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Toxicity of fungal-generated silver nanoparticles to soil-inhabiting *Pseudomonas putida* KT2440, a rhizospheric bacterium responsible for plant protection and bioremediation

Indarchand R. Gupta[a,b], Anne J. Anderson[c], Mahendra Rai[a,d,*]

[a] Nanobiotechnology Laboratory, Department of Biotechnology, S.G.B. Amravati University, Amravati 444602, Maharashtra, India
[b] Department of Biotechnology, Institute of Science, Nipat Niranjan Nagar, Caves Road, Aurangabad 431004, Maharashtra, India
[c] Department of Biology, Utah State University, Logan, Utah 84321, USA
[d] Laboratório de Química Biológica, Instituto de Química, UNICAMP, Cidade Universitária "Zefferino Vaz" Barão Geraldo, CEP 13083-970, Caixa Postal 6150, Campinas, SP, Brazil

HIGHLIGHTS

- This study incorporates the mycosynthesis of AgNPs and their characterisation by various methods.
- A first attempt demonstrating the toxicity assessment of AgNPs on beneficial soil microbe.
- Use of biosensor in *Pseudomonas putida* KT2440, gave accurate antimicrobial results.

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ABSTRACT

Silver nanoparticles have attracted considerable attention due to their beneficial properties. But toxicity issues associated with them are also rising. The reports in the past suggested health hazards of silver nanoparticles at the cellular, molecular, or whole organismal level in eukaryotes. Whereas, there is also need to examine the exposure effects of silver nanoparticle to the microbes, which are beneficial to humans as well as environment. The available literature suggests the harmful effects of physically and chemically synthesised silver nanoparticles. The toxicity of biogenically synthesized nanoparticles has been less studied than physically and chemically synthesised nanoparticles. Hence, there is a greater need to study the toxic effects of biologically synthesised silver nanoparticles in general and mycosynthesized nanoparticles in particular. In the present study, attempts have been made to assess the risk associated with the exposure of mycosynthesized silver nanoparticles on a beneficial soil microbe *Pseudomonas putida.* KT2440. The study demonstrates mycosynthesis of silver nanoparticles and their characterisation by UV–vis spectrophotometry, FTIR, X-ray diffraction, nanosight LM20 – a particle size distribution analyzer and TEM. Silver nanoparticles obtained herein were found to exert the hazardous effect at the concentration of 0.4 μg/ml, which warrants further detailed investigations concerning toxicity.

1. Introduction

Nanotechnology offers new applications in science and technology [1]. Synthesis of various nanomaterials is achieved by physical, chemical and biological methods. However, biological synthesis is a green approach, and therefore, has been a topic of attraction.

Out of the vast array of nanomaterials, silver nanoparticles (AgNPs) play a pivotal role particularly in biomedical applications. The use of silver ions as a bactericide was common upto 1940 [2–5]. The silver ions provide protection against a variety of microbial pathogens [6]. Colloidal silver solutions (CSSs) are effective in pharmacology, human and veterinary medicine, food industry, and water purification [7]. The development of nano-silver as antimicrobial compounds is an active area of research [8,9] because it has higher antimicrobial activity than its bulk counterpart [10].

Microbes and plants are remarkably effective in reducing metal ions to generate nanoparticles [11–18]. Among all microbes, the biosynthesis of nanoparticles by using fungi is preferred because

* Corresponding author at: Nanobiotechnology Laboratory, Department of Biotechnology, S.G.B. Amravati University, Amravati - 444602, Maharashtra, India. Tel.: +91 721 2662208 9x267; fax: +91 721 2662135/2660949.
E-mail addresses: mahendrarai@sgbau.ac.in, pmkrai@hotmail.com (M. Rai).

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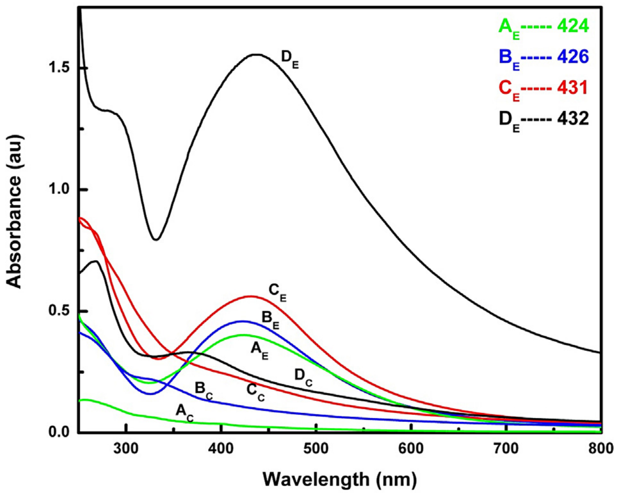

Fig. 1. UV–vis spectra of suspensions of AgNPs synthesised by filtrates of *F. tricinctum* (A), *F. culmorum* (B), *F. moniliforme* (C) and *P. glomerata* (D). The subscript E denotes spectra from the filtrate with Ag ions and C filtrates without added ions. The peak absorbance wavelengths (nm) for the preparations are shown in insert.

the culture is cost-effective and requires simple growth conditions. The fungal mycelia when starved release nitrate reductase into the surrounding medium. This enzyme reduces metal ions to nanoparticles [11,19].

