insciences journal
open access

Insciences J. ***2011****, 1(1),* 65-79; doi:10.5640/insc.010165 ISSN 1664-171X

Full Research Paper

Biosynthesis of Silver Nanoparticles by Fungus *Trichoderma Reesei*

(A Route for Large-Scale Production of AgNPs)

Khabat Vahabi [(1)], G.Ali Mansoori [(2)] and Sedighe Karimi [(3)]

Department of BioEngineering, University of Illinois at Chicago, Chicago, IL 60607-7052 USA

Emails: (1) khabat.v@gmail.com; (2) mansoori@uic.edu; (3) sedighk@gmail.com

Received: 22 February 2011 / Accepted: 11 March 2011/ Published: 28 March 2011

Abstract: One of the requirements for advancement of nanotechnology are the development of reliable experimental protocols for the synthesis of nanomaterials over a range of biological compositions, sizes and high monodispersity. An attractive possibility of green nanotechnology is to use micro-organisms in the synthesis of nanoparticles. Recently, the utilization of biological systems, especially fungi, has emerged as a novel method for the synthesis of nanoparticles. Nanoparticles are considered as fundamental molecular building blocks for nanotechnology. They are the starting points for preparing many nanostructured materials and devices. In this paper we report the extracellular biosynthesis of silver nanoparticles (AgNPs) by using a fungus named *Trichoderma Reesei* (also known as *Hypocrea jecorina*). In the biosynthesis of AgNPs by this fungus, the fungus mycelium is exposed to the silver nitrate solution. That prompts the fungus to produce enzymes and metabolites for its own survival. In this process the toxic *Ag+* ions are reduced to the none-toxic metallic AgNPs through the catalytic effect of the extracellular enzyme and metabolites of the fungus. Absorption UV-Visible light spectroscopy is used to follow up with the reaction process. Fluorescence emission spectroscopy is used to produce detailed information on the progress of reduction of silver nitrate (formation of silver nanoparticles) on the nanosecond timescale. Fourier transform infrared spectroscopy is used for quantitative analyses of the reaction products. Our measurements indicate that extracellular biosynthesis of AgNPs by *Trichoderma reesei* produces AgNPs with the diameters in the range of 5-50 *nm*. *Trichoderma Reesei* is an environmentally friendly fungus, and it is well known for its formation of extracellular enzyme and metabolites in very large amounts, much higher than other fungi. The present process is an excellent candidate for industrial scale production of silver nanoparticles.

Keywords: Extracellular biosynthesis; Extracellular enzyme; Extracellular metabolitem; Fungus; Green nanotechnology; Large-scale production; Silver nanoparticle; *Trichoderma reesei.*

1. Introduction:

Nanotechnology is the application of science and technology to control matter at the molecular level. At the nanoscale level, the properties of matter are significantly different from their macroscopic bulk properties. Nanotechnology is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale [1].

Nanoparticles are viewed as the fundamental building blocks of nanotechnology [1, 2]. They are the starting points for preparing many nanostructured materials and devices. Their synthesis is an important component of the rapidly growing research efforts in nanoscience and nanoengineering [1]. The nanoparticles of a wide range of materials can be prepared by a number of methods. In synthesis and assembly strategies of nanoparticles or nanomaterials, precursors from liquids, solid or gas phase are used [1].

Currently, there is a growing need to using environmentally friendly nanoparticles that do not produce toxic wastes in their process synthesis protocol. To achieve this, we are inclined to shift to benign synthesis processes, which happen to be mostly of biological nature [1]. This is the theme of nanobiotechnology, which has many advantages such as ease with which the process can be scaled up, economic viability, possibility of easily covering large surface areas by suitable growth of the mycelia, etc. Another advantage of nanobiotechnology is the development of reliable processes for the synthesis of nanomaterials over a range of sizes (with good monodispersity) and chemical composition.

It is well known that biological entities like microorganisms and living cells are the best examples of machines that possess operating parts at the nanoscale level and perform a number of jobs ranging from generation of energy to extraction of targeted materials at a very high efficiency [3]. The utilization of such microorganisms like bacteria, fungi, herbal extracts and yeasts in the synthesis of nanoparticles is a relatively recent activity. It is known that certain bacteria, yeasts and now fungi play an important role in remediation of toxic metals through reduction of the metal ions so long as they are not toxic in other ways. For example, environmentally-friendly microorganisms could minimize the toxicity in the process of metallic nanoparticle production by reduction of the metal ions or by formation of insoluble complexes with metal ions (e.g. metal sulfides) in the form of colloidal particles [4]. Accordingly, these environmentally-friendly biological systems may be considered as benign nanofactories. It must be pointed out that many such microorganisms are biologically poisonous to humans, animals and plants, and care must be taken in their choice for production of nanoparticles.

Nanoparticle synthesis is an important component of rapidly growing research efforts in nanoscale science and engineering. Biotechnology approach towards the synthesis of nanoparticles has many advantages, such as ease with which the process can be scaled up, economic viability, possibility of easily covering large surface areas by suitable growth of the mycelia, and its green chemistry nature provided the microorganism medium is safe.

Some of the examples of the use of biological entities in the synthesis of nanoparticles of different chemical compositions include the following:

i. Ribosomes for biosynthesis of gold nanoparticles [5];

ii. Bacteria for production of cadmium sulfide [6, 7], zinc sulfide [8], magnetite [9], iron sulfide [10] and silver [11-13] nanoparticles;

iii. Yeasts for production of lead sulfide and cadmium sulfide [7] nanoparticles;

iv. Production of silver nanoparticles using *Emblica Officinalis* herbal fruit extract [14], production of gold nanoparticles using lemongrass extract [15] and synthesis of nanoparticles of variable morphology using leaves of different plants, sprouts, roots [16] and stems of live alfalfa plants [17].

v. Application of fungi for production of silver nanoparticles, which is the emphasis of the present paper. Production of silver nanoparticles through fungi has several advantages over the above-mentioned approaches. They include tolerance towards high metal nanoparticle concentration in the medium, easy management in large-scale production of nanoparticles, good dispersion of nanoparticle and much higher amounts of protein expressions. Compared to bacterial broth, fungal broth can be easily filtered by filter press or similar commonly used equipment, thus saving considerable investment costs for specialized equipment which may be needed for other methods. As a result, for large-scale production of nanoparticles fungi is preferred over other methods.

