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Research Article

Biosynthesis of zinc oxide nanoparticles using *Aspergillus fumigatus* JCF and its antibacterial activity

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Abstract

Advancement of nanotechnology leads to the synthesis of nanomaterials with a well-defined dimensions and high mono-dispersity. An attractive possibility of green nanotechnology is to use microorganisms in the synthesis of nanoparticles. The present study reports the synthesis of zinc oxide nanoparticles using fungus *Aspergillus fumigatus* JCF. The production of zinc oxide nanoparticles was confirmed by the formation of white aggregates of zinc oxide nanoparticles. The optical properties of the nanoparticles were analyzed using UV–vis spectroscopy. The characteristic functional groups were analyzed using Fourier transform infrared spectroscopy. Particle size and morphology were analyzed using scanning electron microscope. Anti-microbial activity of zinc oxide nanoparticles towards both gram negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa and Escherichia coli*) and gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) were studied using well diffusion method.

Keywords: Zinc oxide nanoparticles; *Aspergillus fumigatus* JCF; Antimicrobial activity; Characterization.

Introduction

Nanotechnology is the act of purposefully manipulating matter at the nanoscale by designing and characterizing, the structures, devices and system (Cheng-Hsien, 2007). One of the most important criteria of nanotechnology involves in developing green and ecofriendly technologies for synthesis of various nanoparticles of variable size, shape, chemical composition and controlled dispersity. Metallic nanoparticles and metal oxide nanoparticles have wide biomedical application not only due to their large surface area to volume ratio but also of their high reactivity in comparison to bulk form (Absaret al., 2005).

Nanoparticles can be synthesized by physical, chemical methods and biological method (Jones et al., 2008). The use of toxic solvents and generation of hazardous by products makes green route synthesis of nanoparticles with high yield, low cost, non-toxic and environmental friendly properties more beneficial. The synthesis of nanoparticles employing microorganisms such as fungi, bacteria, yeast, algae and viruses have attracted much because of their role in remediation of toxic metals through reduction of metal ions and are considered as potential nano-factories (Mann, 1996). Eukaryotic organism such as fungi is an ideal candidate for the synthesis of metal nanoparticles, because of their ability to secrete large amount of enzymes (Castro-Longoria et al., 2010). Other advantages include metal bio accumulation ability, ease in the scale up process, economic viability, and ease in handling the biomass. Fungi give nanoparticles with good mono-dispersity and well defined dimensions (Zeinab et al., 2011).

Zinc oxide nanoparticles have a wide variety of application in many fields including medicine. It is used as chemotherapeutic agents (Wang et al., 2009; Hanley et al., 2008). It is also used as drug carrier and in the treatment of leukemia and carcinoma cancer cells. Apart from this, zinc oxide nanoparticle can be used as photocatalyst in photodegradation of environmental organic and toxic pollutants (Yumaket al., 2011). The advantage of using zinc oxide as antimicrobial agent is that they contain mineral elements essential for human and exhibit strong activity even administrated in small amount (Jeevalekshmi et al., 2012).

The present study was focused on the biosynthesis of zinc oxide nanoparticles using *Aspergillus fumigatus* JCF and characterization of synthesized nanoparticles. The anti-bacterial activity of biosynthesized zinc oxide

nanoparticles was studied towards *K. pneumoniae, P. aeruginosa, E. coli, S. aureus* and *B. subtilis*.

Materials and methods

Fungus used and growth condition

The fungus *A. fumigatus* JCF was isolated previously from vegetable waste. Fungus was characterized and sequence deposited in GenBank. The fungus was subcultured by growing on CzepakDox agar plate for 96 hrs at 32ºC and further refrigerated at 4ºC. All chemical used were analytical grade. *Aspergillus fumigatus* JCF was inoculated in a medium containing glucose - 40g/l, mycological peptone - 10 g/l, KCl - 4 g/l, $MgSO_4$ - 0.5 g/l, $FeSO_4$ - 0.010 g/l and the pH was adjusted to 6.5. The fungal cultures were incubated in an orbital shaker with 150 rpm at 32ºC for 96 hrs. After 96 hrs of incubation, fungal biomass was separated from broth by filtration followed by centrifugation at 3500 rpm at 10ºC for 20 min and the supernatant was used for the synthesis of zinc oxide nanoparticles (Dhoble and Kulkarni 2015).

