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Short Communication

Intracellular Biosynthesis of Gold Nanoparticles by the Fungus Penicillium Chrysogenum

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Abstract:
In this study, the biosynthesis of gold nanoparticles by Penicillium chrysogenum, isolated from Ahar copper mine, was investigated. The gold nanoparticles were synthesized by reducing the aqueous gold ions using the culture supernatant of the filamentous fungi. The UV–vis spectrum displayed a characteristic peak at 532 nm that is very specific for gold nanoparticles. The XRD spectrum confirmed the presence of crystalline gold nanoparticles. Transmission electron microscopy exhibited the intracellular formation of gold nanoparticles in spherical, triangle and rod shapes with the size range of 5 to 100 nm.
Keywords: *Gold nanoparticles, Biosynthesis, Penicillium chrysogenum.*

1. INTRODUCTION

Nanotechnology involves the production, manipulation and use of materials ranging in size from less than a micron to that of individual atoms [1]. Nanoparticles can be synthesized by physical and chemical methods [2]. The problems of these methods are often experienced with low stability of the nanoparticle preparations, difficult control of the crystal growth and aggregation of the particles [3-5]. The development of an eco-friendly and reliable process for the synthesis of gold nanomaterials either intra or extra-cellular using microorganisms such as bacteria Lactobacil sp. and Licheniformis sp., algae Sargassum wightii, fungi Trichothecium sp. and Trichoderma Koningii, actinomycetes Rhodococcus sp and Thermomonospora sp., yeast Yarrowia lipolytica NCIM 3589 is of great importance in the field of nanotechnology [1, 6,7].

Microorganisms such as bacteria, yeast and fungi play an important role as the nanofactories in the remediation of toxic metals through the reduction of the metal ions [8]. Gold nanoparticles have found many applications in many fields such as cancer diagnosis and therapy, drug and gene delivery, DNA and ptoteins determination, catalysis and sensors [9]. In an interesting recent study, Binupriya and co-workers demonstrated that the fungus Rhizopus stolonifer, when placed in aqueous solution of 1mM $HAuCl_4$, resulted in the extracellular formation of gold nanoparticles [10]. The use of fungus Penicillium sp. for the intracellular synthesis of gold nanoparticles has been reported by Zhang and co-workers [11]. In this study, we investigated the use of novel metal resistance fungus, Penicillium chrysogenum isolated from Ahar copper mine, in the synthesis of gold nanoparticles. This fungus is a mold that is widely distributed in nature and is often found on foods and in the environment [12].

2. EXPERIMENTAL

2.1. Media and chemical agents

All the media and chemical agents including Glucose, yeast extract, peptone, malt extract, SDA media and chloroauric acid trihydrate ($HAuCl_4.3H_2O$) were purchased from Sigma Aldrich.

2.2. Synthesis of gold nanoparticles

The Penicillium chrysogenum strain was isolated from the soil of Ahar copper mine (in north-west of Iran) and characterized according to the morphological features and growth parameters on malt extract agar or oatmeal agar mediums. The fungus was cultured in the medium sabourau dextrose agar (SDA) in the petri dishes. The plates were then incubated at 27°C for 3-7 days. The fungal biomass was grown aerobically in 100 ml MYPG medium that was composed of malt extract (0.3٪), yeast extract (0.3٪), peptone (0.5٪) and glucose (1٪) at pH 5.5-6. The culture was incubated in an orbital shaker with agitation of 160 rpm at 27–29°C for 96 hours. The fungus biomass was separated from the culture broth by centrifugation (5000 rpm) at 10°C for 20 min. Then, the biomass was washed extensively with distilled water to remove medium components. The fresh and clean biomass (5 g of wet biomass) was suspended in 100 ml of 1 mM aqueous $HAuCl_4$ solution with pH 2.5 in 250 ml Erlenmeyer flask. The whole mixture was put into a shaker-incubator at 27–29°C for 72 h with the rate of 160 rpm. The reaction was carried out in darkness.

