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Antimicrobial activity of *Aspergillus niger* synthesized titanium dioxide nanoparticles

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ABSTRACT

Nanoparticles synthesised by fungi have various applications and is much effective than the nanoparticles synthesised by other means. The present study is aimed to determine the antimicrobial potential of Titanium dioxide nanoparticles synthesised from the fungus, Aspergillus niger. The synthesised nanoparticles were characterised using UV spectrophotometer, SEM, and XRD. The antibacterial activity of the nanoparticles was tested against Bacillus, Klebsiella, Pseudomonas, and Streptococcus sp. Maximum zone of inhibition was observed against Pseudomonas (21mm). Titanium dioxide nanoparticles were coated on 100% cotton fabric. The cotton fabric impregnated with TiO_2 was tested for its antifungal activity against Aspergillus niger and Trichoderma reesei. Maximum zone of inhibition was found to be 65 mm against T.reesi. Hence, fungi synthesized Titanium dioxide nanoparticles are proven to be effective antimicrobial agent. The characterization study supported to confirm the size of nano-TiO_2.

Keywords: Nano Titanium dioxide, *Aspergillus niger*, antibacterial activity, antifungal activity.

INTRODUCTION

Nanoparticles have been intensively studied in the past decade. Nanosized materials are considered to be an important subject in basic and applied sciences. Biological systems have well organized and controlled physiological processes, and thus, their use in the nanoparticle synthesis is rapidly gaining importance. Nanomaterials of different shapes and sizes have attracted considerable attention because of their unique physicochemical properties compared to the bulk materials. There are a considerable number of opportunities to utilize nanomaterials in modern clinical technology, the new concepts and phenomena, that have appeared in the field of NP research [1]. The number of antibiotic resistant microorganisms is rising along with the incidence of infections by these microorganisms. With this increase in health awareness, many people are focusing on educating and protecting themselves against harmful pathogens. New strategies are therefore needed to identify and develop the next generation of drugs or agents to control bacterial infections. The interaction of nanoparticles with microorganisms and biomolecules, is an expanding field of research, which as yet, is largely unexplored. Until now, several intensive studies have revealed, that some metal oxides like TiO2 and SiO2 [2]. CaO and CeO2 are also able to exhibit bacteriostatic potential [3][4]. Recent advances in the field of nanotechnology, particularly the ability to prepare highly ordered nanoparticulates of any size and shape, have led to the development of new biocidal agents. Several studies have indicated that nanoparticulate formulations can be used as effective bactericidal materials [5]. Nanoparticles with their large surface area to volume ratio have been studied to propose them as candidates for antimicrobial agents. The antibacterial activity has been observed to vary as a function of surface area in contact with the microbe; therefore nanoparticles with large surface area ensure a broad range of reactions with the bacterial surface [6].

MATERIALS AND METHODS

Synthesis of titanium dioxide nanoprticles and characterization

Biomass of *Aspergillus niger* was produced and the cell free filtrate was obtained, which was added to TiO_2 salt in concentration of 0.1 M (optimum salt concentration from our preliminary experiment). The entire mixture was put into shaker (150 rpm) at 28^{o}C and the reaction allowed for a period of 48hrs. The bio trans-formation particles were collected periodically and monitored for characterization. The synthesized nanoparticles were characterized using UV Visible Spectrophotometer in the range of 200 to 800nm. The size of nanoparticles was obtained by Scanning Electron Microscopy. Crystal structure, phase composition, phase purity and mean size of the nanoparticles were analysed by X-Ray diffraction spectroscopy [7] [8].

Antibacterial activity

Antimicrobial screening is generally performed by well diffusion method. *In-vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media. The MHA plates were prepared by pouring 15ml of molten media into sterile Petri plates. A suspension of 0.1% inoculums were swabbed uniformly and the inoculums were allowed to dry for 5 minutes. Wells were punched in the media and different concentrations of Titanium dioxide nanoparticles (10, 20, 30, 40µg) were added to the wells and incubated at 37^{0}C for 24 hours. Kanamycin was used as a positive control. At the end of incubation period, inhibition zones found around the discs were measured with transparent scale in millimetres.

Fabric treatment of titanium dioxide nanoparticles (Dip dry method)

The method of dipping was done by immersing the fabric material (100% Cotton woven) in the treatment bath containing the titanium dioxide nanoparticles for 20 mins at room temperature and then pulled up from the bath, followed by squeezing and the finished fabric was air-dried.

Antifungal activity assessment of TiO_2 coated fabric

The control and sample fabrics (sterilized) were placed in intimate contact with potato dextrose agar, which has been previously swabbed with broth suspension culture of test organisms (*Aspergillus niger and Trichoderma reesei*). At the end of incubation, the zone of inhibition formed around the fabric was measured in millimeters and recorded.

RESULTS AND DISCUSSION

Antibacterial activity of Titanium dioxide nanoparticles

TiO2 nanoparticles were sybthesized by *Aspergillus niger* and characterized by UV-Visible spectroscopy. 73.58 to 106.9 nm particles were observed [8]. Titanium dioxide nanoparticles were tested for their antimicrobial efficacy against bacterial cultures of *Bacillus, Klebsiella, Pseudomonas* and *Salmonella* by well diffusion method. Four concentrations of TiO_2 nanoparticles were used for the study. The results are shown in Table 1. The zone of clearance was significantly large in *Pseudomonas* inoculated petriplate, when compared to plates inoculated with *Bacillus, Salmonella* and *Klebsiella*. Maximum zone of inhibition was recorded against *Pseudomonas* (21 mm) at a concentration of 30 µg. The zones of inhibition in each plate reveal that TiO_2 nanoparticles have good inhibitory action against bacterial species. This may be due to the deactivation of cellular enzymes and DNA, by coordinating to electron-donating groups such as thiols, carbohydrates, amides, indoles, hydroxyls etc. This causes pits in bacterial cell wall which leads to increased permeability and cell death. Our results are in accordance with the previous study [9] [10].