Nanoparticle synthesis

10 ml of 1.0mM $ZnSO_4$ salt was added to 10 ml filtrate by adjusting the pH to 6.5 and incubated in orbital shaker for 150 rpm at 32ºC for 72 hrs. White precipitate deposition at the bottom of the flask indicated the formation of nanoparticles. White aggregate formed at the bottom of the flask was separated from the filtrate by centrifugation at 10,000 rpm for 10 min and lyophilized (Baskar et al., 2013).

Characterization of synthesized zinc oxide nanoparticles

Optical properties of the nanoparticles were analyzed using UV–vis spectroscopy. UV-vis spectrum was recorded on Systronics Double Beam UV-vis spectrophotometer 2201. The characteristics functional groups present in the molecule of synthesized nanoparticles were analyzed using Bruker α-T FT-IR spectrometer. The samples were mixed with KBrand were made into pellets at high pressure using hydraulic press and were scanned in the range of 400 to 4000 cm^{-1}.

Particle size and morphology of the synthesized zinc oxide nanoparticles were analyzed using scanning electron microscope (SEM) under Quanta 200 SEM, magnification range 35 to 30,000.

Antimicrobial Assay

Bacteria such as *K. pneumoniae, P. aeruginosa, E. coli, S. aureus* and *B. subtilis* were used for the study. Antimicrobial activity of zinc oxide nanoparticles towards gram positive and negative bacteria were studied using well diffusion method. Nutrient broth (1.3 g in 100 ml distilled water) was prepared and autoclaved. Each strain was inoculated separately in to the culture broth and incubated over night at 37 ºC. Nutrient agar was prepared and autoclaved. The agar was then poured into sterile plates and allowed to solidify. 50 µl fresh bacterial cultures were spread on the agar plate. Well of 5mm diameter was made in the plates using gel puncher. Wells were loaded with sample (containing zinc oxide nanoparticles). Procedure repeated for all the five bacterial strains. The plates were incubated for 2 days in an incubator at 37ºC. The zone of clearance was checked to find the antimicrobial activity of zinc oxide nanoparticles (Jeevalekshmi et al., 2012).

Results and discussions

Synthesis of zinc oxide aanoparticles

Zinc oxide nanoparticles were synthesized using a reduction of aqueous zinc sulphate solution with cell free filtrates of fungus *A. fumigatus* JCF at room temperature. The color was changed from yellow to white after 48 hrs, indicated the formation of nanoparticles as compared to the control in which no color change (Dhoble and Kulkarni, 2015). The color change was due to the surface plasmon resonances (SPR) effect followed by reduction of zinc ions by the proteins present in the filtrate which resulted in the formation of white aggregates of zinc oxide nanoparticles.

UV-Vis spectrum of zinc oxide nanoparticles synthesized by A. fumigates JCF

The produced zinc oxide was analyzed using UV-VIS spectrum. For the zinc oxide nanoparticles absorption peaks should be between 340-385nm.UV-vis spectroscopic studies shows the SRR, confirm the reduction of metal ions and formation of nanoparticles with peak at 350 nm (Sangeetha et al., 2012). Figure 1 shows the UV-Vis absorption spectra of zinc oxide nanoparticles.

