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Studies on the Inhibitory Activity of Biologically Synthesized and Characterized Zinc Oxide Nanoparticles using *Lactobacillus sporogens* against *Staphylococcus aureus*

Mudit Mishra, Jeny Singh Paliwal, Suneet Kumar Singh, E Selvarajan, C.Subathradevi and V. Mohanasrinivasan*

School of Bio Sciences and Technology, VIT University, Vellore - 14, India.

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In present scenario the science and engineering of nanosystems is one of the most challenging and fastest growing sectors of nanobiotechnology. Biologically synthesised nanoparticles are the most efficient miniaturized functional materials that are constructed and engineered to exert specific functions with enormous ability. Micro-organisms have this extraordinary capacity to form such exquisite nanostructures. This research work reports the biological synthesis of zinc oxide nanoparticles using a probiotic bacteria *Lactobacillus sporogens*. To ascertain the formation of zinc oxide nanoparticles X-Ray Diffractometer (XRD), Fourier Transform Infra-Red Spectroscopy (FTIR) and UV-Visible Spectroscopy were performed. XRD analysis indicated that the zinc oxide nanoparticle has hexagonal unit cell structure with the average particle size of 145.7 nm. The synthesised nanoparticles were found to be effective against *Staphylococcus aureus*.

Key words *Lactobacillus sporogens*. Zinc oxide, Biogenic nanoparticle, Inhibitory activity.

Nanotechnology and nanomaterial's is a very promising technology that provide a broad range of novel uses and also has a huge potential in the field of biomedical and life sciences. The properties of a material are greatly altered because of the increase in surface to volume ratio that leads to increase in the dominance of the behaviour of atoms on the surface to that of in the interior of the particle. There are a variety of ways to synthesize different types of nanoparticles, it could be a physical, chemical, biological and even hybrid methods. Physical and chemical methods are popular methods of synthesising nanoparticles but because of their toxicity levels their application is limited and these methods are costly too. K. M. Reddy et al 2007 demonstrated that the Introduction of ~13 nm ZnO NP kills gramnegative *E. coli* at concentrations 3.4 mM, whereas growth of gram-positive *S. aureus* was prevented at much lower concentrations (1 mM). Importantly, human T-cells are considerably more resistant to NP toxicity than either *E. coli* or *S. aureus*. These findings suggest that ZnO NP may potentially prove useful as nanomedicine based antimicrobial agents at selective therapeutic dosing regimes. Therefore a method was needed for the synthesis of nanoparticles which would be cheaper, non-toxic and eco-friendly. For such purpose microorganisms can be exploited to synthesise nanoparticles. Nanoparticles which are produced by "biogenic" enzymatic process have varied applications and they can be readily used in clinical areas also. Bacteria and fungi have been naturally bestowed with this property of reducing or oxidising metal

* To whom all correspondence should be addressed.
E-mail: v.mohan@vit.ac.in

ions into metallic or oxide nanoparticle functioning as "mini nanofactories" (Park *et al.,* 2009; Wang 2008).

In this research the Gram positive, mesophilic, facultative anaerobe *Lactobacillus* has been used for the biogenic synthesis of Zinc oxide nanoparticles and it is one of the beneficial microbes present in the human intestinal tract and inhibits the growth of pathogenic bacteria. Zinc oxide is an interesting material having a wide spectrum of applications such as that of a semiconductor ($Eg = 3.37eV$), magnetic material, electroluminescent material, UV absorber, piezoelectric sensor, nanostructure varistor, field emulsion displaying material, thermoelectric material, gas sensor, constituents of cosmetics (Wang 2008; Botello-Méndez *et al.,* 2008; Grigorjeva *et al.,* 2008; Daneshvar *et al.,* 2008; Lee *et al.,* 2006; Tong *et al.,* 2004). And apart from these applications zinc oxide nanoparticles can also be used for selective destruction of tumour cells and has a great potential in drug delivery applications (Rasmuessen *et al.,* 2010).

As Zinc oxide nanoparticles have selective cytotoxic action on proliferating cells whether it is benign or malignant (Tacolla *et al.,* 2011). Apart from these many applications, Zinc oxide due to its low toxicity is listed as "Generally Recognised As Safe" (GRAS) by the US Food and Drug Administration (21 CFR 182.8991). As it shows antimicrobial activity it has been integrated into the linings of cans used for packaging of various food materials. Due to high surface area to volume ratio of nanoparticles it allows Zno nanoparticles for better interaction with bacteria. Recent studies have shown that nanoparticles exhibit minimal effects on human cells in comparison to microbes against which they show selective toxicity (Reddy *et al.,* 2007). Here in this research we are also investigating the inhibitory activity of Zinc oxide nanoparticles against a food borne pathogen *Staphylococcus aureus.*

MATERIALS AND METHODS

Materials

Tablets of Lactobacilli were procured from a local pharmacy. The chemicals such as Zinc sulphate and Sodium hydroxide were procured from the Hi-Media laboratory, Mumbai. Deionised water was used through out the experiment.

