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Articles of General Interest

SYNTHESIS OF NANOPARTICLES USING FUNGUS *FUSARIUM OXYSPORUM*

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ABSTRACT

There has been commendable development in the field of biosynthesis of nanoparticles due to their low cost, environment friendly approach. One such biological entity employed in the synthesis is a fungus, *Fusarium oxysporum* that has shown hopes for a sustainable synthesis of many nanoparticles. In this review we will give an overview of the synthesis of nanoparticles using green methods and the use of *Fusarium oxysporum* for the same with will focus on the synthesis of silver and gold nanoparticles using *Fusarium oxysporum*.

Keywords: Biological synthesis, *Fusarium oxysporum*, Eco-friendly synthesis, Nanoparticles, Fungi

INTRODUCTION

Nanotechnology refers the engineering of particles regime of 10^{-9}m. Such particles are of tremendous interest due to the unique optical (Mohanpuria *et al*., 2008), electronic (Mohanpuria *et al*., 2008), magnetic (Janki *et al*., 2013) and biological properties (Ahmad *et al.*, 2003).Their novel behaviour is attributed to the fact that their size is so small that the fundamental behaviour of electrons is altered completely. They have size dependent properties, i.e., their properties can be greatly altered if there is a small change in the size of such nanoparticles. This has opened up a wide market for the possible applications of such nanoparticles. They have been used in the fabrication of sensors (Otari *et al*., 2012; Patra *et al*., 2010), detection of cancer at much faster rates (Patra *et al*., 2010; Lifeng *et al*., 2010), antibacterial agents (Ahamed *et al*., 2010) & catalysts (Lifeng *et al*., 2010) and various drug delivery agents (Ghosh *et al*., 2008). There has been extensive use of TiO_2 (Nakata *et al*., 2012) and ZnO (Hong *et al*., 2007) nanoparticles in the cosmetic industry too.

Many approaches have been used to synthesize nanoparticles, mainly using chemicals (Bhansia *et al*., 2006, Shankar *et al*., 2004; Lee *et al*., 2007). But a fundamental disadvantage of such techniques is that toxic chemicals are used (Bhansia *et al*., 2006; Shankar *et al*., 2004) and there is need to precisely control all the parameters associated with them (Bhansia *et al*., 2006). The chemical methods are expensive and the size distribution of particles is spread across a wide range (Mohanpuria *et al*., 2008).Biological synthesis of nanoparticles has emerged as an important alternative. The inclination towards the use of biological resources stems from the fact that the biological synthesis provides low cost and synthesis of stable nanoparticles, without the use of toxic chemicals (Bhansia *et al*., 2006; Shankar *et al*., 2004). The particles synthesized are stable without the use of any external capping agents and the stability has been shown to extend from few days to months. They also have an advantage of being much more biocompatible than their chemically synthesized counterparts.

Many biological agents like bacteria, viruses, fungi and actinomycetes and plant extracts like *Magnolia kobus* (Song *et al*., 2009), *Diopyros kaki* (Song *et al*., 2009), *Acalypha indica* (Krishnaraj *et al*., 2009), *Azadirachta indica* (Shankar *et al*., 2004) and *Iresine herbstii* (Dipankar *et al*., 2011) many others have been used to synthesize a variety of nanoparticles like silver, gold, platinum (Seyad *et al*., 2012), zinc sulphide (Mirzadeh *et al*., 2012) and copper sulphide (Hosseini *et al*., 2012) have been synthesized. There have also been reports of synthesis of nanoparticles using biological compounds like bovine serum albumin (Kumar *et al*., 2013).

For the biological entities like bacteria, viruses, actinomycetes and fungi, the synthesis of nanoparticles can be broadly classified under two heads; extracellular synthesis (i.e. synthesis of nanoparticles outside the cell) and intracellular synthesis (i.e. synthesis of nanoparticles inside the cell) (Durán *et al.*, 2005). The former is always favourable as there is no need to isolate the nanoparticles from the cellular mass which involves additional steps like cell lysis and purification of nanoparticles (Konishi *et al*., 2005). *Fusarium oxysporum* has been shown to demonstrate extracellular synthesis (Bhainsa *et al*., 2006). It can be found in soil and is also found in association with many important crop plants. They are responsible for *Fusarium* wilt disease in many species of plants. Plants are known to produce reactive oxygen species as a defence mechanism against such pathogens (Thakker *et al*., 2013). So these fungi must produce strong reducing agents to counteract the defence mechanism (Thakker *et al*., 2013). We exploit this property of *Fusarium oxysporum* to synthesize nanoparticles.

EXTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES

Fusarium *oxysporum* secretes a variety of compounds in the growth medium. These enzymes or compounds act as reducing agents for the Ag^+ ions in the AgCl solution (Ahmad *et al*., 2003; Durán *et al*., 2005). Ahmad *et al*. (2003) have elucidated that this reducing agent should be stronger than the reducing agent responsible for gold ions reduction as gold is much more easily reduced than silver. There have been many theories proposed as to the exact mechanism of the synthesis of such nanoparticles. However the mechanism of the synthesis is beyond the scope of this paper and we will focus on the synthesis and the results.

METHODOLOGY

The experiment by Ahmad *et al*. (2003) involved the addition of 10g of Fusarium *oxysporum* in a flask containing distilled water. The mixture is pale yellow in colour. A measured quantity of $AgNO_3$ is added and the mixture is incubated in dark. Small amount of the solution mixture is taken out at regular intervals to measure the UV visible absorbance and the fluorescence value. A dual approach was followed by Nelson Durán *et al*. (2005) where they recorded the UV visible absorbance of the mixture of nanoparticles with the mycelia (Method A). Another approach was followed where they added $AgNO_3$ to only to the fungal filtrate (Method B). The FTIR value of the final solution was recorded to characterise the protein binding with the silver nanoparticles. TEM images give us the shape distribution of the particles (Nelson Durán *et al*., 2010).

RESULTS & DISCUSSIONS

Both the groups reported a colour change from pale yellow (colour of the fungal biomass in water) to a brownish after 72 hours, indicating the reduction of Ag^+ to nanoparticles. This colour change indicated the surface plasmon resonance that happens in the nanoparticles. When the size of the nanoparticles becomes small, only shorter wavelengths are able to interact with the small particles, indicating a blue shift that is evident from the results. Ahmad *et al*., (2003) reported that on filtration of the biomass, the solution had an intense yellow colour. This was a clear evidence of the blue shift. The incubation was done in dark to nullify any possibility of a photochemical reaction affecting the reaction. The UV visible absorbance gives us information about the shape and size of the nanoparticles. A result of the absorbance as reported by Ahmad *et al*., (2003) is shown below.

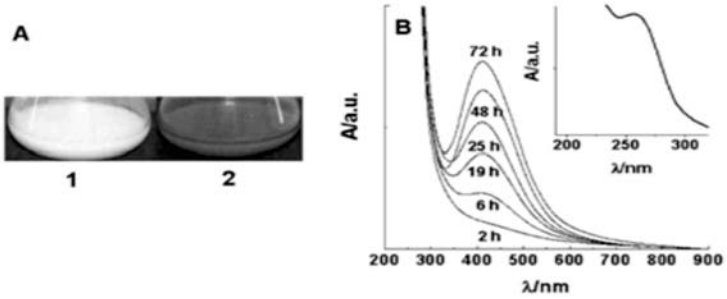

Fig. 1(A): Pictures of the flask containing *Fusarium oxysporum* at the beginning (flask1) and after the completion of the reaction (72h) (flask 2). **Fig 1 (B):** The absorbance of the mixture of the fungal biomass and the nanoparticles in solution at different times during the reaction (Ahmad *et al*., 2003).

The intensity of the peak correlates the amount of nanoparticles produced during the reaction. A sharp peak indicates a narrow distribution of the size of the nanoparticles whereas a broad peak indicates a wide distribution. An interesting finding was that the particles synthesized by the Ahmad *et al*., (2003) were extremely sable even after one month with a very little sign of flocculation. This was easily confirmed with the UV visible spectra (not shown in the paper).