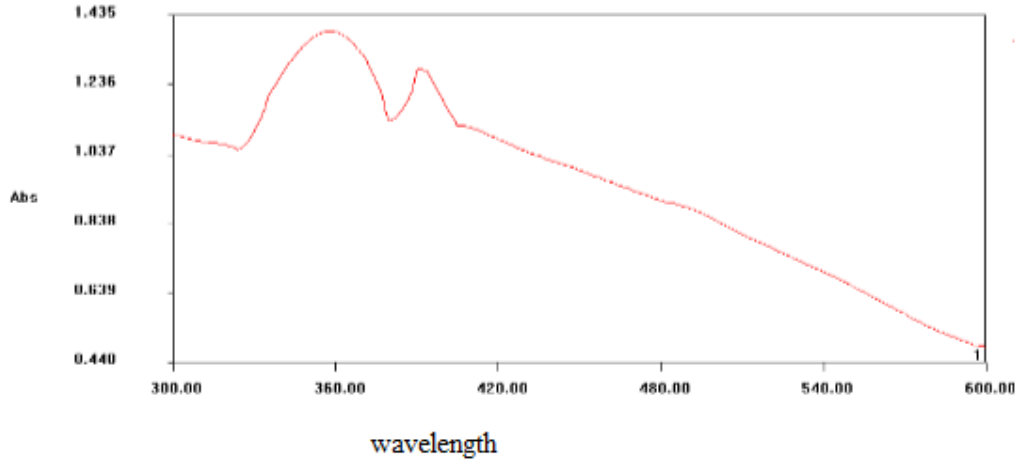

Figure 1. UV-Vis spectra of synthesized zinc oxide nanoparticles

FT-IR spectrum analysis of Zinc Oxide Nanoparticles Synthesized by A. fumigatus JCF

The synthesized zinc nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated with the synthesized nanoparticles. The peaks indicate the characteristics functional group present in the synthesized zinc oxide nanoparticles. It is inferred from Figure 2 that the samples have absorption peaks in the range of 3507, 3423, 2314, 2087, 1666, 1635, 1635, 1118, 992, 967, 794 , 601 and 524 cm^{-1}. The absorption peak at 524 cm^{-1} corresponds to metal-oxygen (ZnO stretching vibrations) vibrational mode. The peak at 1635 cm^{-1} is ascribed to the stretching vibration of N-H bond of primary amine, alkyl C=C stretch, open chain amino group. The peak at 1666 cm^{-1} is ascribed to carbonyl C=O stretch, alkyl C=C stretch, open chain amino group. The peak at 1118 cm^{-1} is ascribed to the stretching vibration of C-N bond of aliphatic amine, aromatic C-H in plane bend. The peak at 3423 cm^{-1} is ascribed to the stretching vibration of O-H bond of alcohols, phenols, aromatic primary amines. The peak at 3507 cm^{-1} is ascribed to the vibration of aromatic primary amines. The presence of these functional makes the synthesized zinc oxide nanoparticles as effective antimicrobial agent (Baskar et al., 2013).