1.1. Mechanism of nanoparticle production through fungi

It is demonstrated that using the dissimilatory properties of an eukaryotic organism such as fungi may be used to biosynthesize and grow nanoparticles. It is shown that certain fungi have the ability of producing extracellular metabolites that serve as agent for their own survival when exposed to such environmental stresses like toxic materials (such as metallic ions), predators and temperature variations [4].

In the biosynthesis of metal nanoparticles by a fungus, the fungus mycelium is exposed to the metal salt solution. That prompts the fungus to produce enzymes and metabolites for its own survival. In this process the toxic metal ions are reduced to the none-toxic metallic solid nanoparticles through the catalytic effect of the extracellular enzyme and metabolites of the fungus.

The presence of hydrogenase in fungi, such as *Fusarium oxysporum* [18]**,** *Trichoderma reesei* [19] and *Trichoderma viride***,** was demonstrated with washed cell suspensions that had been grown aerobically or anaerobically in a medium with glucose and salts amended with nitrate [20]. The nitrate reductase was apparently essential for ferric iron reduction [21]. Many fungi that exhibit these characteristic properties, in general, are capable of reducing *Au* (III) or *Ag* (I) [22]. Besides these extracellular enzymes, several naphthoquinones [23-25] and anthraquinones [26] with excellent redox properties, were reported in *Fusarium oxysporum* that could act as electron shuttle in metal reductions [28-30]. Specifically the following results towards production of nanoparticles have been achieved using fungi:

i. Biosynthesis of magnetite using the fungus *fusarium oxysporum* and *Verticillium species* [31].

ii. Production of gold nanotriangles by actinomycete, which is a bacteria resembling fungi [32];

iii. Intracellular synthesis of gold and silver nanoparticles in *Verticillium* fungal cells [11, 12, 33].

iv. Extracellular production of gold, silver and bimetallic *Au-Ag* alloy nanoparticles by the fungus *Fusarium oxysporum* [34-38]. It has been observed that the exposure of aqueous solutions of metal salts or a mixture of metal salts to *Fusarium oxysporum* resulted in extracellular formation of nanoparticles of dimensions 5–50 *nm* and alloy nanoparticles of dimensions 8–14 *nm* [37-40].

v. Extracellular production of silver nanoparticles using the fungus *aspergillus fumigatus* [41].

vi. Production of silver nanoparticle as a result of the reduced state of pretreated fungus Phoma Species [42].

vii. Extracellular enzymatic reduction of MnO_2, nitrate, selenite and ferric ions using fungus *Trichoderma reesei* [29].

In the present paper we report extracellular production of silver nanoparticles using *Trichoderma reesei* (also known as *Hypocrea jecorina*). In what follows, the main advantages of *Trichoderma reesei* over other fungi are reported.

1.2. Advantages of Trichoderma reesei over other fungi

For industrial applications, fungi should have certain properties which include high production of specific enzymes or metabolite, high growth rate, easy handling in large-scale production and low-cost requirement for production procedures [43]. *Trichoderma reesei* is well known for its formation of extracellular enzyme in very large amounts, up to 100 *g/L* [44], which is much higher compared with other fungi. It is also shown that *Trichoderma reesei* produces a wide variety of extracellular enzymes and metabolites such as industrial production of glucosidase, paracelsin, protein, acetyl xylem asterase, cellobiohydrolase D, cellulose, hemicellulase, cell wall lytic enzyme, β-glucosidase, β-1, 3-glucanase, and glucose at industrial scale [43]. It should also be mentioned that *Trichoderma reesei* is the best studied cellulolytic fungus. It is widely used for the large-scale gene transformation and other biotechnology industries dealing with overexpression of extracellular enzymes [45]. Here we report our experimental study of the extracellular synthesis of silver nanoparticles using *Trichoderma reesei*.

2. Experimental Procedure and Materials

In this study, six different strains of *Trichoderma reesei* were used for experimentation. The fungal inoculates were prepared in potato dextrose agar (PDA) media (a common microbiological media for culturing fungus) at 28°*C* in Petri plates.

For the synthesis of nanoparticles, the fungus was grown in 200 *mL* bottles each containing 100 *mL* of GC medium (composed of 0.5 % glucose and 0.4 % casein hydrolysate) and at 25–28°*C* under continuous mixing condition by a magnetic stirrer (rotary shaker IKA KS 260 basic) at 150 *rpm* for 72 hours. The reason to use the GC medium is because the growth yield of *Trichoderma reesei* is greater in glucose-casein hydrolysate broth than in other media. Casein hydrolysate is a mixture of amino acids and peptides produced by enzymatic or acid hydrolysis of casein.

The mycelial (vegetative part of the fungus) mass was then separated from the culture broth by sterile paper filter, and the settled mycelia were washed thrice with sterile distilled water. The harvested mycelial mass was then used for the synthesis of silver nanoparticles.

2.1. Biosynthesis of silver nanoparticles

In a typical biosynthesis production scheme of silver nanoparticles, 10*g* of *Trichoderma reesei* fungus wet biomass was mixed with a 100 *ml* aqueous solution of 1 *mM* silver nitrate ($AgNO_3$). Then the mixture was placed in a 100 *rpm* rotating shaker at 28 °*C* for 120 hours duration. In this process silver nanoparticles were produced through reduction of the silver ions to metallic silver.