Biosynthesis of Zinc oxide nanoparticles

Sterilized 50 ml MRS broth was prepared and tablet containing spores of Lactobacilli was added. The culture solution was incubated at 37°C for 24 h. To the 250 ml flask, 50 ml each of 0.1 M zinc sulphate and 0.4 M sodium hydroxide were added. And then this mixture was added to the 50 ml of culture solution followed by vigorous shaking and then heating at 40°C for 15 minutes. After this the flask was kept in microwave oven for 1-2 minutes, followed by 1 h cooling that allowed the nanoparticles to settle down. The formation of nanoparticles was confirmed by observing the white colour deposition at the bottom of flasks. After this deionised water was added to nanoparticles and they were transferred to the centrifuge tubes and centrifugation was done at 3000 rpm for 10 minutes and this was repeated a number of times. After every centrifugation the pellet was washed properly with deionised water. Finally the pellet was collected in a small plate and it was kept for drying in oven at 40°C for 8 h till it was totally dry and Zno nanoparticle was obtained in powdered form.

Characterization

Determination of Absorption Maxima for the Zinc oxide nanoparticles using UV-Visible Spectroscopy

For the initial characterization of biologically synthesised ZnO nanoparticles UV-Visible spectroscopy was carried out. The reduction of ZnO nanoparticles was monitored from 200-600 nm on the Hitachi double beam spectrophotometer after dispersing the nanoparticles with deionised water.

X-Ray Diffractometer (XRD) of Zinc oxide nanoparticles

To ascertain the formation of Zno nanoparticles they were characterized by X-ray diffraction (XRD Technique using an X-Ray diffractometer (Bruker Germany, D8 Advance, 2.2 KW Cu Anode, Ceramic X-Ray) with CuKα radiation (λ=1.5406A and operating at 35kV and 30 mA, XRD θ-2¸ patterns were recorded in the 2θ range 0-70° at the scan rate of 2.4°/min

Fourier Transform Infrared Spectroscopy (FTIR) of Zinc oxide nanoparticles

By employing the FTIR spectrophotometer structural information about the various

vibrational modes can be obtained. Zno nanoparticles were embedded in KBr matrix and pellets were prepared using hydraulic press. The FTIR spectra were recorded by scanning the samples in the range of frequency 400-4000 cm^{-1} at the resolution of 4 cm^{-1}.

Method for Inhibitory Activity

Materials used for the inhibitory activity of Zinc oxide nanoparticles were nutrient broth, zinc oxide nanoparticle sample; culture of *Staphylococcus aureus* NCIM No.2079 was collected from the National Collection of Industrial Microorganisms (National Chemical Lab) Pune, India. Method employed for evaluating the inhibitory activity was Growth inhibition in liquid medium.

Growth Inhibition In liquid medium

The inhibitory effect of zinc oxide nanoparticle was studied in liquid nutrient medium. Stock solution of ZnO nanoparticles was prepared by dispersing zinc oxide nanoparticles with autoclaved deionised water by ultrasonication. Aqueous dispersions of zinc oxide nanoparticles of concentration 20 mM were made. The flasks were marked as blank that contained only the nanoparticle, control (standard) that had the bacterial culture only and the test which contained the nanoparticles and *Staphylococcus aureus* cultures both. The freshly prepared inoculums were allowed to grow in the presence of zinc oxide nanoparticle of concentration 20mM to observe the bacterial growth pattern at 310k (37°C) and 150 rpm. In liquid medium growth of *Staphylococcus aureus* growth was indexed by measuring the optical density (O.D). Optical density measurements were carried out at » max = 600nm after every one hour interval up to 24 hours. Graph was plotted to interpret the results.

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

As the size of the particles is greatly reduced so the optical properties of Zno nanoparticles become immensely important. Here we investigated the radiative absorptions of the nanoparticles using the UV-Visible spectroscopy. Fig. 1 shows the optical absorption region due to Zno nanoparticles appearing in the range of 300-350 nm. This region indicates the size distribution of nanoparticles. To measure the band gap the peak position is ascertained by differentiating (ie. d(Abs)/dE) the experimental plot in the range of 250-400 nm to get a peak position of approximately 315 nm which corresponds to the band gap of 3.93 eV. This represents the blue shift in the band gap of zno nanoparticles from its bulk band gap energy 3.20 eV which is less (Hu and Chen 2008). The increase in the band gap is due to the decrease in the crystallite size which is attributed to size confinement.

