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Research Article

Biological Approach of Zinc Oxide Nanoparticles Synthesis by Cell Free Extract of *Spirulina Platensis*

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Abstract

The present study explores biological synthesis of ZnO nanoparticles (ZnO NPs) using the cell free aqueous extract of Spirulina platensis. Biosynthesized ZnO NPs were characterized by UV-Vis spectroscopy, SEM, TEM, XRD and FTIR studies and finally tested for antibacterial activity. Bio-synthesis using extract of S. platensis showed the formation of well scattered, highly stable, spherical ZnO NPs with an average size of 40-50 nm. The size and morphology of the nanoparticles were confirmed by SEM and TEM analysis. FTIR and UV-Vis spectra showed that proteins and peptides are mainly responsible for the formation and stabilization of ZnO NPs. Furthermore, the synthesized nanoparticles exhibited good antibacterial activity against pathogenic gram-negative i.e. Escherichia Coli- MTCC-9721, Proteus vulgaris- MTCC-7299, Klebsiella pneumonia- MTCC-9751 and gram-positive i.e. Staphylococcus aureus- MTCC-9542, S. epidermidis- MTCC- 2639, Bacillus cereus- MTCC-9017 bacteria. The ZnO NPs had shown maximum zone of inhibition (ZOI) i.e. 34.8±1.65 in P. vulgaris. Use of such a biological method provides a simple, cost-effective alternative template for the synthesis of nanomaterials in a large scale that could be great use in biomedical applications.

Keywords: *Zinc nanoparticles, Spirulina platensis, Antibacterial activity, and Biosynthesis*

Introduction

Nanotechnology is evolving as a rapidly growing area with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level. Zinc oxide (ZnO) is listed as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). As a food additive, it is the most commonly used in the battlements of cereal-based foods. Because of its microbicidal properties, ZnO has been incorporated into the linings of food cans in packages for meat, fish, corn, and peas to preserve colours and to prevent spoilage in cosmetics, food additives, biosensors, and pharmaceuticals [Sharma *et al* 2009, Huang *et al* 2010, Rasmussen *et al* 2010, Sharama *et al* 2014]. Nano-sized particles of ZnO have more prominent antimicrobial properties than large particles, since the nanoparticle (less than 100 nm) and enhances the bactericide activity due to its large surface area and the presence of vacancies and uncoordinated atoms at corners and edges allow for better interaction with bacterial surface (Jones *et al* 2008). Reddy *et al* (2007) elucidate that zinc Oxide nanoparticles (ZnO NPs) have selective toxicity to bacteria but exhibit minimal effects on human cells. ZnO nanoparticles have been shown to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria, including major foodborne pathogens (Jones *et al* 2008, Liu *et al* 2009, Rajgovind *et al* 2015).

ZnO NPs also used as photo-catalyst in degrading pesticide carbetamide, herbicide triclopyr, pulp mill bleaching wastewater, 2-phenylphenol, phenol, blue 19, and acid red 14. The photocatalytic activity of ZnO NPs is because it has higher reaction rates, more active sites, and more effective in generating hydrogen peroxide (H_2O_2) [Rao *et al* 2009].

It is necessary to understand the mechanism of action of ZnO NPs against bacteria to make better use of ZnO nanoparticles in food products and contribution in the development of powerful, nontoxic, antimicrobial derivatives. Few studies have suggested that antibacterial activity may be from the disruption of cell membrane permeability (Brayner *et al* 2006). Another prospect could be the induction of intercellular reactive oxygen species (ROS) (Sawai 2003).

Chemical and physical methods of synthesis of ZnO NPs are costly and require extensive labour and time. Furthermore, in this processes large quantities of secondary waste by product are generated resulting

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from the addition of chemical agents for the reduction. Many techniques including oxidation of metallic zinc powder [Xiao *et al* 2008], molecular beam epitaxy [Yang *et al* 2008], pulsed laser deposition [Zhu *et al* 2010], sputtering [Sohal *et al* 2010], chemical vapor deposition [Chou *et al* 2011] and hydrothermal synthesis [Wu *et al* 2011] have been used to prepare ZnO NPs in different shape and size for various applications.