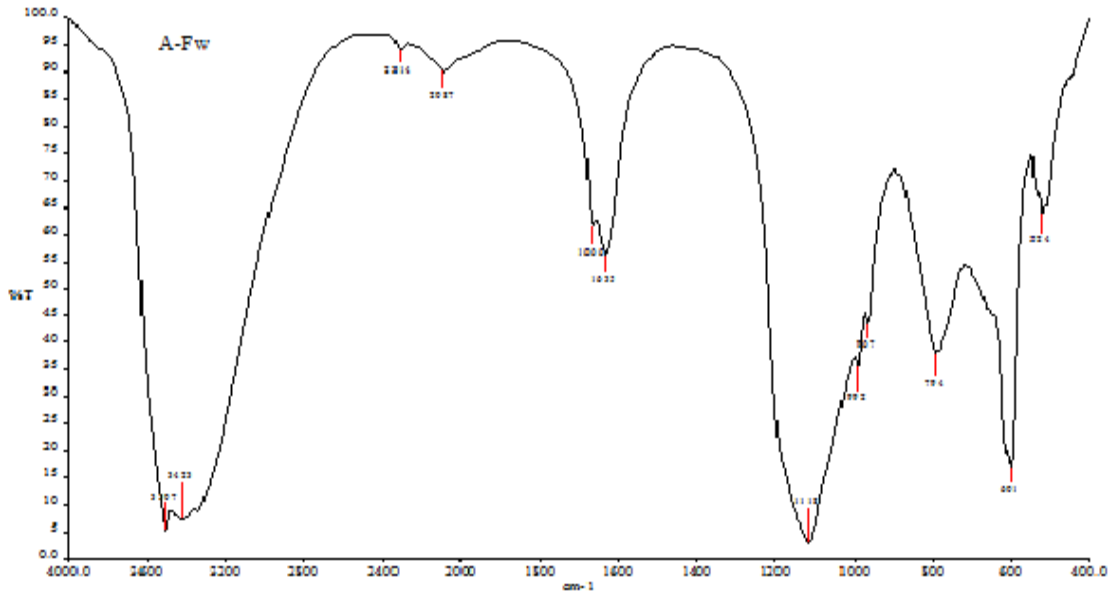

Figure 2. FT-IR spectra of synthesized zinc oxide nanoparticles

Structural characterization of zinc oxide nanoparticles using SEM

The SEM images of zinc oxide nanoparticles synthesized by *A. fumigatus* JCF are shown in the Figure 3. It can be inferred that the particles are spherical shaped with an average size of 60-80 nm (Baskar et al., 2013).

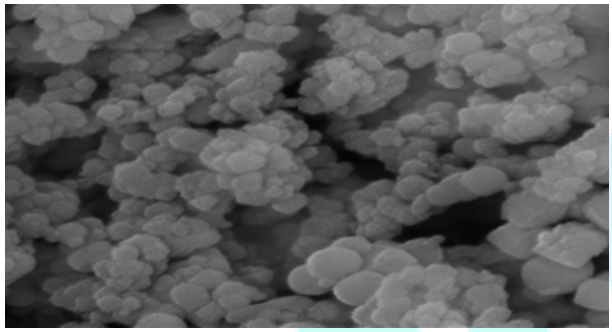

Figure 3. SEM image of synthesized zinc oxide nanoparticles

Antimicrobial activity of zinc oxide nanoparticles synthesized by A. fumigatus JCF

The effect of zinc oxide nanoparticles on *K. pneumoniae, P. aeruginosa, E. coli, S. aureus* and *B. subtilis* were studied using well diffusion method.

The zone of inhibition was observed in all the plates from which it is inferred that zinc oxide nanoparticles have antimicrobial activity. The antimicrobial activity of zinc oxide nanoparticles is mainly due to the generation of highly reactive species like OH^-, H_2O_2, and O_2^{2+}. H_2O_2 penetrate the cell membrane. OH^- and O_2^{2+}damage the cell membrane and cell wall from outside. The effect of zinc oxide nanoparticles on growth of bacterial strain is shown in Figure 4. The values of zone of inhibition obtained from the assay are presented in the Table 1.

Among gram positive bacteria, the diameter of inhibition zone produced by zinc oxide nanoparticles against *S. aureus* showed significant increase as compared to *B. subtilis*. Among Gram negative bacteria, the diameter of inhibition zone produced by zinc oxide nanoparticles against *K. pneumonia* showed significant increase as compared to *E. coli* and *P. aeruginosa*. Zinc oxide nanoparticles exhibit strong antibacterial activity on both Gram positive and Gram negative bacteria (Jones et al., 2008; Sawai, 2003).