ISSN 1664-171X

2.2. Characterization of nanoparticles

The reduction of silver ions was routinely monitored by visual inspection of the solution as well as by measuring the UV-Visible spectra of the solution by periodic sampling of aliquots (2 *mL*) of the aqueous component. The UV-Vis spectroscopy measurements were recorded on a Shimadzu dual-beam spectrophotometer (model UV-1601 PC) operated at a resolution of 1 *nm*. The fluorescence measurements were carried out on a Perkin-Elmer LS 50B luminescence spectrophotometer.

In order to perform Fourier transform infrared spectroscopic (FTIR) studies of the results the films of nanoparticles were produced on *Si*(111) substrates by drop-coating the metal nanoparticle solution. The FTIR system used in this study was a Shimadzu FTIR-8201 PC instrument. It was run in the diffuse reflectance mode at a resolution of 4 cm^{-1}.

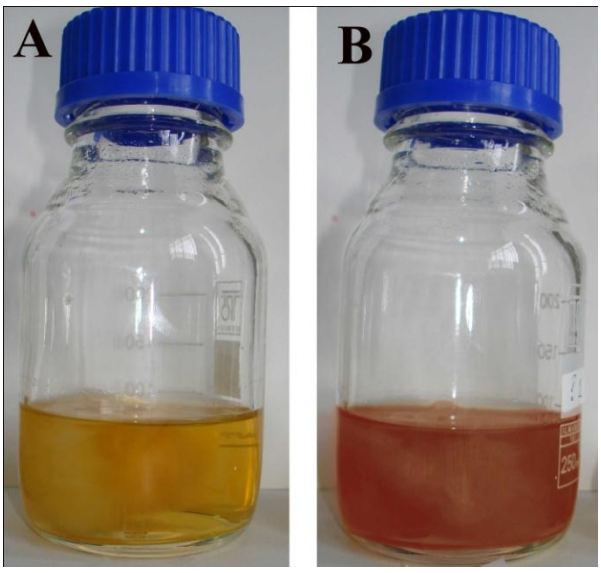
The nanoparticle films were also formed on carbon coated copper grids (40 μm × 40 μm mesh size) and transmission electron microscopy (TEM) images of the films were scanned on a JEOL 1200 EX instrument operated at an accelerated voltage of 120 *kV*.

3. Results and Discussion

3.1. Extracellular Synthesis of Silver Nanoparticles

Figure 1A shows a bottle of the fungal cells after removal from the culture medium and before immersion in 1 *mM AgNO3* solution. The pale yellow color of the fungal cells can clearly be observed in Figure 1A. A picture of the bottle containing the fungal cells after immersion in 1 *mM AgNO3* solution for 72 hours is shown in Figure 1B. It can be observed that the previous pale yellow color of the reaction mixture is changed to the brownish color after 72 hours of reaction. The appearance of a yellowish-brown color in solution containing the biomass is a clear indication of the formation of silver nanoparticles in the reaction mixture. The color of the solution is due to the excitation of surface plasmon vibrations (essentially the vibration of the group conduction electrons) in the silver nanoparticles.

Figure 1: Picture of bottles containing *Trichoderma reesei* biomass before (A) and after (B) exposure to Ag+ ions for 72 h.



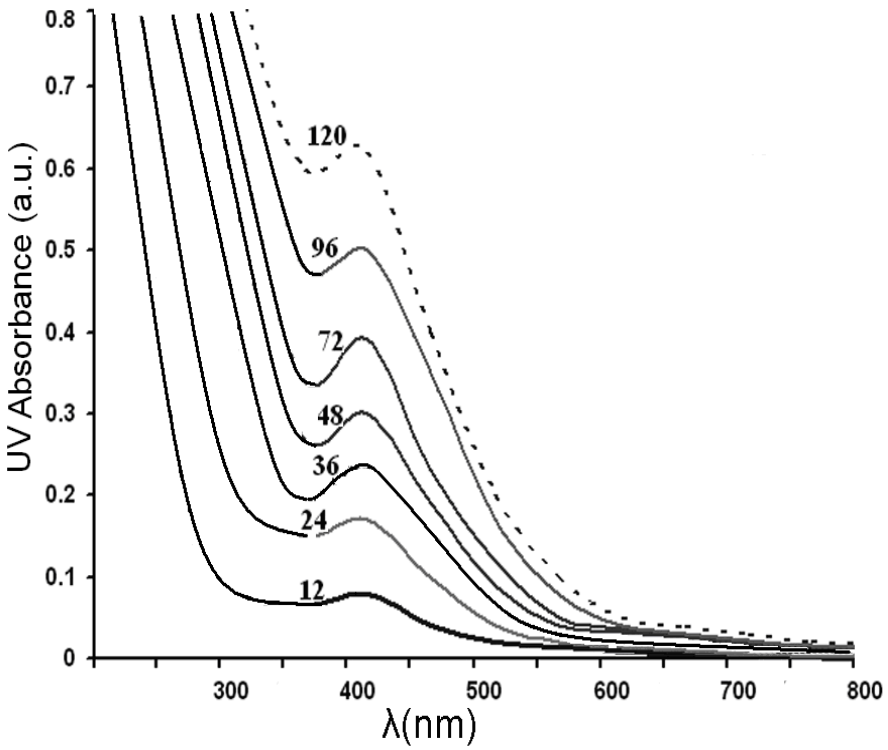
3.2. Optical Spectroscopy Measurements

Optical spectroscopy is widely used for the characterization of nanomaterials. In the present study we use three different spectroscopy techniques to fully characterize the silver nanoparticles we have produced. They include absorption UV-Visible light spectroscopy, fluorescence emission spectroscopy and Fourier transform infrared spectroscopy.