Table 1: Antibacterial activity of TiO_2 NPs

S. No	Concentration of TiO_2NP (µg)	Zone of Inhibition (mm)			
		Bacillus	*Klebsiella*	*Pseudomonas*	*Streptococcus*
1.	10	16	15	18	12
2.	20	18	17	20	15
3.	30	16	16	21	16
4.	40	17	19	19	18
5.	Control(Kanamycin)	19	24	22	18

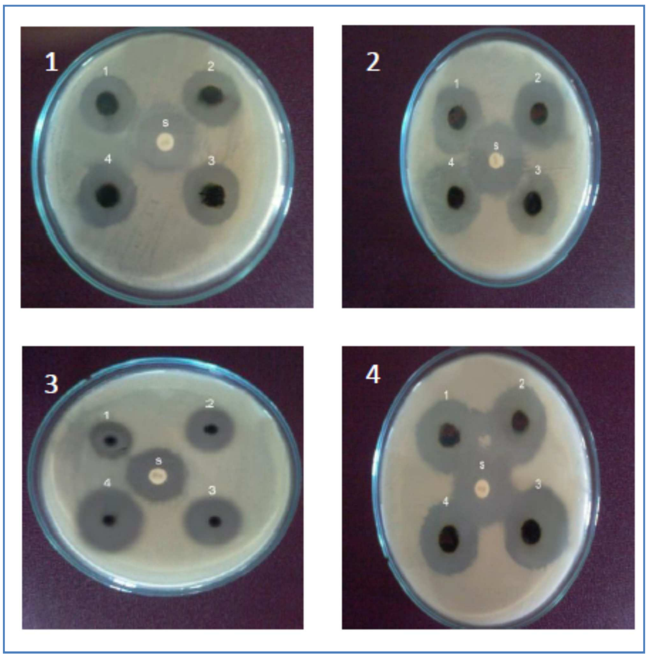

Figure 1 : Zone of inhibition in the plates treated with TiO_2 nanoparticles *(1) Bacillus, (2) Klebsiella, (3) Pseudomonas* and *(4) Streptococcus*

Antifungal activity of tio_2 treated fabric

The antifungal activity of the fabric treated with titanium dioxide nanoparticles against *Aspergillus niger* and *Trichoderma reesei* is represented in Table 2. A clear area of uninterrupted growth, underneath and along the side of the test material indicates antifungal effectiveness of the fabric. It can be noted that a strong antifungal activity against *Aspergillus niger* and *Trichoderma reesei* is manifested. The antifungal activity was slightly higher against *Trichoderma reesei* than *Aspergillus niger* (Figure 2).

Table 2 : Antifungal activity of TiO_2

S.NO	Sample	Zone of inhibition (mm)	
		A. niger	*T. reesei*
1	Titanium dioxide nano particles finished 100% cotton	60	65

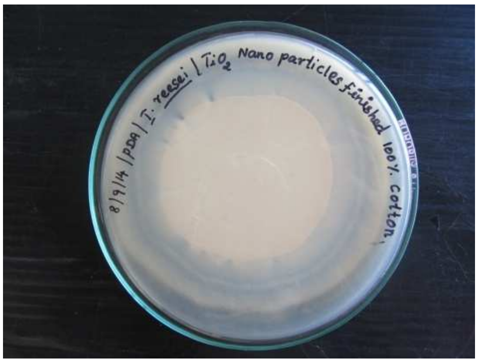

Plate A: *T. reesi*

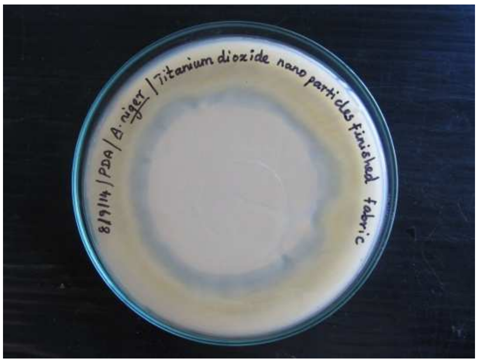

Plate B: *A.niger*

Figure 2: Antifungal activity of TiO2 Nanoparticles

According to several studies, it is believed that the metal oxides carry positive charge, while the microorganisms carry the negative charges and this causes electromagnetic attraction between microorganisms and the metal oxides, which leads to oxidation and finally death of microorganisms [11]. A strong binding of nanoparticles to the outer membrane of microorganisms causes the Inhibition of active transport, Dehydrogenase and Periplasmic enzyme activity and Inhibit the DNA, RNA and protein synthesis, due to which cell lysis occurs.

CONCLUSION

Highly pure TiO_2 NPs were prepared by *Aspergillus niger*. TiO_2 NPs exhibited excellent antimicrobial activity against both bacterial species and fungi. They might have potential for external uses as antibacterial agents in surface coatings on various substrates to prevent microorganisms from attaching, colonizing, spreading, and forming biofilms in indwelling medical devices. This study suggests that antimicrobial activity of TiO_2 NPs in different species of bacterial should be further investigated.

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