XRD Analysis of Zinc oxide nanoparticles

Fig 2 depicts the broadening of XRD characteristic lines for Zno which reflects the formation of crystalline Zno nanoparticle. By recording the full width of half maxima (FWHM) of any one of the most prominent peaks using the software Origin 8 Evaluation, the average size of the nanocrystallites is determined using the Debye Scherrer equation (D=dl)b Cos y), k=0.99) for spherical particles). (Cullity 1978; Azaroff 1968) The prominent peaks and planes confirm the formation of hexagonal zinc oxide phase (JCPD 00-36-0451). The lattice parameters are a=3.24982 and here a=b and. The estimated average size of ZnO nanocrystal is 145.7 nm.

FTIR Analysis of Zinc oxide nanoparticles

As the surface to volume ratio for the nanoparticles is higher than their bulk counterparts because more atoms/molecules are arranged on the surface of nanoparticles therefore the surface chemistry of these particles are of great interest (Cao 2004). To assess the presence or absence of the various vibrational modes of Zno nanoparticles FTIR Spectroscopy was performed.

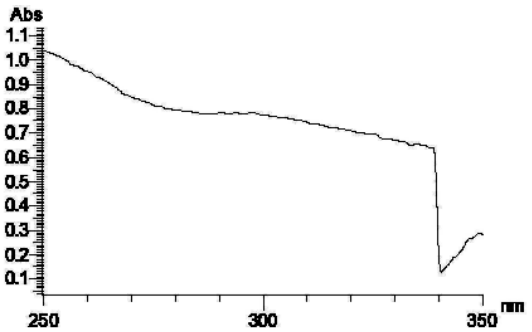

Fig. 1. UV–visible absorption spectra of the ZnO nanoparticles

Fig 3 shows an intense wide peak at 3396.9 cm^{-1} and 1480 cm^{-1} appear typically due to the stretching and banding modes of hydroxyl (O-H) group of H, O (Nakamoto 1997). The presence of these peaks clearly indicates that despite the heating and drying of samples moisture could not be completely removed from the samples. The bands at 2925.47 cm^{-1} correspond to the aliphatic CH2 stretch (antisymmetric). The bands at 1645 cm^{-1} and 1028.30 cm^{-1} corresponds to C=C stretching and –C–O– and –C–C– stretch. The peak at 462.25 cm^{-1} is the characteristic or the signature absorption of ZnO bond (He 2005; Nyquist 1997). Thus the FTIR results confirm the formation of ZnO nanoparticles.

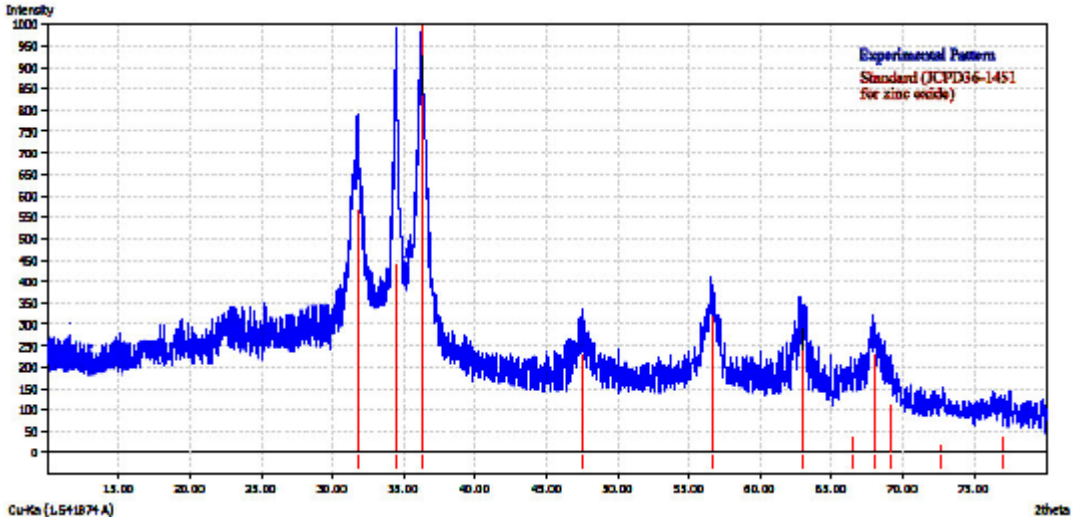

Fig. 2. X-ray diffraction pattern of ZnO particle in powdered form.

