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Effect of biosynthesized silver nanoparticles on the growth and some biochemical parameters of *Aspergillus foetidus*

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ABSTRACT

The biosynthesis of silver nanoparticles (AgNPs) has been carried out by using the extracellular filtrate of the fungal strain, *Aspergillus foetidus*. The synthesized nano particles have been characterized following different biophysical techniques. The results obtained from the studies of antifungal activities of AgNPs were found to be of utmost significance. Growth of *A. foetidus* both in liquid and solid CD media have been monitored in presence of the nanoparticles (0–40 ppm). Medium free dry biomass of the fungi was collected and emphasis has been laid on the assay of catalase activity, lipid per oxidation, thiol and protein content estimation and leakage of thiol, protein content. Growth of fungi in the presence of AgNPs was significantly inhibited in a dose dependent manner. Adherence of AgNPs on the fungal strain was realized by field emission scanning electron microscopy, and energy-dispersive X-ray spectroscopy, which indicated presence of silver nanomaterials in the surface of fungal mycelium. The growth of the fungus was found to be remarkably decreased with the increase in concentration of AgNPs and a considerable change in the activities and contents of all of the biochemical parameters considered indicated a plausible mechanistic mode of action of biosynthesized AgNPs establishing its antifungal activity.

1. Introduction

Since ancient times, silver and its compounds are known to be very effective antimicrobial agents [1–3]. In particular, because of the recent development in research on metal nanoparticles, nano silver particles have received immense attention as a possible antimicrobial agent [4–7]. Therefore, the synthesis of uniform nanosized silver particles with particular requirements in respect of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products [8,9]. Biological methods have been developed for the synthesis of these nanoparticles which eliminate the use of toxic chemicals during their synthesis process [10,11]. Silver nanoparticles have been widely used due to their physicochemical properties [12]. Among the all metallic nanoparticles, AgNPs have drawn prime attention because of their versatile applications [13–19]. AgNPs has been used in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. and it possesses low toxicity to human cells, high thermal stability and low volatility [20]. Even at low concentration AgNPs are capable of exhibiting antibacterial and antifungal activities. Hence, the demand of AgNPs as potent antimicrobial agents is gradually increasing. Silver has been utilized for much applicative purpose in pure free metallic or compound form due to possession of antimicrobial activity against pathogens, but at low doses it is less toxic to humans [21,22]. Silver ions are highly reactive, leading to inhibition of microbial respiration and metabolism as well as physical damage [23,24]. Moreover, it has been suggested that silver ions intercalate into bacterial DNA once entering the cell, which prevents further proliferation of the pathogen [25]. Though AgNPs has been used as an antimicrobial agent but it has some risks as the exposure to AgNPs can induce argyria and it can be toxic to mammalian cells [26].

The fungal strain *A. foetidus* had been used for the biosynthesis of AgNPs and the same had been characterized using different biophysical and biochemical techniques in our previous report [27,28]. Biosynthesized AgNPs were found to be effective antifungal agent [29–32]. The sizes of the biosynthesized nanoparticles are in the range of 20–50 nm and there are also few small particles of ~5 nm.

In general, *Aspergillus* species conidia dimension are in the range of 2–7 μm and cell wall thickness of *Aspergillus* species varies

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from 85 to 315 nm [33]. Though exact value of membrane pore diameter is not known but it may be only few nanometers and we do belief that not all but some the biosynthesized nanoparticles can cross the cell membrane and can shows the toxic effect. According to literature approximately 16 h after spore germination, the first phenotypic evidence of hyphal specialization within the colony becomes readily apparent and a new spore is formed and the asexual cycle can be reinitiated within about 24 h after the original spore germinates [34]. The growth and diameter of the fungus after apical extension of the conidiophore stalk ceases, the tip begins to swell and reaches a diameter of 5–10 mm after 96 h of growth.

Even though we are well familiar with the use of AgNPs as conventional antifungal agents but reports on the mechanistic aspect of this antifungal effect were not plenty. Previously, Dorau et al. [35] reported that AgNPs exhibit antimicrobial activity due to the formation of insoluble compounds by inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane bound enzymes and lipids resulting in lysis of the cell. Kim et al. [36] reported AgNPs may exert an antifungal activity by disrupting the cell membrane structure. We have used *A. foetidus* as a source of reducing agent and as well as stabilizing/capping agent for the biosynthesis of AgNPs. It is well known that AgNPs shows good antimicrobial activity and we have also tested the antimicrobial activity of our biosynthesized AgNPs. We have planned the present work and for this study we can select any type of strain but we did choose the same strain which we had used for the synthesis purpose because our aim is to show the effect of growth and some biochemical parameter of microbes in presence of nanoparticles. Now, in the featured work we have focused upon studying the effect of different concentrations of our biosynthesized AgNPs on the growth of *A. foetidus* in both liquid and solid media and also stressed on elucidating some intracellular biochemical parameters of *A. foetidus* under the same condition.