AgNPs synthesized by fungi possess significant antimicrobial activity [11,12,20–27]. Efficacy of AgNPs produced by *Fusarium acuminatum* [19] was demonstrated against multidrug-resistant *Staphylococcus aureus, S. epidermidis, Salmonella typhi,* and *Escherichia coli.*

The production and application of nanomaterials increase the likelihood of environmental threat to microbes essential for ecosystem functions including biogeochemical cycles, degradation of pollutants, and the basis of food webs and soil health. *Pseudomonas putida* strain KT2440 is an example of the ubiquitous pseudomonads that thrive in the rhizosphere [28], providing plant protection [29], and participating in bioremediation of xenobiotic compounds [30]. *P. putida* KT2440 is a plasmid-free derivative of the original *P. putida* mt-2 isolate [31]. The isolate typifies many uses in biocatalysis [32]. Gajjar et al. [10] demonstrated dose dependent changes in energy status and culturability in a strain of *P. putida* KT 2440 engineered to act as an energy biosensor when treated with physically prepared AgNPs.

The aim of the present study was to evaluate the activity of mycosynthesized AgNPs against *P. putida* KT 2440. We have synthesized nanoparticles by filtrates of *Fusarium culmorum*, *Fusarium moniliforme*, *Fusarium tricinctum* and *Phoma glomerata*. In this study, we used biosensor to test the sensitivity of *P. putida* KT 2440 to mycosynthesized AgNPs.

2. Experimental

2.1. Fungi used for the synthesis of AgNPs

The fungal isolates *F. tricinctum (*MTCC-1978*), F. culmorum* (MTCC-1893) *F. moniliforme* (MTCC-7375), and *P. glomerata* (MTCC-2710) were used for the synthesis of AgNPs. These isolates were procured from the microbial type culture collection (MTCC),

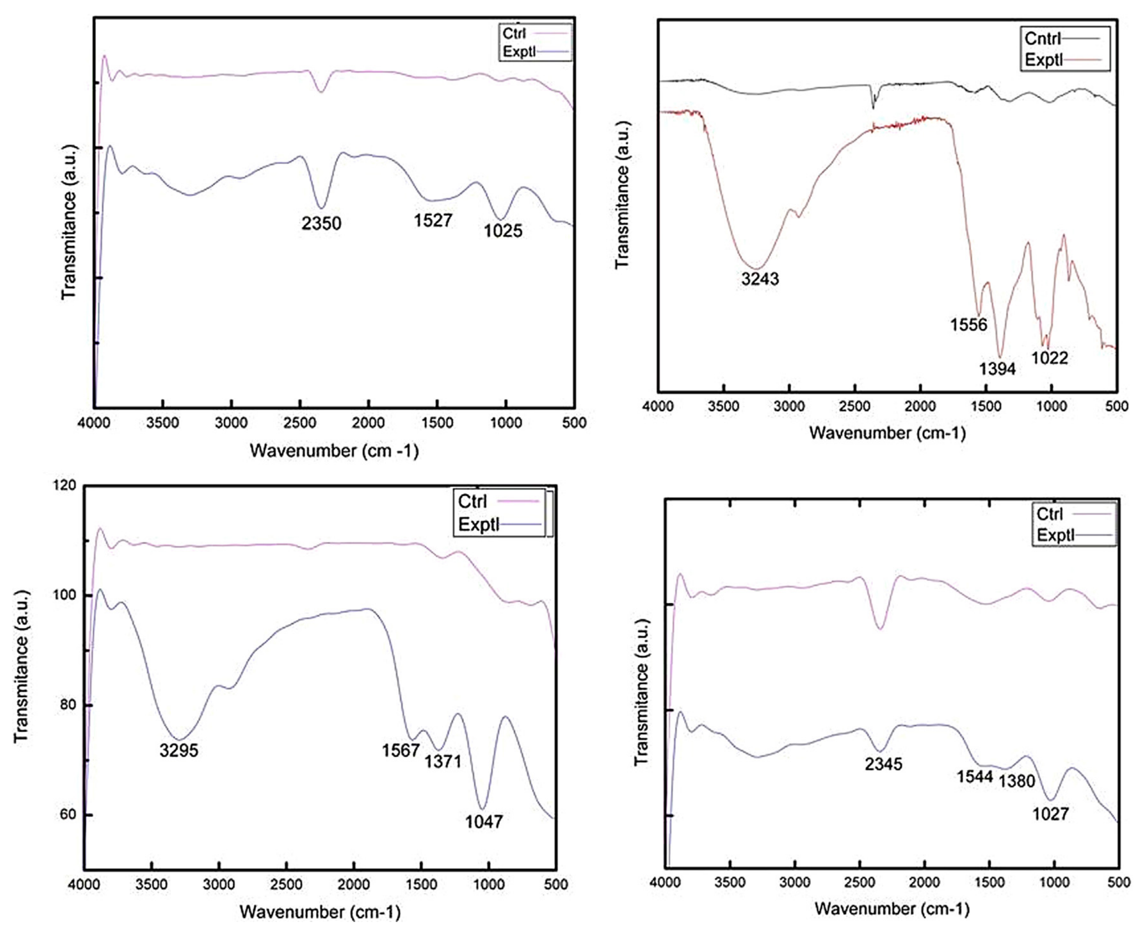

Fig. 2. FT-IR spectra for AgNPs prepared from filtrates from: (A) *F. tricinctum*, (B) *F. culmorum*, (C) *F. moniliforme*, and (D) *P. glomerata*.