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Mycosynthesis of silver and gold nanoparticles: Optimization, characterization and antimicrobial activity against human pathogens

M.D. Balakumaran[a,*], R. Ramachandran[a], P. Balashanmugam[a], D.J. Mukeshkumar[b], P.T. Kalaichelvan[a]

[a] Centre for Advanced Studies in Botany, School of Life Sciences, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India
[b] Department of Microbiology, Asan Memorial College of Arts & Science, Jaladampet, Chennai 600 100, Tamil Nadu, India

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ABSTRACT

This study was aimed to isolate soil fungi from Kolli and Yercaud Hills, South India with the ultimate objective of producing antimicrobial nanoparticles. Among 65 fungi tested, the isolate, Bios PTK 6 extracellularly synthesized both silver and gold nanoparticles with good monodispersity. Under optimized reaction conditions, the strain Bios PTK 6 identified as *Aspergillus terreus* has produced extremely stable nanoparticles within 12 h. These nanoparticles were characterized by UV–vis. spectrophotometer, HR-TEM, FTIR, XRD, EDX, SAED, ICP-AES and Zetasizer analyses. *A. terreus* synthesized 8–20 nm sized, spherical shaped silver nanoparticles whereas gold nanoparticles showed many interesting morphologies with a size of 10–50 nm. The presence and binding of proteins with nanoparticles was confirmed by FTIR study. Interestingly, the myco derived silver nanoparticles exhibited superior antimicrobial activity than the standard antibiotic, streptomycin except against *Staphylococcus aureus* and *Bacillus subtilis*. The leakage of intracellular components such as protein and nucleic acid demonstrated that silver nanoparticles damage the bacterial cells by formation of pores, which affects membrane permeability and finally leads to cell death. Further, presence of nanoparticles in the bacterial membrane and the breakage of cell wall were also observed using SEM. Thus, the obtained results clearly reveal that these antimicrobial nanoparticles could be explored as promising candidates for a variety of biomedical and pharmaceutical applications.

1. Introduction

Decreasing the size of nanoparticles has pronounced effect on the physical properties that are significantly different from their corresponding parent materials. The strong relationship between size and properties of nanoparticles has offered countless opportunities for many scientific breakthroughs. The extremely surprising activities of nanoparticles have enormous potential for modern technology emphasizing their use in human wellbeing (Heiligtag and Niederberger 2013; Mullai et al., 2013). In recent times, the green chemistry procedure which utilizes microorganisms and plants for nanoparticles preparation has turned as a viable alternative to conventional physicochemical methods since it is facile, rapid, cost-effective, and environmentally benign. Use of microorganisms as cell factories for producing nanoparticles has received much scientific attention over the past decade. Recently, the microbe mediated synthesis method has emerged as a burgeoning area of research in the field of nanobiotechnology (Narayanan and Sakthivel, 2010).

Currently, an exhaustive study on biological synthesis of nanoparticles has been carried out using a wide array of microorganisms such as algae, bacteria, actinomycetes, fungi, yeasts, and viruses (Narayanan and Sakthivel, 2010; Thakkar et al., 2010). Among them, fungi are more advantageous because the fungal mycelial mesh can withstand flow pressure, agitation and other conditions in bioreactors or other chambers compared to plant materials and bacteria. They are fastidious to grow, easy to handle and synthesize nanoparticles (Gade et al., 2008; Thakkar et al., 2010). The fungal mediated extracellular synthesis method has attracted a great deal of interest owing to its simplicity, no further downstream processing, and lesser time consumption in contrast to intracellular synthesis (Mishra et al., 2011). In addition, the size and shape of extracellularly synthesized nanoparticles can also be manipulated by controlling pH, temperature, substrate concentration (metal ions), and reaction time (Krishnaraj et al., 2012; Sathishkumar et al., 2010). Although nanoparticles with controlled

* Corresponding author.
E-mail address: dakshinbala@gmail.com (M.D. Balakumaran).

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size and shape could be achieved through intracellular synthesis, product harvesting and recovery are laborious and expensive (Fayaz et al., 2010; Thakkar et al., 2010). Therefore, the extracellular synthesis methods could be ideally used for large scale production of nanoparticles for several industrial applications (Mishra et al., 2011). Importantly, fungi have also secreted fairly large amount of proteins and secondary metabolites extracellularly and hence, the fungal biomass could reduce the metal ions more easily leading to the rapid formation of nanoparticles (Gade et al., 2008). Because of these advances over other methods, the myco-based extracellular synthesis method is often considered a better resource for higher productivity of nanoparticles (Du et al., 2011; Muhsin and Hachim, 2014).

Many researchers have reported the extracellular synthesis of both silver and gold nanoparticles using a variety of fungi. In *Fusarium oxysporum*, the reduction of Ag^+ ions has occurred through the release of reductase enzymes (Ahmad et al., 2003). Silver nanoparticles have rapidly been produced using a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment of South India (Kathiresan et al., 2009). A fast growing and non-pathogenic fungus, *Trichoderma viride* has been used for silver nanoparticles preparation (Fayaz et al., 2010). Du et al. (2011) have reported that the culture supernatant of *Penicillium* species supported the extracellular production of gold nanoparticles within a minute. Interestingly, a few researchers have used isolated fungal components for extracellular production of nano-sized silver and gold particles. For instance, Apte et al. (2013) have prepared silver and gold nanostructured materials by employing L-DOPA-melanin isolated from *Yarrowia lipolytica*. Very recently, a nitrate reductase enzyme purified from *F. oxysporum* was also successfully used for the production of biologically active silver nanoparticles (Gholami-Shabani et al., 2014).