2.3. Characterization of gold nanoparticles

The characterization of gold nanoparticles was carried out by UV–vis spectrophotometer, XRD and TEM analyses. UV–vis spectrophotometer (shimadzu, UV Pharma spec 1700 with a resolution of 0.72 nm) was employed for absorption spectra measurements of the synthesized nanoparticles in the range of 400–800 nm. It is possible to release intracellular gold nanoparticles via reaction with suitable detergents [13]. The XRD measurement of the bioreduced chloroauric acid solution was carried out using a Philips PW 1800. 5 g of the biomass was put into a petri dish and dried in 50°C for 24 h in the avan before the analysis [2, 10]. A drop of the fungus containing the gold nanoparticles was placed on the carbon-coated copper grids for transmission electron microscopy (Philips model EM 208S) operating at 200 kV [6].

3. RESULTS AND DISCUSSION

The detailed study on the fungal (Penicillium sp.) biosynthesis of gold nanoparticles was carried out in this work. By mixing Penicillium chrysogenum

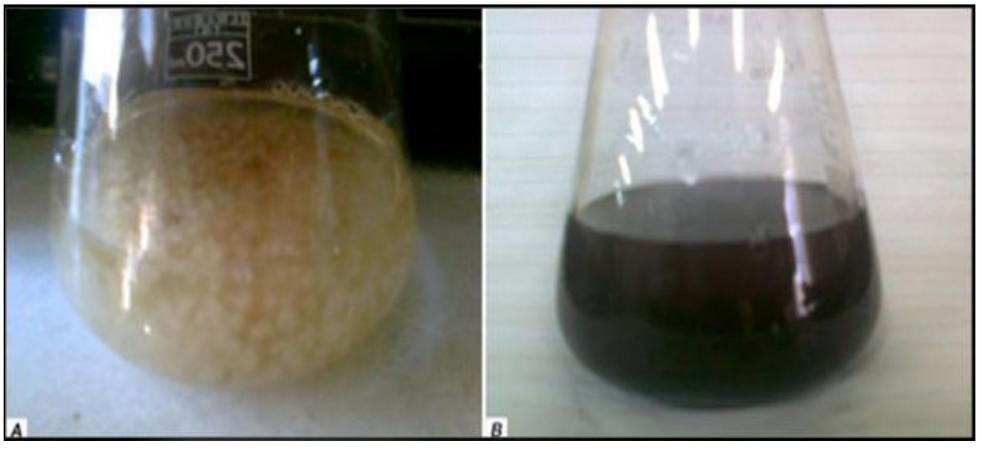

Figure 1: *Penicillium chrysogenum biomass before the reaction (a) and after 12 h of the reaction (b) with the solution of 1 mM $HAuCl_4$.*

with the aqueous solution of chloroaurate ions, the color of the biomass changed from yellow to purple after 12 hours. The color change is a signal for the formation of gold nanoparticles (Figure 1).

The UV-vis spectrum of the suspension purple showed an strong surface plasmon resonance at 532 nm. This is a characteristic property for gold nanoparticles (Figure 2).

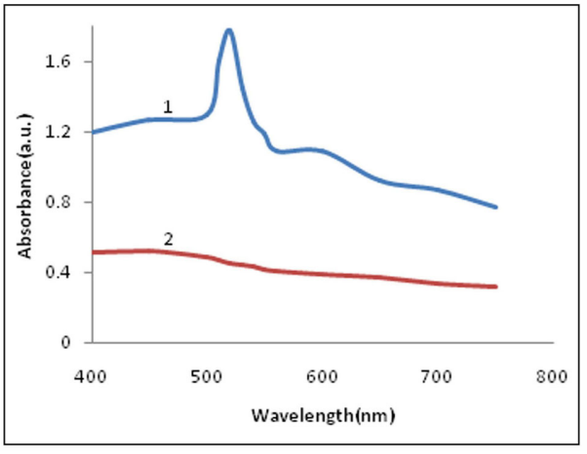

Figure 2: *UV-vis spectra recorded as a function of reaction aqueous solution of 1 mM $HAuCl_4$ with the fungal biomass before (2) and after (1) synthesis gold nanoparticles.*

One of the important ways to characterize nanoparticles is evaluating the XRD spectrum of the sample. The XRD pattern clearly showed that the gold nanoparticles have been formed by the bioreduction of gold ions by the fungus [Figure 3]. The XRD spectrum resulted in four intense peaks in the spectrum, (38.269), (44.600), (64.678), (77.549) and (82.352), which agree to the Bragg's reflection of gold nanoparticles (14).

TEM micrographs approved the intracellular production of gold nanoparticles by Penicillium chrysogenum (Figure 4). The nanoparticles have been formed in spherical, triangles and rod shapes in the size range of 5 to 100 nm. The nanoparticles formation proceeds via an intracellular mechanism, but there are still some questions regarding the details of the process.