Growth Inhibition in liquid medium to determine inhibitory activity of Zinc oxide nanoparticles

Optical density (O.D) measurements were indexed and turbidity was observed to evaluate the inhibitory activity of ZnO nanoparticles. Growth of *Staphylococcus aureus* (NCIM 2079) was inhibited by the ZnO nanoparticles in the liquid medium. Here the Fig. 4 clearly shows the difference in turbidity in the two flasks. The test flask which contained both the nanoparticles and the organism was found to be less turbid in comparison to the standard (control). The standard (control) that had only the organism was turbid and O.D. readings indicated that there was growth. Graph shown in Fig. 5 was plotted to determine the growth pattern of *Staphylococcus aureus* (NCIM 2079) in the presence and absence of nanoparticles.

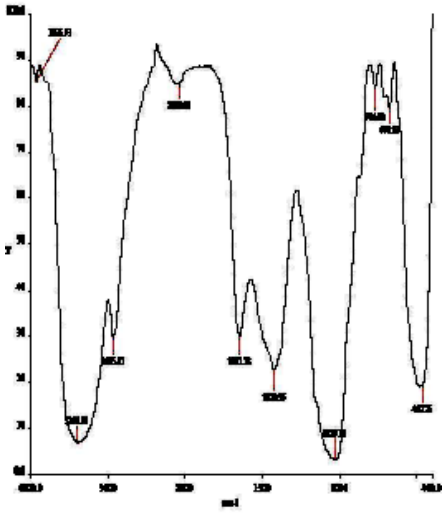

Fig. 3. FTIR spectrum of the ZnO nanoparticles

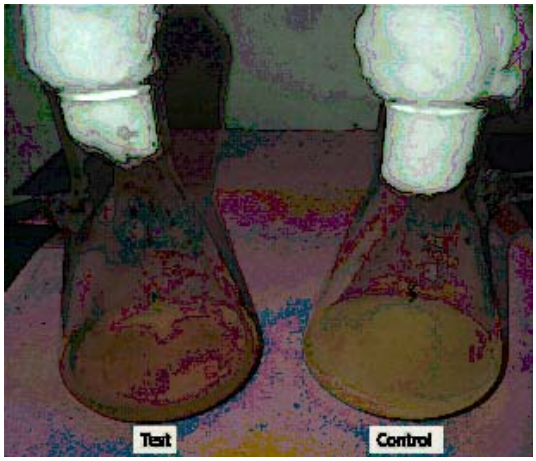

Fig. 4. (left) Test showing no turbidity due to the inhibitory action of ZnO nanoparticles and (right) Standard control showing turbidity after 24 hour incubation at 310 K (37°C)

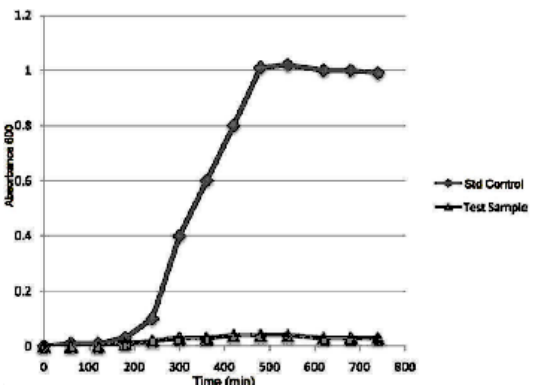

Fig. 5. Graph representing the growth pattern of *S. aureus*. The dotted line with hollow squares show the standard control (nutrient broth + microorganism) and the solid line with hollow circles denote the test sample (nutrient broth + nanoparticle + microorganism)

CONCLUSION

ZnO nanoparticles were biologically synthesized using the probiotic microbe *Lactobacillus sporogens*. Employing a biological process to synthesize inorganic nanoparticles is one of the most efficient and eco-friendly way and also inexpensive. Biogenic nanoparticles have remarkable properties that clearly distinguish them from those that are produced from either chemical or physical methods. The obtained ZnO nanoparticles had an average particle size of 145.7nm.The formation of pure zinc oxide nanoparticles was also ascertained by the metal-oxygen vibration reflected in the FTIR spectrum. The crystal phase and structural attributes were determined through XRD. ZnO nanoparticles inhibited the growth of *Staphylococcus aureus* a gram positive food borne pathogen. This clearly indicates if further research is done to evaluate the inhibitory activity of Zinc oxide nanoparticles against other pathogens promising results could be obtained.

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