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Microbial biosynthesis of nontoxic gold nanoparticles

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ABSTRACT

We study the extracellular biosynthesis of gold nanoparticles (GNPs) using the fungal species *Aspergillus foetidus*. The formation of GNPs were initially monitored by visual observation and then characterized with the help of various characterization techniques. X-ray diffraction (XRD) results revealed distinctive formation of face centered cubic crystalline GNPs. From field emission scanning electron microscopy (FESEM) the morphology of the nanoparticles were found to be roughly spherical and within the size range of 30–50 nm. The spherical and polydispersed GNPs in the range of 10–40 nm were observed by transmission electron microscopy (TEM) analysis. It was established that alkaline pH, 1 mM gold salt concentration and 75 °C temperature were the respective optimum parameter for biosynthesis of GNPs. Cell cytotoxicity of GNP was compared with that of normal gold salt solution on A549 cell. The A549 cell growth in presence of GNPs was found to be comparatively less toxic than the gold ion.

1. Introduction

Developing a safe, reliable, and eco-friendly method for the synthesis of nanoparticles imposes immense challenge to researchers presently. Nanotechnology, the powerful tool for the creation of new objects in nanoscale dimensions, is a cutting edge technology having important applications in modern biomedical research [1–4]. As the dimension of nanoscale devices is similar to cellular components such as DNA and proteins [5,6], the tools developed through nanotechnology may be utilized to detect several diseases at the molecular level [7]. In particular, for applications in bio-nanotechnology, nanomaterials need to be biocompatible. Nanoparticles can be synthesized by following various physical and chemical processes [8]. The problems, often experienced with these methods are showing low stability of the nanoparticles, toxicity of chemicals, difficulty in controlling the crystal growth and aggregation of the particles [9–11]. Now a days, green synthesis of nanoparticles has drawn much more attention in the rapidly growing area of nanoscience and nanotechnology [12–16]. Utilization of cheap nontoxic chemicals, eco-friendly solvents and renewable materials are some of the key factors critical for the green synthetic process of nanomaterials. In this context, biological synthesis of nanoparticles as an emerging highlight mediating intersection of nanotechnology and biotechnology has received increasing attention. Biological systems like plants [17,18], bacteria [19], algae [20,21] and fungi [22–24] have been widely used to synthesize a range of inorganic nanomaterials. Many microbes are known to produce highly structured metallic nanoparticles with very similar properties to that of chemically synthesized nanomaterials, while having precise control over size, shape and monodispersity [25]. Fungi are advantageous over bacteria and algae, due to their high metal tolerance and bioaccumulation ability [26]. Additional benefits of using fungi in nanoparticle synthesis are downstream processing, easy to handle biomass, and economic viability. Supplemented to that, fungi are extremely efficient secretor of extracellular enzymes.

Among the all available metallic nanoparticles (NPs) now a days, GNPs has shown exceptional interest owing to its unique optical properties in conjugation with its low or no immediate toxicity toward biological material [27]. Presently, biosynthesis of GNPs has received a lot attention and emerged to be an active research area in the field of nanotechnology. Gold nanoparticles has derived tremendous interest over the last few decades as it possessing some remarkably novel properties such as intense plasmon resonance, electrical, magnetic, thermal conductivity, chemical and bio-stability, catalytic activity, antimicrobial activity, anti-HIV activity, anti-angiogenesis activity, anti- malarial agent, and anti-arthritic activity [28,29]. Gold nanoparticles are very useful due to their stability under atmospheric conditions, resistance to oxidation and biocompatibility [9,30,31]. These unique properties of

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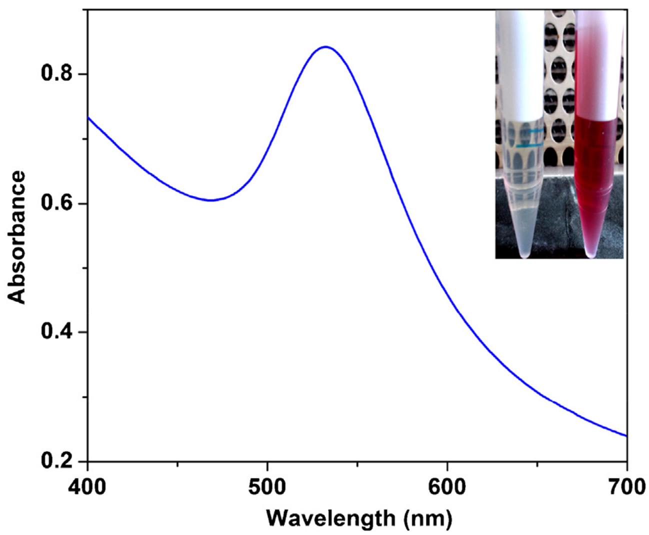

Fig. 1. UV–vis spectrum of *A. foetidus* mediated biosynthesized GNPs, Fig. 1 inset: The cell free filtrate of *A. foetidus* before (left) and after (right) exposure with $HAuCl_4$ (1 mM).

