Pollmann K, Raff J, Merroun M, Fahmy K, Selenska-Pobell S (2006) Metal binding by bacteria from uranium mining waste piles and its technological applications. Biotechnol Adv 24: 58-68.

36. Sleytr UB, Messner P, Pum D, Sára M (1993) Crystalline bacterial cell surface layers. Mol Microbiol 10: 911-916.

37. Baco-Carles V, Datas L, Tailhades P (2011) Copper Nanoparticles Prepared from Oxalic Precursors. ISRN Nanotechnology: 1-7.

38. Ramanathan R, Field MR, O'Mullane AP, Smooker PM, Bhargava SK, et al. (2013) Aqueous phase synthesis of copper nanoparticles: a link between heavy metal resistance and nanoparticle synthesis ability in bacterial systems. Nanoscale 5: 2300-2306.

39. Parikh RY, Singh S, Prasad BLV, Patole MS, Sastry M, et al. (2008) Extracellular Synthesis of Crystalline Silver Nanoparticles and Molecular Evidence of Silver Resistance fromMorganellasp.: Towards Understanding Biochemical Synthesis Mechanism. Chem Bio Chem 9: 1415-1422.

40. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar S, et al. (2001) Fungus-Mediated Synthesis of Silver Nanoparticles and Their Immobilizaation in the Mycelial Matrix- A Novel Biological Apporach to Nanoparticle Synthesis. Nano Letters 1: 515-519.

41. Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27: 76-83.

42. Ummartyotin S, Bunnak N, Juntaro J, Sain M, Manuspiya H (2012) Synthesis of colloidal silver nanoparticles for printed electronics. Comptes Rendus Chimie 15: 539-544.

43. Ahmad A, Mukherjee P, Mandal D, Senapati S, Khan MI, et al. (2002) Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, Fusarium oxysporum. J Am Chem Soc 124: 12108-12109.

44. Vahabi K, Mansoori GA, Karimi S (2011) Biosynthesis of Silver Nanoparticles by Fungus Trichoderma Reesei (A Route for Large-Scale Production of AgNPs). Insciences Journal: 65-79.

45. Sanghi R, Verma P (2009) Biomimetic synthesis and characterisation of protein capped silver nanoparticles. Bioresour Technol 100: 501-504.

46. Eustis S, El-Sayed MA (2001) Why gold nanoparticles are more precious than pretty gold Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. Chemical Society Reviews 35: 209-217.

47. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, et al. (2001) Bioreduction of AuCl(4)(-) Ions by the Fungus, Verticillium sp. and Surface Trapping of the Gold Nanoparticles Formed D.M. and S.S. thank the Council of Scientific and Industrial Research (CSIR), Government of India, for financial assistance. Angew Chem Int Ed Engl 40: 3585-3588.

48. Castro-Longoria E, Moreno-Velázquez SD, Vilchis-Nestor AR, Arenas-Berumen E, Avalos-Borja M (2012) Production of platinum nanoparticles and nanoaggregates using Neurospora crassa. J Microbiol Biotechnol 22: 1000-1004.

49. Riddin TL, Gericke M, Whiteley CG (2006) Analysis of the inter- and extracellular formation of platinum nanoparticles by Fusarium oxysporum f. sp. lycopersici using response surface methodology. Nanotechnology 17: 3482-3489.

50. Bharde A, Rautaray D, Bansal V, Ahmad A, Sarkar I, et al. (2006) Extracellular biosynthesis of magnetite using fungi. Small 2: 135-141.

51. Sun S, Zeng H (2002) Size-controlled synthesis of magnetite nanoparticles. J Am Chem Soc 124: 8204-8205.

52. Thapa D, Palkar VR, Kurup MB, Malik SK (2004) Properties of magnetite nanoparticles synthesized through a novel chemical route. Materials Letters 58: 2692-2694.

53. Spadaro D, Gullino ML (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Protection 24: 601-613.

54. Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, et al. (2008) Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum. Nanotechnology 19: 075103.

55. Nevalainen H, Suominen P, Taimisto K (1994) On the safety of Trichoderma reesei. J Biotechnol 37: 193-200.

56. Narayanan KB, Sakthivel N (2011) Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Adv Colloid Interface Sci 169: 59-79.

57. Iravani S, Zolfaghari B (2013) Green synthesis of silver nanoparticles using Pinus eldarica bark extract. Biomed Res Int 2013: 639725.