Increasing incidence of microbial resistance to clinically approved classes of antibiotics and the continuing emphasis on health care costs have made a strong push towards the development of new and effective antimicrobial agents (Goffeau, 2008). The remarkable antimicrobial activity of silver nanoparticles has been well-demonstrated against a broad spectrum of Gram-positive and Gram-negative bacteria including multi drug resistant human pathogens (Ingle et al., 2008). Silver nanoparticles, biologically synthesized from *Cryphonectria* sp., showed potent antibacterial activity against multidrug resistant *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Candida albicans* and also significantly enhanced the bactericidal activity of standard antibiotics (Dar et al., 2013). More recently, Mishra et al. (2014) have reported that gold nanoparticles derived from *Trichoderma* sp. showed their potential as a new generation antimicrobial agents. In addition, gold nanoparticles synthesized from *Penicillium brevicompactum* have also displayed *in vitro* cytotoxic activity against mouse mayo blast cancer cells (C_2C_{12}) (Mishra et al., 2011).

At this juncture, as a part of our continuing search to identify microorganisms with the potential to synthesize nanoparticles with amazing biological properties, interest has spurred on soil microbes residing in forest areas. To the best of our knowledge, only a few soil fungi have been reported to synthesize nanoparticles (Gade et al., 2008; Jain et al., 2011; Li et al., 2012; Muhsin and Hachim, 2014; Salunkhe et al., 2011). Indeed, soil fungi are relatively unexplored as a potential resource of novel bio-reductants for the extracellular synthesis of silver and gold nanoparticles. Even though a large number of studies have focused on the antimicrobial activity of biosynthesized nanoparticles, the search for new nanoparticles with distinct physicochemical and biological properties remains at the forefront of current nanobiotechnological research (Elbeshehy et al., 2015). Therefore, the present study was aimed to isolate soil fungi from the forest areas of Kolli and Yercaud Hills, South India with the ultimate objective of producing antimicrobial nanoparticles.

2. Material and methods

2.1. Chemicals

Silver nitrate and gold chloride were purchased from HiMedia Laboratories Pvt., Ltd, Mumbai, India. The human pathogenic microorganisms were obtained from the Microbial Type Culture Collection (MTCC) centre, Chandigarh, India.

2.2. Study area

In this present study, a total of 20 soil samples were collected from the different forest areas of Kolli Hills, Namakkal (11.42N and 78.57416E) and 18 soil samples were collected from Yercaud Hills, Yercaud (12.309N and 78.343E) in the Province of Tamil Nadu, South India. The soil samples were collected in sterile polypropylene bags and were brought to the laboratory for isolation of fungi.

2.3. Isolation of soil fungi

Ten grams of soil samples were suspended in 100 mL of sterile water and these suspensions were considered as 10^{-1} dilution. Serial dilutions were done and 10^{-4}, 10^{-5}, and 10^{-6} dilutions were used for obtaining pure culture using potato dextrose agar (PDA) medium. The medium was also amended with chloramphenicol (25 μg/mL) to minimize bacterial contamination. Following serial dilution, the Petri plates were incubated at room temperature (28 ± 2 °C) for 7 days. The Petri dishes were observed at regular intervals from the second day onwards for the fungal growth. Individual colonies of fungi were isolated and maintained on PDA slants.

2.4. Identification of soil fungi

The morphological identification of fungal isolates was determined by bright field microscopy observations of lacto phenol cotton blue stained fungal specimen at 40× magnification. The isolated fungal strains were identified on the basis of spore morphology down to genus level by standard mycological monographs (Nag Raj, 1993; Sutton, 1980). In this present study, a total of 65 different fungal isolates were obtained and identified.

2.5. Screening of soil fungi for mycogenic synthesis of silver and gold nanoparticles

All the 65 fungi were screened for the biogenic synthesis of both silver and gold nanoparticles. To prepare the biomass, the fungi were grown aerobically in potato dextrose broth (PDB) and were incubated at 27 °C for 7 days. After incubation, the profusely grown fungal mat was washed extensively using sterile double distilled water to remove the traces of medium components. Typically, 10 g (wet weight) of fungal mat was brought in contact with 100 mL sterile double distilled water in an Erlen Meyer flask and was kept under shaker condition (120 rpm) for 48 h at 27 °C. Then, the mycelial free filtrate was obtained by passing it through Whatman filter paper No. 1. The filtrate was reacted with known quantity of silver nitrate to yield an overall Ag^+ ion concentration of 10^{-3} M and the reaction was carried out in dark at room temperature. Concurrently, the mycelial free extract and silver nitrate solution were maintained as controls and the change in color was observed up to 48 h. For gold nanoparticle synthesis, gold chloride was added in the place of silver nitrate and the same conditions were provided (Fayaz et al., 2010).