The growing need of environmental friendly nanoparticles, researchers are using green methods for the biosynthesis of various metal nanoparticles. A well-controlled synthesis process at prominent temperature is needed for the economical use of ZnO in bactericide applications such as water treatment and other environmental applications. Herein, we are reporting, for the first time to the best of our knowledge, a direct, simple, efficient and environmental benign synthesis method for ZnO nanoparticles aqueous extract of *Spirulina platensis* as a reducing and capping agent. The synthesized ZnO NPs were examined as bactericide agent against pathogenic gram-negative i.e. *Escherichia Coli*- MTCC-9721, *Proteus vulgaris*- MTCC-7299, *Klebsiella pneumonia*- MTCC-9751 and gram-positive i.e. *Staphylococcus aureus*- MTCC-9542, *S. epidermidis*- MTCC- 2639, *Bacillus cereus*- MTCC-9017 bacteria.

Materials and methods

Microorganism and Culture condition

The experimental organism *S. platensis* was isolated from Jal mahal, Jaipur, Rajasthan (India) and cultivated in Zarrouk's medium (Zarrouk, 1966) under 30±2°C temperature and illuminated with white fluorescent lamps at a light intensity of 2,000 lux (Sharma *et al* 2014).

Preparation of microalgal Extract

Typically, 5 g (dry weight) *S. platensis* biomass was suspended in 100 ml of double distilled sterile water and boiled for 15 min at 100°C in an Erlenmeyer flask. After boiling, the mixture was cooled and centrifuged at 10,000 rpm for 15 min. Supernatant was collected and was stored at 4 ºC for further analysis.

Biosynthesis of Zinc oxide nanoparticles

In the extracellular synthesis process of ZnO NPs, add 2 ml of pure aqueous extract added drop wise into the 100ml of 1mM of Zinc nitrate solution in 250 ml conical flask. Then the solution was kept under constant stirring using a magnetic stirrer to completely dissolution of zinc nitrate. After complete dissolution of zinc nitrate, the solution was heated at 100°C for 30 min under constant mechanically stirring. The colour change was noted and nanoparticles formation was monitored using UV-vis Spectrophotometer. The synthesized ZnO NPs were centrifuged at 15,000 rpm for 20 min at 4 ºC, and collect the pellet. The pellet was washed with distilled water for several times to remove impurities and 90% ethanol to get pure ZnO NPs powder.

Characterization of prepared ZnO nanoparticles

The Characterization of ZnO NPs was carried out by surface plasmon resonance band using a UV-Visible Spectroscopy 1800 of Shimadzu, Kyoto, Japan. Crystalline structure of AgNPs were analyzed by XRD-6000 instrument of Shimadzu at 30 kV and 20 mA current with Cu Ka (I = 1.54 A °). All X-ray diffraction technicalities carried out under the exploratory circumstances in the angular extent $3° \leq 2\theta \leq 50°$. Micrograph of AgNPs was obtained by scanning electron microscope of S-4500, Hitachi, Chiyoda-ku, Japan. TEM micrograph of the ZnO NPs was observed using the TEM instrument of JEOL JSM 100^{cx}. TEM device conducted at an increasing voltage of 200kv. The FTIR spectrum was recorded on a Perkin Elmer FT-IR system Spectrum GX model. All measurements were carried out in the range of 400– 4,000 cm^{-1} at a resolution of 4 cm^{-1}.

Antibacterial activity of ZnO NPs

A turbid liquid sample of each bacterial strain with an OD of McFarland of 0.5 (1×108 CFU/mL) was prepared in an isotonic NaCl (0.85%) solution. Furthermore, this solution was diluted ten times (1×107 CFU/mL) and used as inoculums. The MHA plates were inoculate with test disk and Gentamicin (10µg/disc) acquired by Hi-Media, Mumbai, used as control. The zone of inhibition (ZOI) observed at the surrounding area of 'ZnO NPs (100 µg/ml) solution after incubation at 37°C for 24 hours. The experiments were done four replicate and mean values of ZOI were reported.