An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different chemical compositions, sizes, shapes and controlled dispersity. Gold nanoparticles have potential applications in various fields, such as catalysis, sensor technology, biological labelling photonics, electronics, biomedicine and optics [7]. A green chemistry synthetic route has been used for gold nanoparticles synthesis. The use of fungi in the synthesis of nanoparticles is a relatively recent addition to the list of microorganisms possessing nanoparticle biosynthesis "ability". Application of fungi to produce nanoparticles is potentially exciting because of their ability to secrete the large amounts of enzymes [13]. Many fungi produce inorganic materials either intra- or extracellularly. Liangwei Du and co-workers have shown that the fungus fungus Penicillium sp. 1-208 when placed in a concentrated aqueous solution of $HAuCl_4$, resulted in the reduction of the Au^{3+} ions, rapid extra-/ intracellular biosynthesis of gold nanoparticles [15]. The extracellular synthesis of silver nanoparticles by a marine fungus Penicillium fellutanum has been described by Kathiresan and coworkers [16]. It was shown in this work that reaction of aqueous chloroaurate ions with the fungus biomass Penicillium chrysogenum isolated from the soil of

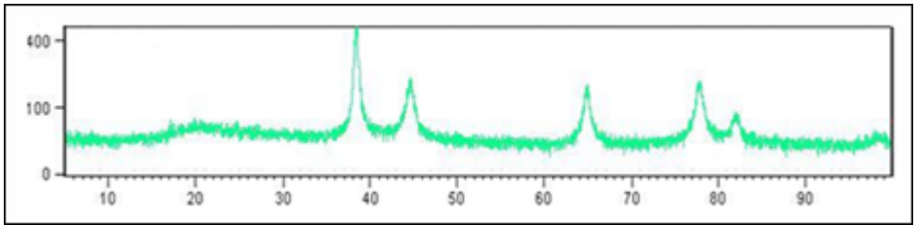

Figure 3: *XRD pattern of the produced gold nanoparticles by Penicillium chrysogenum.*

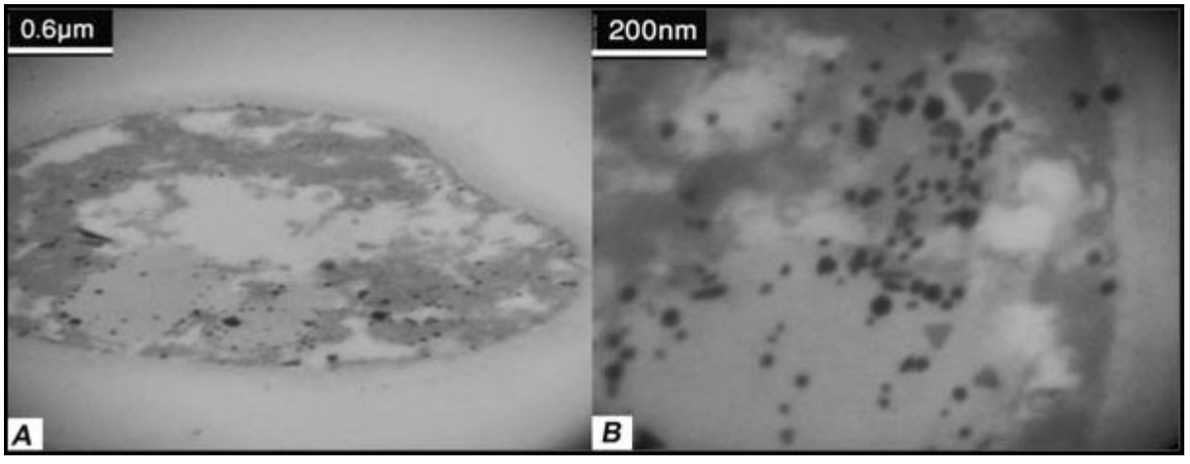

Figure 4: *TEM images indicate the intracellular synthesis of gold nanoparticles with different shapes*

Ahar copper mine leads to the formation of gold nanoparticles intracellular. To our knowledge, this is the first study on the synthesis gold nanoparticles potential of Penicillium chrysogenum in vitro. The results showed that this fungus is a good candidate for the simple and clean synthesis of gold nanoparticles.

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