3.2.1. Ultraviolet-Visible (UV-Vis) Spectroscopy

We use UV-Vis spectroscopy to follow up with the reaction process. The spectra recorded from the Trichoderma reesei reaction vessel at different reaction times are reported in Figure 2. The time at which the aliquots were removed for measurement is indicated next to the respective curves. The strong surface plasmon resonance centered at ca. 414-420 nm, is characteristic of colloidal silver. This peak increased from 414 to 420 nm as the reaction proceeded. The spectra clearly show the increase in intensity of silver solution with time, indicating the formation of increased number of silver nanoparticles in the solution. According to this figure, there is no appreciable change in the UV-Vis spectra of the reaction product after 72 hours indicative of the fact that reaction came to equilibrium at about 72 hours. It should be pointed out that the reaction was allowed to proceed for about one month. Interestingly, the solution was extremely stable even after a month of reaction, with no evidence of aggregation of particles.

Figure 2: UV-Vis spectra recorded at various times (12, 24,…120 hours) after the start of the reaction of 100 ml of 1 mM AgNO3 solution with 10 g of *Trichoderma reesei* wet biomass.



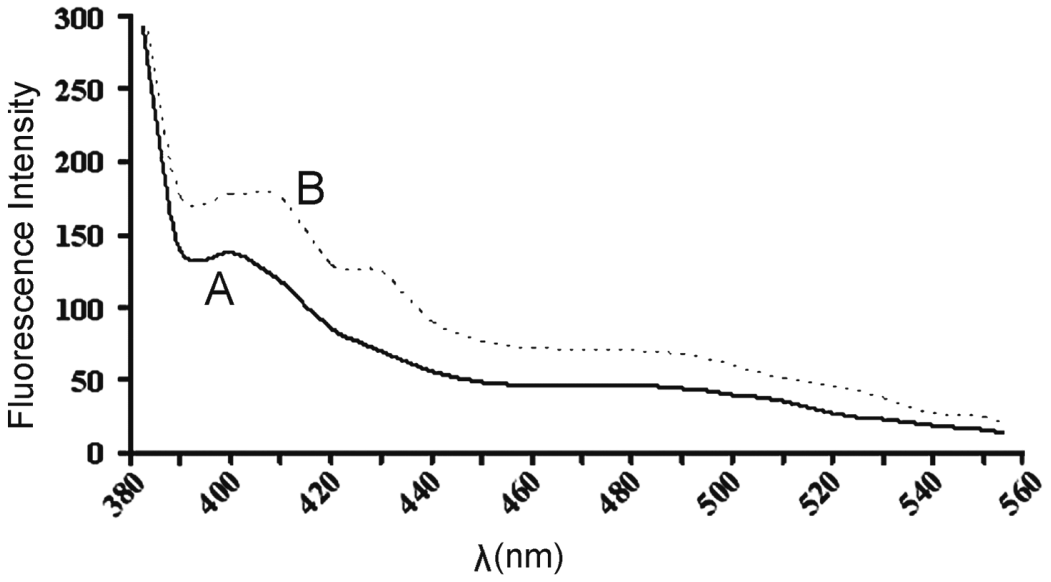
3.2.2. Fluorescence Emission Spectroscopy

Fluorescence spectroscopy is one of the widely used spectroscopic techniques in the fields of nanobiotechnology [34, 38], biochemistry and molecular biophysics today [46]. Fluorescence spectroscopy can provide detailed information on the behavior of macromolecules on the nanosecond timescale [46].

In this technique, light of some wavelength is directed onto a specimen, prompting the transition of the electron from the ground to excited state, which then undergoes a non-radiative internal relaxation, and the excited electron moves to a more stable excited level. After a characteristic time in the excited state, the electron returns to the ground state by emitting the characteristic wavelength in the form of light. This emitted energy can be used to provide qualitative and sometimes quantitative information about chemical composition, structure, impurities, kinetic process and energy transfer [47].

In the process of dissociation of silver nitrate, it appears that a reductase enzyme (nitrate reductase) is responsible for the reduction of Ag+ ions and the subsequent formation of metallic silver nanoparticles. The same observation was reported with another fungus, Fusarium oxysporum, and it was pointed out that nitrate reductase was responsible for the reduction of Ag+ ions and the subsequent formation of silver nanoparticles [29]. In Figure 3 we report the nitrate reductase through the reaction of nitrite with 2,3-diaminophthalene (DAN-reagent). The emission spectrum exhibits two major peaks of fluorescence intensity at 405 nm and 490 nm, corresponding to the emission maximum of 2,3-diaminonapthotriazole and excess DAN-reagent, respectively. The intensity of these two bands increased with the addition of a 0.1% KNO_3 solution, confirming the presence of nitrate reductase.

Figure 3: Fluorescence emission spectra for the reaction of nitrite with 2,3-diaminophthalene. In the emission spectra the curves A and B were, respectively: fungal filtrate and fungal filtrate and 0.1% KNO_3 solution. The maximum excitation wavelength was at 375 nm.