58. Mittal AK, Chisti Y, Banerjee UC (2013) Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv 31: 346-356.

59. Bar H, Bhui DK, Sahoo GP, Sarkar P, De SP, et al. (2009) Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and Surfaces A: Physicochemical and Engineering Aspects 339: 134-139.

60. Lukman AI, Gong B, Marjo CE, Roessner U, Harris AT (2011) Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using Medicago sativa seed exudates. J Colloid Interface Sci 353: 433-444.

61. Mallikarjuna K, Narasimha G, Dillip GR, Praveen B, Shreedhar B, et al. (2011) Green Synthesis of Silver Nanoparticles Using Ocumum Leaf Extract and their Characterization. Digest Journal of Nanomaterials and Biostructures 6: 181-186.

62. Kasthuri J, Kathiravan K, Rajendiran N (2008) Phyllanthin-assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. Journal of Nanoparticle Research 11: 1075-1085.

63. Park Y, Hong YN, Weyers A, Kim YS, Linhardt RJ (2011) Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles. IET Nanobiotechnol 5: 69-78.

64. Raveendran P, Fu J, Wallen SL (2003) Completely "green" synthesis and stabilization of metal nanoparticles. J Am Chem Soc 125: 13940-13941.

65. Laudenslager MJ, Schiffman JD, Schauer CL (2008) Carboxymethyl chitosan as a matrix material for platinum, gold, and silver nanoparticles. Biomacromolecules 9: 2682-2685.

66. Cai J, Kimura S, Wada M, Kuga S (2009) Nanoporous cellulose as metal nanoparticles support. Biomacromolecules 10: 87-94.

67. Ma Y, Li N, Yang C, Yang X (2005) One-step synthesis of amino-dextran-protected gold and silver nanoparticles and its application in biosensors. Anal Bioanal Chem 382: 1044-1048.

68. Saha S, Pal A, Kundu S, Basu S, Pal T (2010) Photochemical green synthesis of calcium-alginate-stabilized Ag and Au nanoparticles and their catalytic application to 4-nitrophenol reduction. Langmuir 26: 2885-2893.

69. Kemp MM, Kumar A, Mousa S, Park TJ, Ajayan P, et al. (2009) Synthesis of gold and silver nanoparticles stabilized with glycosaminoglycans having distinctive biological activities. Biomacromolecules 10: 589-595.

70. Song JY, Kim BS (2009) Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng 32: 79-84.

71. Shankar SS, Rai A, Ankamwar B, Singh A, Ahmad A, et al. (2004) Biological synthesis of triangular gold nanoprisms. Nat Mater 3: 482-488.

72. Shankar SS, Rai A, Ahmad A, Sastry M (2004) Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci 275: 496-502.

73. Song JY, Kwon EY, Kim BS (2010) Biological synthesis of platinum nanoparticles using Diopyros kaki leaf extract. Bioprocess Biosyst Eng 33: 159-164.

74. Parker HL, Rylott EL, Hunt AJ, Dodson JR, Taylor AF, et al. (2014) Supported palladium nanoparticles synthesized by living plants as a catalyst for Suzuki-Miyaura reactions. PLoS One 9: e87192.

75. Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Toiani HE, et al. (2002) Formation and growth of Au nanoparticles inside live alfalfa plants. Nano Letters 2: 397-401.

76. Daniel H, Frank L, Steven M, David R, Vivek S (2003) Plastic-Compatible Low Resistance Printable Gold Nanoparticle Conductors for Flexible Electronics. Journal of the Electrochemical Society 150: G412-G417.

77. Stuchinskaya T, Moreno M, Cook MJ, Edwards DR, Russell DA (2011) Targeted photodynamic therapy of breast cancer cells using antibody-phthalocyanine-gold nanoparticle conjugates. Photochemical & Photobiological Sciences 10: 822-831.

78. dos Santos CA, Seckler MM, Ingle AP, Gupta I, Galdiero S, et al. (2014) Silver nanoparticles: therapeutical uses, toxicity, and safety issues. J Pharm Sci 103: 1931-1944.

79. Zain NM, Stapley AG, Shama G (2014) Green synthesis of silver and copper nanoparticles using ascorbic acid and chitosan for antimicrobial applications. Carbohydr Polym 112: 195-202.

80. Chen J, Lim B, Lee EP, Xia Y (2009) Shape-controlled synthesis of platinum nanocrystals for catalytic and electrocatalytic applications. Nano Today 4: 81-95.

81. Bratlie KM, Lee H, Komvopoulos K, Yang P, Somorjai GA (2007) Platinum nanoparticle shape effects on benzene hydrogenation selectivity. Nano Lett 7: 3097-3101.