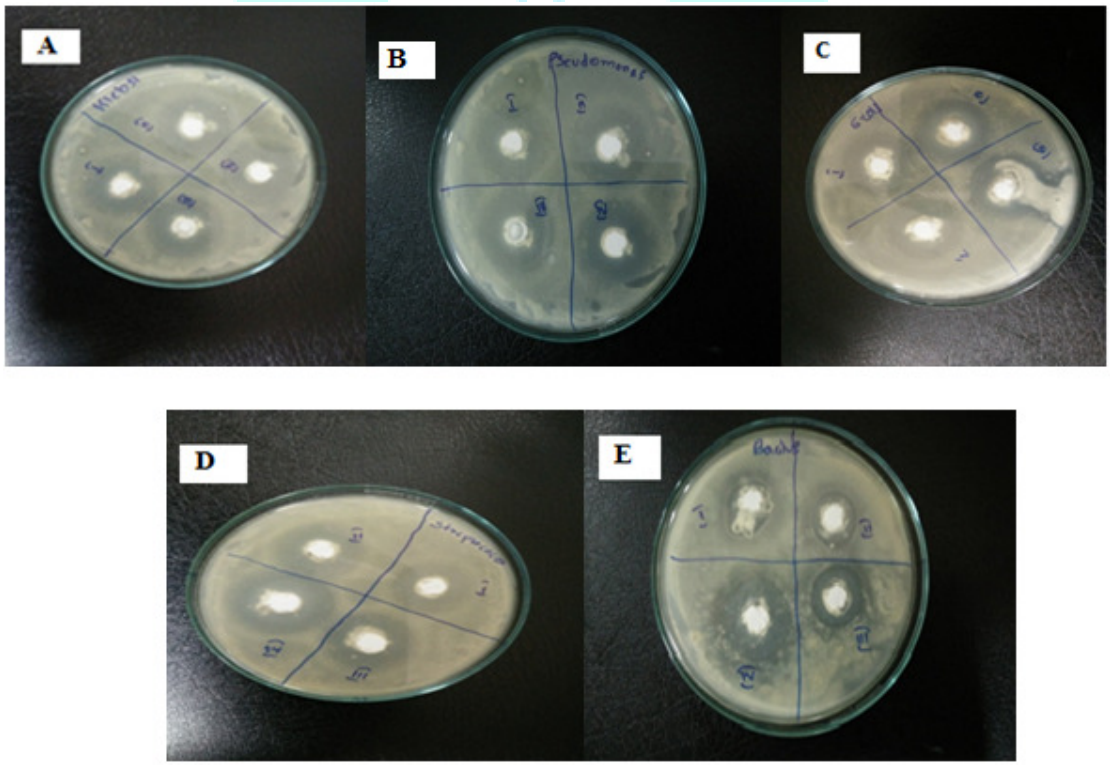

Figure 4. Antimicrobial activity of synthesized zinc oxide nanoparticles against (A*) K. pneumonia, (B) P. aeruginosa, (C) E. coli, (D) S. aureus,(E) B. subtilis* at different concentration

Table 1. Antibacterial activity of *A. fumigatus* JCF synthesized zinc oxide nanoparticles at different concentration (I-250 µg/ml), (II-500 µg/ml), (III-750 µg/ml), (IV-1000 µg/ml) against pathogenic bacterial species

Label	Bacteria	Zone of Inhibition (mm)			
		I (250 µg/ml)	II (500 µg/ml)	III (750 µg/ml)	IV (1000 µg/ml)
A	*K. pneumonia*	25	27	29	30
B	*P. aeruginosa*	20	23	25	27
C	*E. coli*	20	22	23	25
D	*S. aureus*	20	25	28	30
E	*B. subtilis*	15	17	19	20

Conclusions

The above study demonstrated the synthesis of ZnO nanoparticles using *A. fumigatus* JCF. Zinc oxide nanoparticles synthesized by *A. fumigatus* JCF was confirmed with a peak have shown peak at 350 nm. Zinc oxide nanoparticles of spherical structure were analyzed using SEM. The functional groups present in the zinc oxide nanoparticles was confirmed by FT-IR analysis. Antimicrobial study revealed that synthesized zinc oxide nanoparticles are effective antimicrobial agent against pathogenic microorganisms.

Conflict of Interest

Authors declare there are no conflicts of interest.

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