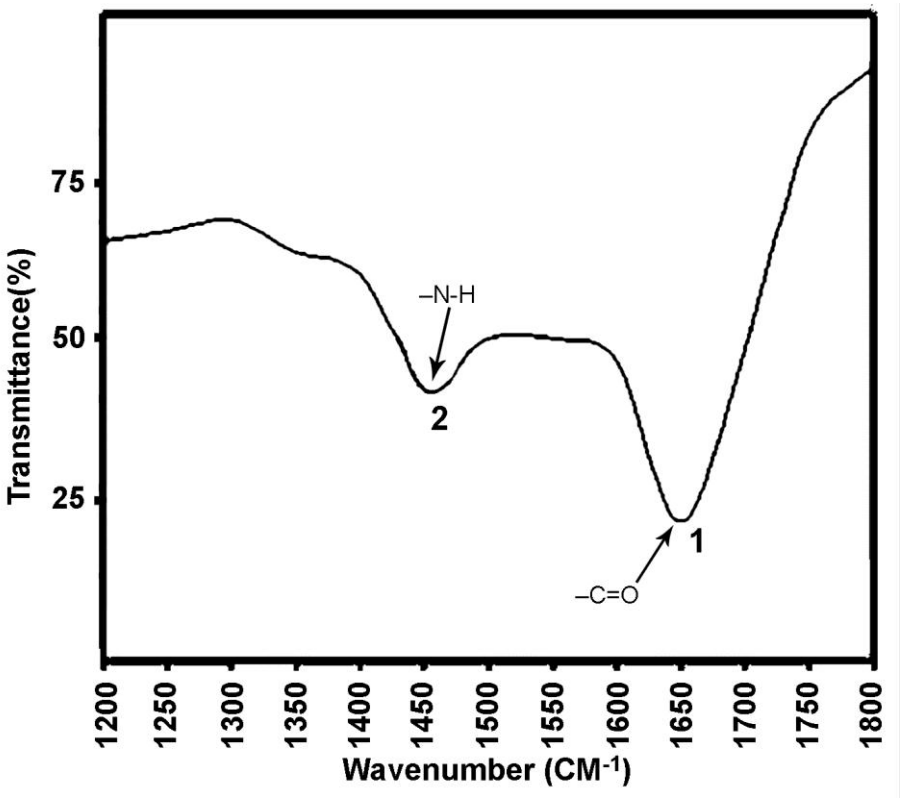


3.2.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint" [27]. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. Because the strength of the absorption is proportional to the concentration, FTIR can be used for quantitative analyses. The FTIR measurement can also be utilized to study the presence of a protein molecule in the solution, as the FTIR spectra in the 1400–1700 cm^{-1} region provides information about the presence of "*C=O*" and "*N-H*" groups [35]. The main goal of FTIR in this study is to determine the chemical functional groups in the sample [27, 35].

The amide linkages between amino acid residues in polypeptides and proteins give rise to well known signatures in the infrared region of the electromagnetic spectrum. The positions of the amide I and II bands in the FTIR spectra of proteins are a sensitive indicator of conformational changes in the protein-secondary structure [33, 44]. Figure 4 shows the FTIR spectrum recorded from a drop-coated film of the silver nanoparticle-fungus reaction mixture on *Si*(111) substrate. The spectrum shows the presence of three bands (Figure 4). The bands at 1650-**(1)** and 1450-**(2)** cm^{-1} are due to *–C=O* and *N-H* stretch vibrations present in the amide linkages of the proteins, respectively. The positions of these bands are close to that reported in literature for native proteins [33, 44]. Thus, the FTIR measurement indicates that the secondary structure of proteins is not affected because of its interaction with Ag^{+} ions or nanoparticles.

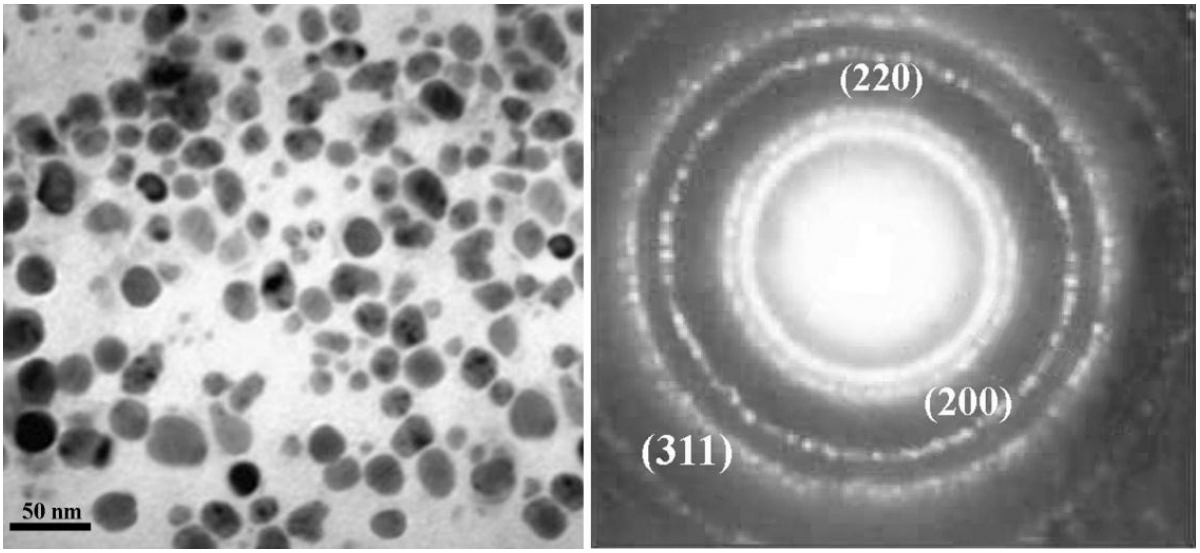
Figure 4: FTIR spectrum recorded from a drop-coated film of an aqueous solution incubated with *Trichoderma reesei* and reacted with *Ag+* ions for 72 hours. The amide bands are identified in the Figure. (A figure 4 FTIR spectrum is showed in the range of 1200 to 1800 cm^{-1}.



3.3. Transmission Electron Microscopy

The representative TEM picture recorded from the silver nanoparticle film deposited on a carbon coated copper TEM grid is shown in Figure 5. This picture shows individual silver nanoparticles as well as a number of aggregates. The morphology of the nanoparticles is highly variable. Under observation of such images, these assemblies were found to be aggregates of silver nanoparticles in the size range 5–50 *nm*. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. The separation between the silver nanoparticles seen in the TEM image could be due to capping by proteins and would explain the UV-Vis spectroscopy measurements, which is characteristic of well-dispersed silver nanoparticles. The silver nanoparticles are crystalline, as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregates (Figure 5 right).

Figure 5: (Left) TEM micrograph recorded from a drop-coated film of an aqueous solution incubated with *Trichoderma reesei* and reacted with Ag+ ions for 72 hours. (Right) Selected area of electron diffraction pattern recorded from one of the silver nanoparticles shown in the left Figure. The diffraction rings have been indexed with reference to the fcc silver.