The fluorescence absorbance is used to characterize the interactions of protein with the nanoparticle (Nelson Durán *et al*., 2010). This characterises that the proteins present in the solution and those bound to the nanoparticle are in naive form (Nelson Durán *et al*., 2010). There is no effect on the tertiary structure of the protein. A clear absorption at 270 nm indicated the presence of tryptophan and tyrosine residues (Ahmad *et al*., 2003).

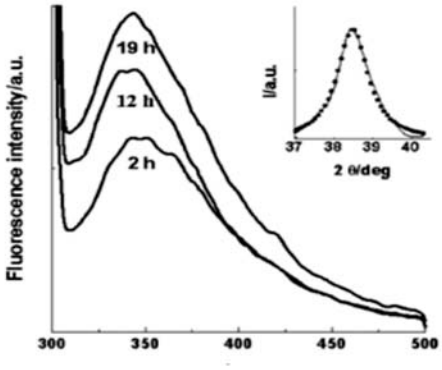

Fig. 2: Ahmad *et al*. (2003) reported fluorescence spectroscopy of the fungal biomass-nanoparticle mixture. The inset shows the (111) Braggs reflection for the silver nanoparticle film growth *Fusarium oxysporum*

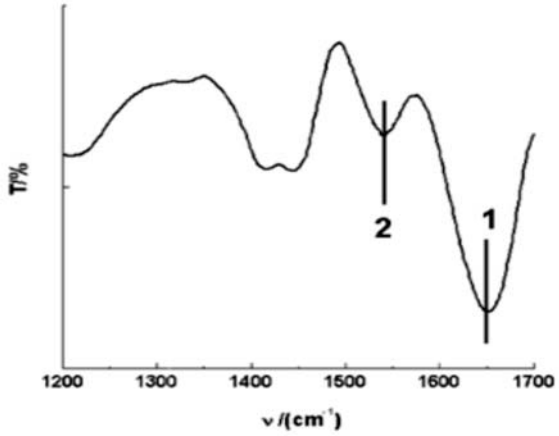

Fig. 3: The FTIR spectra recorded by Ahmad *et al*. (2003). Three bands were identified at 1650 cm^{-1} (1), 1540 cm^{-1} (2) and 1450 cm^{-1}.

The FTIR spectra present on the drop coated film of silver nanoparticles- fungal biomass reaction showed three major bands at 1650 cm^{-1} (1), 1540 cm^{-1} (2) and 1450 cm^{-1}. These bands are shown in the graph (Ahmad *et al*., 2003).

The first 2 bands of 1650 and 1540 cm^{-1} are reported as amide I and amide II bands due to amide linkages in proteins. The FTIR results pointed out that there was no effect on the secondary structure of protein due to the reaction with Ag^{+} ions or binding with silver particles. The third peak of 1450^{-cm} is of methyl scissoring vibrations from proteins in solution (Ahmad *et al*., 2003).

While Ahmad *et al*. (2003) reported a wide distribution in the particle morphology with sizes ranging from 5-50 nm and having mostly spherical and triangular shape, Durán *et al*. (2005) reported relative smaller size distribution of 20-50 nm and having spherical shape using various strains of *Fusarium oxysporum*. This result is much better than the usual chemical methods employed that may have a much larger particle distribution and much less stability.

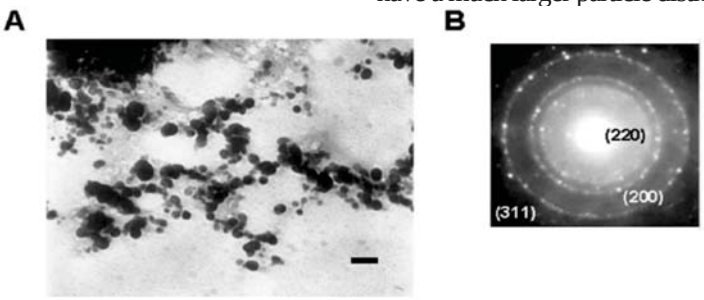

Fig. 4: The TEM images reported by Ahmad *et al*. (2003) (A) The TEM micrograph recorded from a dip coated film of an aqueous solution incubated with *Fusarium oxysporum* and reacted with Ag^{+} ions for 72 hours. The scale bar corresponds to 100nm. (B) Selected area of electron diffraction pattern recorded from one of the silver nanoparticles shown in A

