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Investigations on gold nanoparticles biosynthesis potential of marine derived fungi

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ABSTRACT

The objective of the work was to explore potential of marine-derived fungi for biosynthesis of gold nanoparticles (GNPs). Three marine-derived fungal isolates viz. *Aspergillus candidus*, *Aspergillus flavus and Aspergillus niger* have been examined for their ability to biosynthesize GNPs. All the test isolates were challenged with different concentrations (0.25, 0.5, 1, 2 and 3mM) of gold (III) chloride under static condition at 27^0C for 72h. The isolates could biosynthesize spherical GNPs mostly extracellularly. In certain cases, mode of synthesis extra/intracellular was observed to be dependent on gold ion concentrations. While there is no much information available on exploitation of marine-derived fungi for biosynthesis of metal nanoparticles, present findings suggest marine-derived fungi as potential resource for developing cost-effective green technology for GNP synthesis.

KEYWORDS

Biosynthesis;
Extracellular;
Gold nanoparticles;
Green technology;
Marine-derived fungi.

INTRODUCTION

Gold nanoparticles, having potential applications in the areas like catalysis, diagnosis, drug delivery, imaging, photonics and targeted killing of cells[1-5] are in great demand. While physico-chemical synthesis protocols are expensive, polluting nature and even have restricted use in clinical and pharma applications, biosynthesis of gold nanoparticles offers a promising option to meet the requirement.

A number of microorganisms have been examined for nanoparticle biosynthesis. While researches on nanobiotechnology in future may increasingly depend on marine microbes having ability to grow under extreme conditions, it has been suggested that initiatives should be taken for utilization of these resources in the area of nanobiotechnology[6] However, marine microbes are comparatively less explored for biosynthesis of gold nanoparticles and only few reports exist[7,8]. Harnessing fungi for such purposes is more advantageous[7].

The present study is carried out with a view to examine gold nanoparticles biosynthesis potential of three marine –derived aspergilli. The data showed that all the test fungi could biosynthesize gold nanoparticles mostly extracellularly. In certain cases, mode of synthesis extra/intracellular was observed to be dependent on gold ion concentrations.

MATERIALS AND METHODS

Isolation and identification of test fungus

The test fungi were isolated from waters of Bhavnagar coast (Lat. 21º 45' N and Long. 72º. 14' E), Gulf of Khambhat, West Coast of India. The isolates were grown and maintained on potato dextrose agar (PDA) medium[9] and stored at 4 ºC until use. The medium was prepared in aged seawater and distilled water at a ratio of 3:1[10]. Identification of the test strains was carried out by its macroscopic and microscopic characteristics and confirmed identification as *Aspergillus candidus, Aspergillus flavus and Aspergillus niger* was carried out by the Agharkar Research Institute, Pune, India.

Biosynthesis and characterization of gold nanoparticles

One ml inoculum (spore suspension approximately 10^6/ml) was inoculated in 250 ml Potato Dextrose medium (prepared in 75% 'aged' seawater). The inoculated flasks were incubated at room temperature for 4 days. After incubation period, fungal biomass was separated from the medium by filtration and washed extensively with sterile distilled water.

Approximately 5g fungal biomass of each test isolate was challenged with gold chloride of different concentrations, 0.25mM, 0.5mM, 1mM, 2mM and 3mM. The gold chloride solutions were prepared in sterilized deionized water. The inoculated flasks were incubated at 27^0C for 72h under static condition. Negative and positive controls were also run along with the experimental ûasks.

Two ml sample from each flask was withdrawn at predetermined time intervals and the spectra were recorded in the range of 380 to 800nm at a resolution of 1 nm using UV–visible spectrophotometer (Elico BL-198). The experiments were carried out in triplicates [7].

Transmission electron microscopic (TEM) images of biosynthesized gold nanoparticles (mounted on carbon-coated copper grids) were obtained on JEOL (Model GEM 200).

RESULTS

All the test flasks containing biomass of *Aspergillus candidus* and gold chloride solution showed gradual colour change (pink-purple) in the reaction mixture within 18h, except the test flask containing 1mM gold chloride. In the flask containing 1mM gold chloride, biomass exhibited purple-violet coloration. Figure 1 shows spectrophotometric data of the biosynthesized GNPs. Figure 2 (a) shows TEM image of the GNPs biosynthesized by *A. candidus*.

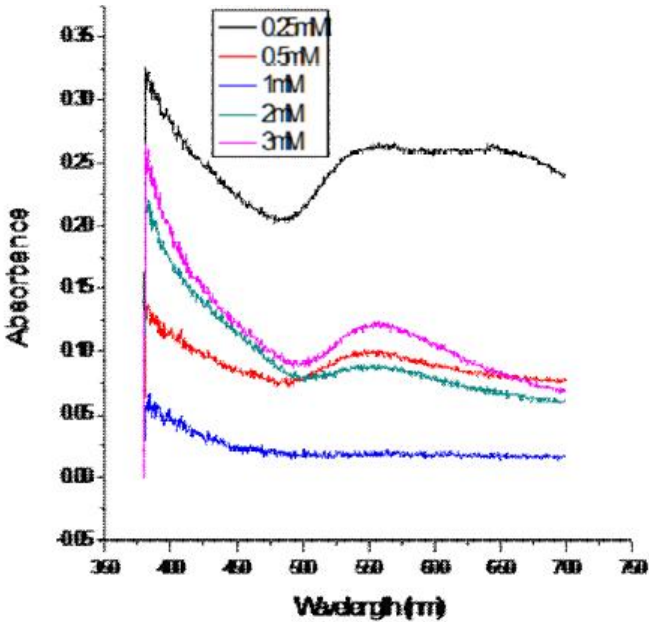

Figure 1 : Uv spectra of GNPs biosynthesized by *Aspergillus candidus* at different gold chloride concentrations

The test fungus *A. flavus* could also synthesize GNPs, here also, the biosynthesis was extracellular within 24h (data not shown).