4. Conclusions

In this research, we have shown for the first time the use of *Trichoderma reesei* in the extracellular synthesis of silver nanoparticles. In the biosynthesis of metal nanoparticle by a fungus, enzymes are produced which reduce a salt to its metallic solid nanoparticles through the catalytic effect. Compared to other filamentous fungus, the *Trichoderma reesei* is considered to be the most efficient extracellular enzyme producer, and has a long history in the production of industrial enzymes [44].

Extracellular secretion of enzymes offers the advantage of obtaining large quantities in a relatively pure state, free from other cellular proteins associated with the organism, and can be easily processed by filtering of the cells and isolating the enzyme for nanoparticles synthesis from cell-free filtrate. Our measurements indicate that extracellular biosynthesis of silver nanoparticle by *Trichoderma reesei* produces AgNPs with the diameters in the range of 5-50 *nm*. In Table 1 we compare the size ranges, methods of AgNP produced through various fungi, together with the environmental, biological and economical implications of the use of each fungus. According to this table biosynthesis of silver nanoparticles by fungus Trichoderma Reesei is preferred from the points of view of safety, economy and the large-scale production potential.

As discussed above, we can biosynthesize silver nanoparticles on a large scale through *Trichoderma reesei*, which is a major advantage over other fungus methods. It should be mentioned that *Trichoderma reesei* is not known to be harmful to humans. According to previous studies on *Trichoderma reesei*, the production of extracellular enzyme and nanoparticles in this fungus is more efficient than other fungi. It is also shown that *Trichoderma reesei* has easier and cheaper cultivation requirements and higher growth rates on both industrial and laboratory scales, thereby having a lower cost in large-scale production. It should be pointed out that large-scale production of silver nanoparticles by other techniques, such as chemical vapor deposition, irradiation, and liquid solution reduction, usually produces particles larger than a few micrometers in size. These other techniques also involve lower production yields and higher expenses [14, 27, 33] compared to large-scale biosynthesis through *Trichoderma reesei*. Because of the significant commercial value of the findings reported in this paper a patent application [54] is submitted on this subject.

Acknowledgements

The authors appreciate the very helpful review of this paper and comments by Prof. Thomas W. George.

Table 1: Comparison of *Trichoderma reesei* with other fungi in regard to AgNP sizes produced, methods of production, and large-scale industrial use implications.

Fungus	AgNP size [*nm*]	Method	Comments	References
Trichoderma reesei	5 - 50	Extracellular	Environmentally and biologically safe, large-scale produced, economical. An industrially important cellulolytic filamentous fungus because of the ease of its downstream processing. Large-scale use quite likely.	Present report
Aspergillus clavatus	10 - 25	Extracellular	Tremorgenic neurotoxicosis in cattle, causes neurological syndrome in dairy cattle. Large-scale production is feasible.	[48]
Aspergillus flavus	8.92 ± 1.61	Extracellular	A plant, animal, and human pathogen that produces the carcinogen, aflatoxin. Its industrial use may be prohibited to control the threat of this fungus and its toxin to human and animal health.	[49]
Aspergillus fumigatus	5 - 25	Intra- & Extracellular	Not safe - Cause disease in immuno-compromised individuals, can produce genotoxic and cytotoxic mycotoxins laboratory scale use only.	[41]
Cladosporium cladosporioides	10 - 100	Extracellular	Can elicit chronic allergy and asthma to humans. Large-scale production is not feasible.	[50]
Filamentous fungus Penicillium sp.	52 - 104	Extracellular	Led to discovery of antibiotics. Large-scale production may be achieved, but it will be much more expensive than *Trichoderma reesei.* Size of AgNPs are rather large.	[51]
Fusarium oxysporum	5 - 50	Extracellular	Infect a variety of hosts causing various diseases. Its large-scale use may be prohibited.	[34-36, 44]
Fusarium semitectum	10 - 60	Extracellular	Often isolated from plants with complex disease and also known to be toxigenic. Its large-scale use may be prohibited.	[52]
Neurospora crassa	~ 11	Intra- & Extracellular	A potentially carcinogen fungus. Used for genetic research. Large-scale industrial use not recommended.	[53]
Phoma species	71.06 ±3.48	Intracellular	Pathogens to plants and humans. Its adverse health factors include allergen, irritant, hypersensitivity pneumonitis, dermatitis. Large-scale use unlikely.	[42]
Verticillium species	25 ±12	Extracellular	Its adverse health factors include allergen, irritant, hypersensitivity pneumonitis, dermatitis. Its industrial use may be prohibited.	[23]

Correction after Publication:

This article was published online on March 28, 2011. Line number 8 of Table 1 page 75 has been modified. The correct version was published on February 04, 2013.

References

[1] Mansoori, GA , 2005 Principles of Nanotechnology – Molecular-Based Study of Condensed Matter in Small Systems, World Scientific Pub. Co., Hackensack, NJ.

[2] Mansoori GA, George TF, Zhang G and Assoufid L, 2007 *Molecular Building Blocks for Nanotechnology*, Springer, New York.

[3] Goodsell DS 2004 *Bionanotechnology: Lessons from Nature*, Wiley-Liss, Hoboken, New York.

[4] Mehra RK, Winge DR 1991 Metal Ion Resistance in Fungi: Molecular Mechanisms and their Regulated Expression. *J. Cell. Biochem*, 45 30-40.

[5] Pavel IS 2005 Assembly of Gold Nanoparticles by Ribosomal Molecular Machines. PHD thesis, The University of Texas at Austin.

[6] Smith PR, Holmes JD, Richardson DJ, Russell DA, Sodeau JR 1998 Photophysical and Photochemical Characterization of Bacterial Semiconductor Cadmium Sulfide Particles. *J. Chem. Soc., Faraday Trans*, 94 1235-1241.