Extracellular synthesis was confirmed by Nelson Durán *et al*. (2005) in both its methods: the one where the $AgNO_3$ was added to the fungal biomass and one where the $AgNO_3$ was added to the fungal biomass extract, both showed the colour change to yellowish-brown indicating the formation of nanoparticles. Hence it was concluded that the reducing agents secreted by the fungus were responsible for the reduction of the Ag^+ ions. Similar work was done by Ahmad *et al*. (2003) too. The reducing agents were identified as nitrate dependent reductive and an extracellular shuttle for ions (Nelson Durán *et al*., 2005).

Geometry and Morphology

Namita Soni *et al*. (2011) reported the effect of temperature, pH, time and concentration on the morphology of the particle. When the temperature was varied from 25°C-30°C, it was observed that at lower temperatures (25°C), the size of the particles were small whereas at higher temperatures (30°C), larger particles were formed. An increase in the size was reported with a decrease in the pH. Increase in time and concentration also followed the trend and produced bigger particles with a decrease in the increase in time and concentration respectively.

EXTRACELLULAR SYNTHESIS OF GOLD NANOPARTICLES

The use of gold nanoparticles has attracted attention due to their stability under atmospheric conditions, resistance to oxidation and biocompatibility (Thakker *et al*., 2013). Both wet chemical (Lee *et al*., 2007) and biological routes have been employed to do synthesize gold nanoparticles but , to the best of our knowledge, there have not been many reports on the synthesis of gold nanoparticles using *Fusarium oxysporum*, although there have been reports of synthesis using leaf extracts of other plants and using other bacteria. These synthesis have been both extracellular (Thakker *et al*., 2013; Kalishwaralal *et al*., 2009) and intracellular (Konishi *et al*., 2006). One of the species, *Fusarium oxysporum* f.sp. cubense JT1 (FocJT1), was isolated from banana plants, where it causes panama disease (Thakker *et al*., 2013).

METHODOLOGY

The *Fusarium oxysporum* f.sp. cubense JT1 (FocJT1) is first grown in potato dextrose agar and then inoculated in potato dextrose broth and incubated at 27°C for 21 days. The broth was then divided into two flasks; one served as the control and the other served as the experimental flask. The experimental flask broth is incubated with 10mM $HAuCl_4$.The samples were periodically withdrawn from the experimental and control flasks at intervals of 0m, 30m,60m, 90m, 24h and 48h respectively (Thakker *et al*., 2013).

The particle size was also analysed using the particle size analyser after filtering the nanoparticle-fungus reaction solution using Whatman filter paper followed by filtering using 0.2µm filter to remove any spores (Thakker *et al*., 2013).

RESULTS AND DISCUSSIONS

A change in the colour of the experimental flask was observed. The colour changed from pale yellow (before the reaction) to purple after the completion of the reaction. It has been shown in Figure 5 (Thakker *et al*., 2013).

Fig. 5: The (a) Control and the (b) Experiment flask (incubated with FocJT1).

The clear blue shift in the colour of the biomass is due to the formation of nanoparticles. The gold nanoparticles have novel properties. This blue shift is due to the surface plasmon resonance that takes place when the size of the particle becomes so small that it cannot interact with the longer wavelengths and only shorter wavelengths are able to interact with the particles. The UV visible spectra of both the control and the experimental broth were recorded in 370 and 340-700nm range (Thakker *et al*., 2013). A broad peak is obtained between 500 to 580 nm and was found to increase with time. An important distinction of this method was that the peak started developing in 60 min of the reaction which was much faster than the conventional reactions that take 24 to 120 hours (Thakker *et al*., 2013). This contributes a major development in the apparent use of such manufacturing techniques nanoparticles in the commercial scale.