GNPs can potentially be used in a diverse range of industrial applications. Gold nanoparticles have strong affinity for S atoms provide GNPs the ability to have their surface modified in a variety of ways with S-containing compounds for diagnosis, sensing, and environmental applications [32–36]. Surface plasmon resonance (SPR) property of GNPs has been applied as sensors for detection of many targets including biological, environmental and military molecules [37–40]. In comparison with metallic nanoparticles, GNPs possess properties such as dielectric function, electrical conductivity, and inert property to oxidation to allow them to be used in applications as sinter inks, selective coatings, data storage, single electron conductivity, and quantum devices [41–44]. Extracellular biosynthesis of GNPs using different fungi like *Verticillium* sp [45], *Fusarium oxysporum* [46], *Aspergillus niger* [47], *Colletotrichum* sp [48], *Aspergillus clavatus* [25], *Trichothecium* sp [49] has already reported. Although bulk gold has been reported to be safe [50], but for biocompatibility and environmental impact GNPs are need to be studied well if they are to be manufactured on a large scale for in vivo usage [51]. So, further investigation and examination is needed in using GNPs as potential agents for therapeutic use. Earlier report indicated size of the GNPs plays a crucial role in inducing cytotoxicity in HeLa cells [52].

In this context, we present biosynthetic process for the production of GNPs using live cell filtrate of the fungal strain *Aspergillus foetidus*. Although the mentioned microbe i.e. *A. foetidus* was earlier investigated by our group for the biosynthesis of silver nanoparticles and its application [53–57], but there is no reports available regarding the biosynthesis of GNPs using cell filtrate of *A. foetidus*. The main objective of the present study is to develop the clean, non-toxic and eco-friendly method for obtaining GNPs and standardization of physical parameters for its synthesis, we also observed cytotoxic effect of biosynthesized GNPs on eukaryotic cell line (Human lung cancer cell line A549) as a model.

2. Experimental

2.1. Organism

A. foetidus MTCC8876 isolated from Kalyani waste water treatment Centre, Kalyani, West Bengal, Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India was used for the study of extracellular biosynthesis of GNPs.

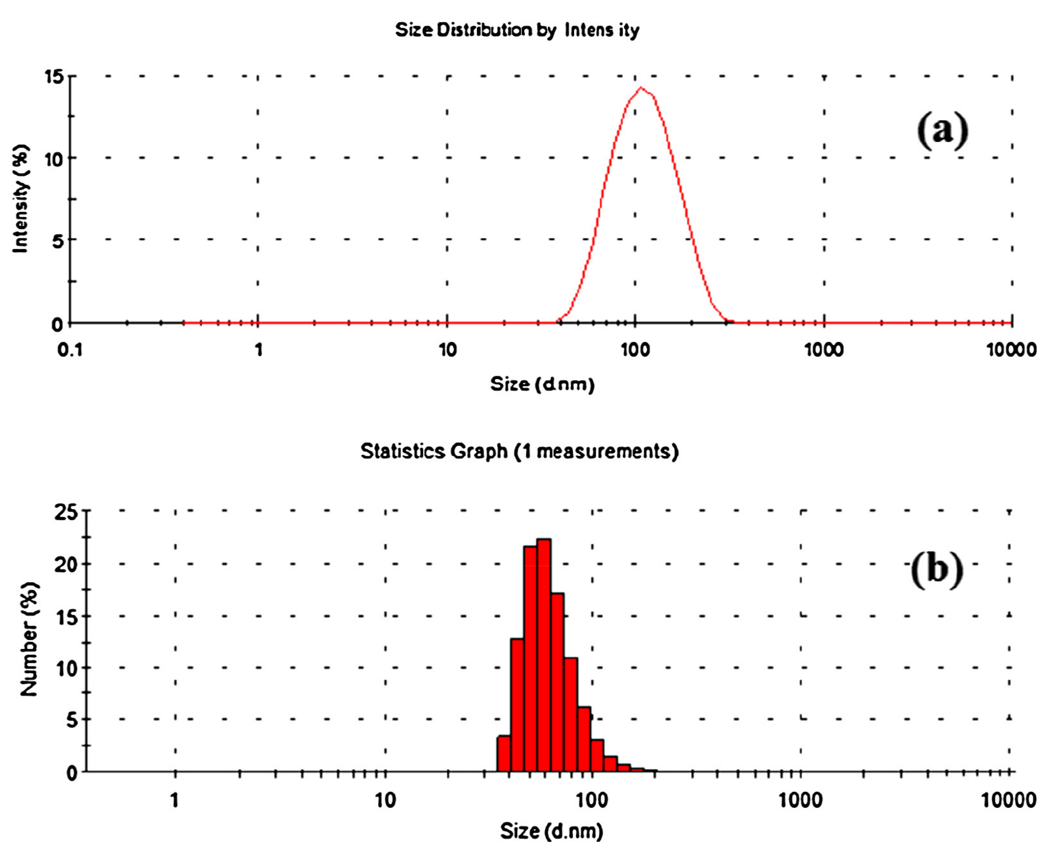

Fig. 2. Dynamic light scattering of biosynthesized GNPs showing the size distribution of nanoparticles by intensity and number in figure (a) and (b), respectively.