[7] Kowshik M, Deshmukh N, Vogel W, Urban J, Kulkarni SK, Paknikar KM 2002 Microbial Synthesis of Semiconductor CdS Nanoparticles, their Characterization, and their Use in the Fabrication of an Ideal Diode. *Biotechnol. Bioengineer*, 78 583-587.

[8] Labrenz M, Druschel GK, Thomsen-Ebert T, Gilbert B, Welch SA, Kemner KM, Logan GA, Summons RE, De Stasio G, Bond PL, Lai B, Kelly SD, Banfield JF 2000 Formation of Sphalerite (ZnS) Deposits in Natural Biofilms of Sulfate-Reducing Bacteria. *Science*, 290 1744-1747.

[9] Lovley DR, Stolz JF, Nord GL, Phillips EJP 1987 Anaerobic Production of Magnetite by a Dissimilatory Iron-Reducing Microorganism. *Nature*, 330 252-254.

[10] Watson JHP, Cressey BA, Roberts AP, Ellwood DC, Charnock JM 2000 Structural and Magnetic Studies on Heavy-Metal-Adsorbing Iron Sulphide Nanoparticles Produced by Sulphate-Reducing Bacteria *J. Magn. Mater*, 214 13-30.

[11] Kowshik M, Vogel W, Urban J, Kulkarni SK, Paknikar KM 2002 Extracellular Synthesis of Silver Nanoparticles by a Silver-Tolerant Yeast Strain MKY3. *Adv. Mater.*, 14 812-815.

[12] Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO 2002 Biomimetic Synthesis and Patterning of Silver Nanoparticles. *Nat Mater*, 1 169-172.

[13] Klaus T, Joerger R, Olsson E, Claes G, Granqvist R 1999 Silver-Based Crystalline Nanoparticles, Microbially Fabricated. *Microbiology Appl Phys Sci*, 96(24) 13611–13614.

[14] Balaprasad A, Chinmay D, Ahmad A, Sastry M 2005 Biosynthesis of Gold and Silver Nanoparticles Using *Emblica Officinalis* Fruit Extract, Their Phase Transfer and Transmetallation in an Organic Solution. *J Nanosci Nanotechnol*, 5(7) 1665-1671.

[15] Shankar S, Rai A, Ankamwar B, Singh A, Ahmad A, Sastry M 2004 Biological Synthesis of Triangular Gold Nanoprisms. *Nature Materials*, 3 482-488.

[16] Shankar SS, Ahmad A, Sastry M 2003 Geranium Leaf Assisted Biosynthesis of Silver Nanoparticles *Biotechnol. Prog.* 19 1627-1631.

[17] Gardea JL, Torresdey E, Gomez JR, Peralta-Videa JG, Parsons H, Troiani M, Yacaman J: 2003 Alfalfa Sprouts A Natural Source for the Synthesis of Silver Nanoparticles. *Langmuir*, 19 1357-1361.

[18] Gilbert B, Zhang H, Huang F, Finnegan MP, Waychunas GA, Banfield JF 2003Special Phase Transformation and Crystal Growth Pathways Observed in Nanoparticles. *Geochem. Trans*, 4 20-25.

[19] Rautio J, Smit BA, Wiebe M, Penttilä M, Saloheimo M 2006 Transcriptional Monitoring of Steady State and Effects of Anaerobic Phases in Chemostat Cultures of the Filamentous Fungus *Trichoderma Reesei*. *BMC Genomics*, 7 247-249.

[20] Chovanec P, Kalinak M, Liptaj T, Pronayova N, Jakubik T, Hudecova D, Varecka L 2005 Study of *Trichoderma Viride* Metabolism under Conditions of the Restriction of Oxidative Processes. *Can. J. Microbiol*, 51(10) 853-862.

[21] Ottow JCG, Von Klopotek A 1969 Enzymatic Reduction of Iron Oxide by Fungi. *Appl. Microbiol*, 18 41-43.

[22] Lloyd JR 2003 Microbial Reduction of Metals and Radionuclides. *FEMS Microbial. Rev*, 27 411-425.

[23] Medentsev AG, Alimenko VK 1998 Naphthoquinone Metabolites of the Fungi. *Photochemistry*, 47 935-959.

[24] Duran N, Teixeira MFS, De Conti R, Esposito E 2002 Ecological-Friendly Pigments from Fungi. *Crit Rev Food Sci Nutr*, 42 53-66.

[25] Bell AA, Wheeler MH, Liu J, Stipanovic RD, Puckhaber LS, Orta H 2003 United States Department of Agriculture-Agricultural Research Service Studies on Polyketide Toxins of *Fusarium Oxysporum f sp Vasinfectum:* Potential Targets for Disease Control. *Pest Manag Sci*, 59 736-747.

[26] Baker RA, Tatum JH 1998 Novel Anthraquinones from Stationary Cultures of *Fusarium Oxysporum*. *J Ferment Bioeng*, 85 359-361.

[27] Senapati S 2005 Biosynthesis and Immobilization of Nanoparticles and their Applications. Ph.D. thesis, University of Pune.

[28] Misko TP, Schilling RJ, Salvemini D, Moore WM, Currie MG 1993 A Fluorometric Assay for the Measurement of Nitrite in Biological Samples. *Anal Biochem*, 214 11-16.

[29] Klittich CJR, Leslie JF 1988 Nitrate Reduction Mutants of *Fusarium Moniliforme* (*gibberella-fujikuroi*). *Genetics*, 118 417-423.

[30] Kumar CV, McLendon GL 1997 Nanoencapsulation of Cytochrome c and Horseradish Peroxidase at the Galleries of Alpha-Zirconium Phosphate. *Chem Mater*, 9 863-870.

[31] Bharde A, Rautaray D, Bansal V, Ahmad A, Sarkar I, Mohammad Yusuf S, Sanyal M, Sastry M 2006 Extracellula Biosynthesis of Magnetite using Fungi. *Small*, 2(1) 135-41.