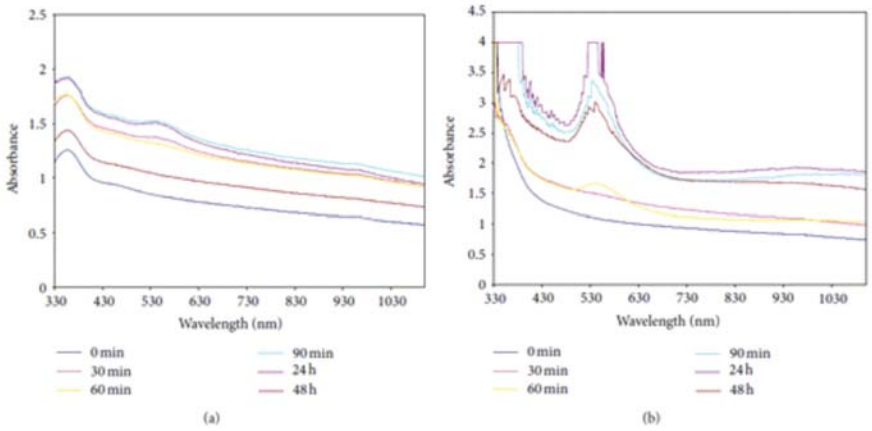

Fig. 6: (a) The UV visible spectra of the control and (b) the experimental flask broth augmented with 10mM $HAuCl_4$ at various time intervals.

The particle size of 22nm was reported with an exceptional stability (Thakker *et al*., 2013). Antimicrobial activity was also reported against *Pseudomonas sp*. (Thakker *et al*., 2013). As an advantage, the synthesis of gold nanoparticles was found to be extracellular (Thakker *et al*., 2013).

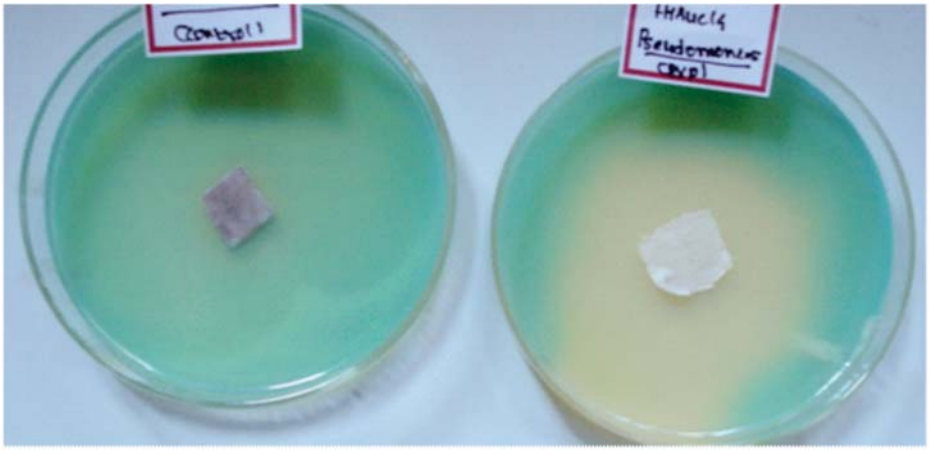

Fig. 7: Antimicrobial activity of (a) control (b) experimental against *Pseudomonas sp.*

CONCLUSIONS

The use of such nanoparticles is now expanding with the growing use in various sectors like optical (Mohanpuria *et al*., 2008), electronic (Mohanpuria *et al*., 2008), magnetic (Janki *et al*., 2013) and biological fields. Biological synthesis of nanoparticles presents a viable alternative to

the costly chemical techniques. Recently synthesis of CuS and platinum nanoparticles has also been reported using *Fusarium oxysporum*. Apart from *Fusarium oxysporum*, use of other biological entities to synthesize nanoparticles presents a hope of a bigger market for the biologically derived nanoparticles, particularly due to their exceptional stability that comes as an innate advantage.

The possible need of the hour is to scale up such process for industries without significantly affecting the efficiency and repeatability of such processes. Also further studies need to be done to see the compatibility of such nanoparticles in the industrially manufactured devices. Most of the studies done are on the synthesis at the lab level. There are barely any reports on the functional application of such nanoparticles. Further, there has been a recent shift in the synthesis of nanoparticles using plant and seed extracts, with more number of recent papers focussing on such green synthesis.

We can conclude with the fact the hope of further research being conducted for some viable industrial application of such nanoparticles.

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