2. Materials and methods

2.1. Source of microorganism

The selected strain was identified from the Institute of Microbial Technology (IMTECH), Chandigarh and deposited as *Aspergillus foetidus* MTCC 8876. The same strain was used for the study of extracellular biosynthesis of AgNPs and to study the effect of AgNPs on the same strain.

2.2. Composition of growth media

Czapek–Dox (CD) broth medium was prepared as described by Raper and Thom (1949), [37] containing (per liter) KH_2PO_4 (1 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g), KCl (0.5 g), $FeSO_4$ (0.01 g), $ZnSO_4$ (0.01 g), glucose (40 g). The pH of the medium was adjusted to 6.8 before autoclaving by addition of suitable amount of 0.1 M NaOH solution. The medium was solidified with 2% agar as solid CD medium (CDA). All the plates were allowed to incubate at 30 °C in an incubator for 96 h for the fungal growth.

2.3. Biological extracellular synthesis of silver nanoparticles

2.3.1. Cell filtrate preparation

Spore suspension of *A. foetidus* (10^{11}conidia in one liter) was inoculated to liquid CD medium and incubated at 28 ± 2 °C for 96 h in an orbital shaker (120 rpm). The harvested biomass (~10 g/L) of *A. foetidus* was filtered and washed thrice with sterile distilled water to remove any medium component. 10 g of biomass was taken in a conical flask and 100 mL of Milli-Q deionized water was added. The mixture was agitated at 150 rpm and incubated at 28 ± 2 °C for 72 h and after filtration using Whatmann no.1 filter paper the live cell filtrate (LCF) was produced [38,39]. Then the LCF was used to reduce the Ag^+ to Ag°.

2.3.2. Biosynthesis of silver nanoparticles

A. foetidus has been exploited as the potential source for the extracellular biosynthesis of AgNPs. For biosynthesis of AgNPs at a 1 mM final concentration $AgNO_3$ was added together with 50 mL of cell filtrate in a 250 mL Borosil flask and agitated at 28 ± 2 °C in dark [27].

Green synthesis of nanoparticles has evolved as a cost effective, eco-friendly and an unparalleled alternative to chemical synthesis. The beauty of biosynthesis lies in the fact that this process does not require addition of a reducing agent and an external capping agent in order to confer stability to the biosynthesized nanoparticles as both the tasks are mediated by cellular proteins. The extracellular live cell filtrate so obtained during nanosynthesis has been used as an abundant source of protein. After processing the biosynthesis procedure has been optimized and estimation of concentration of biosynthesized nanoparticle has also been executed as reported in our earlier work [28]. Synthesized AgNPs have been characterized following biophysical and biochemical methods as mentioned in our earlier report [27]. UV–vis spectroscopic analysis, FTIR spectroscopic analysis, Particle size (DLS) analysis, Zeta potential measurement, AFM analysis, TEM & EDX analysis and Nitrate reductase assay. The detailed method of characterization, standardization of physiochemical parameters for the biosynthesis of AgNPs and its antifungal activity as well as estimation of concentration of biosynthesized nanoparticle have been described in our previous report [27,28,40].

2.4. Growth of the A. foetidus strain in liquid CD medium

A. foetidus was grown in a shaking incubator under aerobic condition. Liquid CD broth was used for the growth of the fungus; 1000 mL of sterile CD medium was transferred into a series of 500 mL conical flasks. The conical flasks were inoculated with spore suspension of the test strain (10^{11} conidia per liter) and shaken at 150 rpm at 30 °C in an orbital shaker. For enzymatic and biomolecular studies, five different concentrations of AgNPs 0, 5, 10, 20, 30, 40 ppm were added separately to the growth media. Biomass was harvested after 120 h growth period, filtered, washed with sterile de-ionized water and pre-weighed. Thereafter the biomass was stored at −20 °C until used.

2.5. Growth of the A. foetidus strain in solid CD medium

A. foetidus was grown in solid CD medium containing different concentrations of AgNPs (0–40 ppm) following point inoculation technique. All the plates were prepared in duplicate and incubated at 30 °C for one week.

2.6. Changes in pH of the spent media

The initial pH of the CD broth was maintained at 5.0 after autoclaving, followed by the addition of AgNPs for different treatment groups. The media were inoculated with the fungal spores and the biomass was harvested after a 120 h growth period. The pH of the spent media was measured for each treatment group.

2.7. Microscopic studies of the fungus

Lacto phenol–cotton blue staining technique was followed to stain the freshly grown mycelia and the morphology of the fungal strain in case of control and treated sample was analyzed under phase contrast microscope (Carl Zeiss).