[32] Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M 2003 Extracellular Biosynthesis of Monodisperse Gold Nanoparticles by a Novel Extremophilic Actinomycete, *Thermomonospora* sp. *Langmuir*, 19(8) 3550.

[33] Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Ramani R, Parischa R, Ajaykumar PV, Alam M, Sastry M, Kumar R 2001 Bioreduction of AuCl4-ions by the Fungus, *Verticillium sp.* And Surface Trapping of the Gold Nanoparticles formed. *Angew Chem Int Edu*, 40 3585-3588.

[34] Durán N, Marcato, PD, Alves OL, Souzaand G, Esposito E 2005 Mechanistic Aspects of Biosynthesis of Silver Nanoparticles by Several Fusarium Oxysporum Strains. *Journal of Nanobiotechnology*, 3:8 doi:10.1186/1477-3155-3-8.

[35] Senapati S, Ahmad A, Khan MI, Sastry M, Kumar R 2005 Extracellular Biosynthesis of Bimetallic Au-Ag Alloy Nanoparticles. *Small,* 1(5) 517-20.

[36] Souza GIH, Marcato PD, Duran N, Esposito E 2004 Utilization of *Fusarium Oxysporum* in the Biosynthesis of Silver Nanoparticles and its Antibacterial Activities. In *IX National Meeting of Environmental Microbiology* Curtiba, PR (Brazil) Abstract pag. 25.

[37] Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan MI, Kumar R, Sastry M 2002 Extracellular Synthesis of Gold Nanoparticles by the Fungus *Fusarium Oxysporum*. *Chem Biochem*, 3 461-463.

[38] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M 2003 Extracellular Biosynthesis of Silver Nanoparticles using the Fungus *Fusarium Oxysporum*. *Colloid Sorf B,* 28 313-318.

[39] Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parischa R, Ajayakumar PV, Alam M, Kumar R, Sastry M 2001 Fungusmediated Synthesis of Silver Nanoparticles and their Immobilization in the Mycelial matrix: A Novel Biological approach to Nanoparticle Synthesis. *Nano Let*, 1 515-519.

[40] Sastry M, Ahmad A, Islam NI, Kumar R 2003 Biosynthesis of Metal Nanoparticles using Fungi and Actinomycete. *Urent Sci*, 85 162-170.

[41] Bhainsa KC, D'Souza SF 2006 Extracellular Biosynthesis of Silver Nanoparticles using the Fungus Aspergillus Fumigatus. *Colloids Surf B Biointerfaces*. 47(2) 160-164.

[42] Chen JC, Lin ZH and Ma XX 2003 Evidence of the Production of Silver Nanoparticles via Pretreatment of Phoma sp.3.2883 with silver nitrate. *Lett Appl Microbiol*, 37 105–108

[43] Durand H, Clanet M, Tiraby G 1988 Genetic Improvement of Trichoderma Reesei for Large Scale Cellulase Production. *Enzyme Microb Technol,* 10 341–346.

[44] Oksanen T, Pere J, Paavilainen L, Buchert J, Viikari L 2000 Treatment of Recycled Kraft Pulps with *Trichoderma Reesei* Hemicellulases and Cellulases. *J Biotechnol*, 78(1) 39–44.

[45] Archer DB, Jeenes DJ, Mackenzie DA 1994 Strategies for Improving Heterologous Protein Production from Filamentous Fungi. *Antonie Leeuwenhoek*, 65 245–250.

[46] Joseph R, Lakowicz Sf, Grattonnll E, Cherek H, Badri P, Maliwal S, Gabor Laczko SS 1984 Determination of Time-resolved Fluorescence Emission Spectra and Anisotropies of a Fluorophore-Protein Complex Using Frequency-Domain Phase-modulation Fluorometry. The *Journal of Biological* Chemistry, 259 (17) 10967-10972.

[47] Lakowicz, JR 1983 *Principles of Fluorescence Spectroscopy,* Plenum Press, New York.

[48] Verma VC, Kharwar RN and Gange AC 2010 Biosynthesis of Antimicrobial Silver Nanoparticles by the Endophytic Fungus Aspergillus Clavatus. *Nanomedicine*, 5(1) 33-40.

[49] Vigneshwaran, N, Ashtaputrea, N.M, Varadarajana, P.V., Nachanea, R.P., Paralikara, K.M. and Balasubramanyaa, R.H. 2007 Biological Synthesis of Silver Nanoparticles using the Fungus Aspergillus Flavus, *Materials Letters*, 61(6), 1413-1418.

[50] Balaji DS, Basavaraja S, Deshpande R, Bedre Mahesh D, Prabhakar BK and Venkataraman A 2009 Extracellular Biosynthesis of Functionalized Silver Nanoparticles by Strains of Cladosporium Cladosporioides Fungus. *Colloids and Surfaces B: Biointerfaces*, 68(1), 88-92.

[51] Hemath Naveen KS, Kumar G, Karthik L, Bhaskara Rao KV 2010 Extracellular Biosynthesis of Silver Nanoparticles using the Filamentous Fungus Penicillium sp. *Archives of Applied Science Research*, 2(6) 161-167.

[52] Basavaraja S, Balaji SD, Lagashetty A, Rajasab AH and Venkataraman A 2008 Extracellular Biosynthesis of Silver Nanoparticles using the Fungus Fusarium Semitectum. *Materials Research Bulletin*, 43(5) 1164-1170.

[53] Castro-Longoria E, Vilchis-Nestor AR and Avalos-Borja M 2011Biosynthesis of Silver, Gold and Bimetallic Nanoparticles using the Filamentous Fungus Neurospora Crassa. *Colloids and Surfaces B: Biointerfaces*, 83(1) 42-48.

[54] Mansoori GA, SYNTHESIS OF NANOPARTICLES BY FUNGI, 2010, Patent application number: 20100055199, IPC8 Class: AA01N5916FI, USPC Class: 424618, www.faqs.org/patents/app/20100055199.