In case of *A. niger*, gradual change in coloration of the reaction mixure was observed within 18h. Uv spectra of biosynthesized GNPs by *A. niger* are shown in Figure 3 while Figure 2(b) shows TEM micrograph of the GNPs. The test fungi produced GNPs in the size range of 9-37nm.

DISCUSSION

Coloration of the reaction mixture indicates extracellular biosynthesis of GNPs. When exposed to 1mM gold chloride, coloration of *A. candidus* biomass was observed indicating intracellular mode of GNP biosynthesis. Hence, all the three test fungi in general exhibited extracellular biosynthesis and *A. candidus* showed a different behaviour (intracellular biosynthesis) in certain case. Concentration dependent change in mode of

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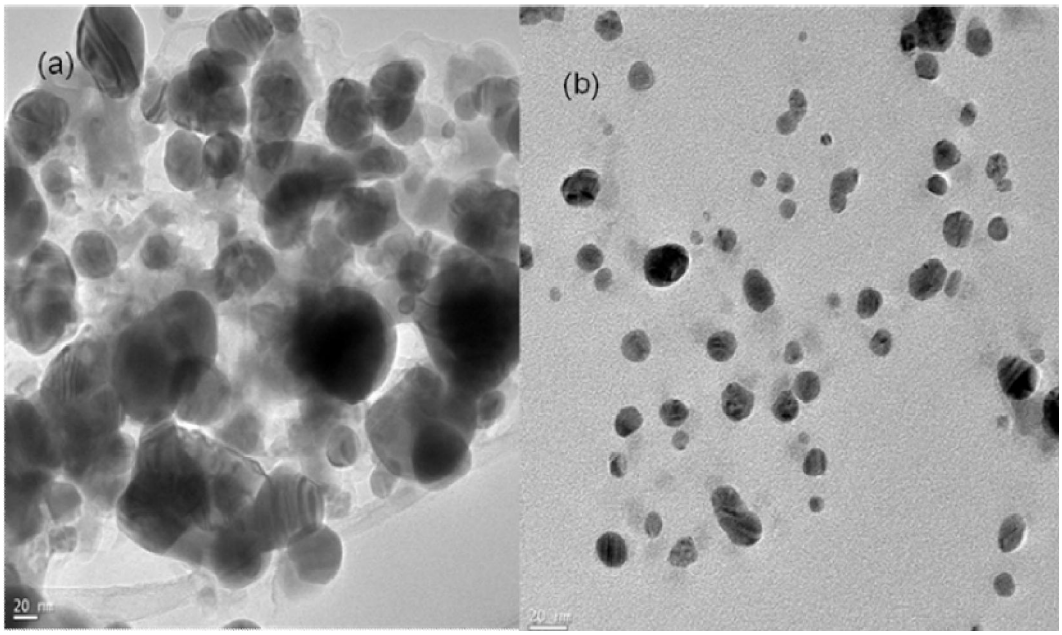

Figure 2 : TEM images of GNPs biosynthesized by (a) *Aspergillus candidus* and (b) *Aspergillus niger*

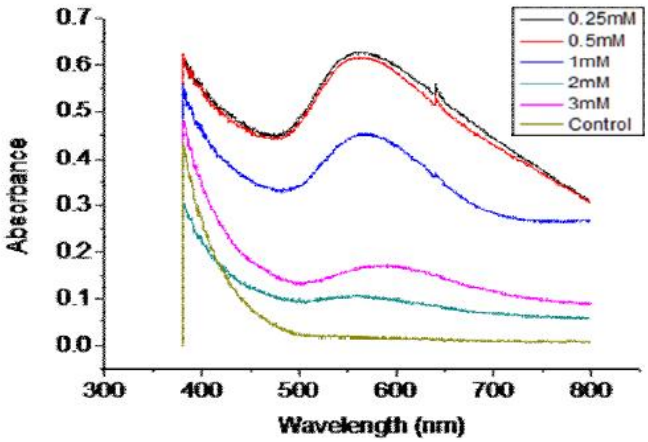

Figure 3 : Uv spectra of GNPs biosynthesized by *A. niger* at different gold chloride concentrations

biosynthesis has been observed by Vala[7]. Ahmad et al.[11] reported intra- and extracellular synthesis of gold nanoparticles by fungus Trichothecium. Sheikhlou et al [12] observed simultaneous intra-extracellular biosynthesis of gold nanoparticles by *Rhizopus oryzae*. UV–Vis spectrophotometry is one of the most extensively used techniques for characterization of gold nanoparticles, The observed surface plasmon resonance band (SPR) in Figures 1 and 3 is characteristic of GNPs, confirming the biosynthesis in all cases. Transmission electron microscopy offers a powerful tool for determination of shape and size of nanoparticles[13]. The TEM data (Figure 2(a,b)) revealed spherical shape of the particles. Spherical GNP production by marine-derived *Rhizopus oryzae* has been reported by Vala[7]. Shivshankar et al.[14] used geranium leaves (*Pelargonium graveolens*) and its endophytic fungus *Colletotrichum* sp. and reported that gold nanoparticles synthesized using the fungus were essentially spherical in shape while the particles grown using the leaves exhibited a variety of shapes.

The present study demonstrates the marine-derived fungi as potential resource for developing cost-effective green technology for biosynthesis of gold nanoparticles.

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