The ZnO NPs prepared by *S. platensis* were used to evaluate antibacterial activity against Gram (-) and Gram (+) Bacteria (*Escherichia Coli*- MTCC-9721, *Proteus vulgaris*- MTCC-7299, *Klebsiella pneumonia*- MTCC-9751, *Staphylococcus aureus*- MTCC-9542, *S. epidermidid*-MTCC- 2639, *Bacillus cereus*- MTCC-9017,) on MHA plates by Kirby- Bauer disk diffusion method (Bauer and Kirby 1966).

Results and Discussion

In this study, extracellular synthesis of ZnO NPs has been revealed from cell free aqueous extracts of *S. platensis*. These extract when interacted with the zinc nitrate solution forms a turbid white solution due to the reduction of the zinc ion to ZnO NPs followed by a precipitate indicating the biotransformation of ionic zinc to reduced zinc, and the subsequent formation of ZnO NPs in an aqueous medium. Characteristic peak observed at 251 nm in the UV visible spectra indicates the presence of ZnO NPs (Figure 3a) due to the

excitation of surface plasmon vibrations in ZnO NPs [Nagarajan S and Kuppusamy 2013].

The morphological characteristic of biosynthesised ZnO NPs were studied by scanning electron microscope, using an instrument of Hitachi S-4500. The SEM images showed that most of the particles are spherical in shape and do not create big agglomerates which indicates that they were in the direct contact, but stabilized by a capping agent (Figure 3a). The TEM images revealed that ZnO NPs in the range of 40-50 nm (Figure 3b).

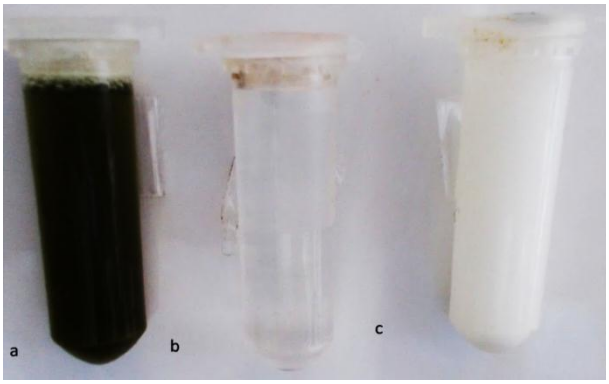


Figure 1: The Pictures show the (a) *S. platensis* extracts, (b) $ZnNO_3$ solution and (c) ZnO NPs solution

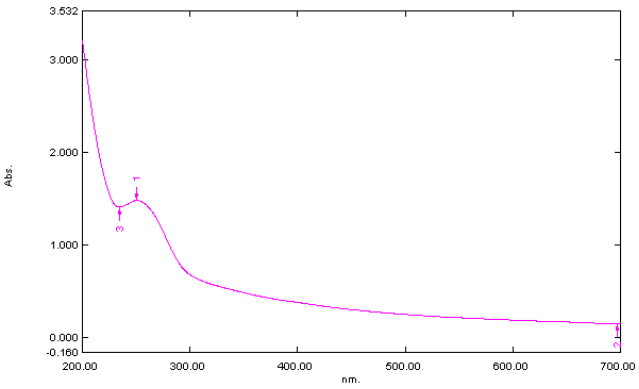
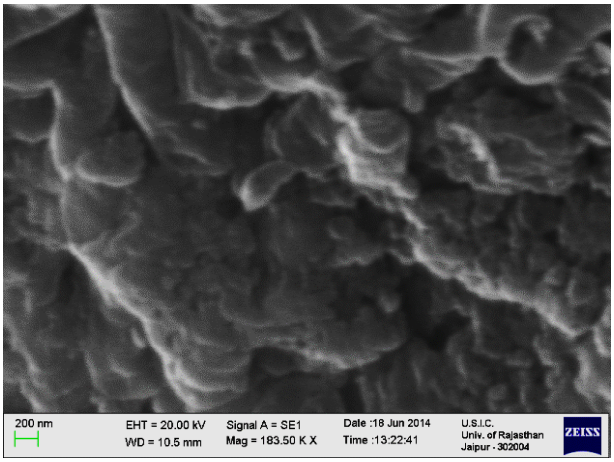


Figure 2: UV–vis absorption spectra of ZnO NPs synthesized from *S. platensis*



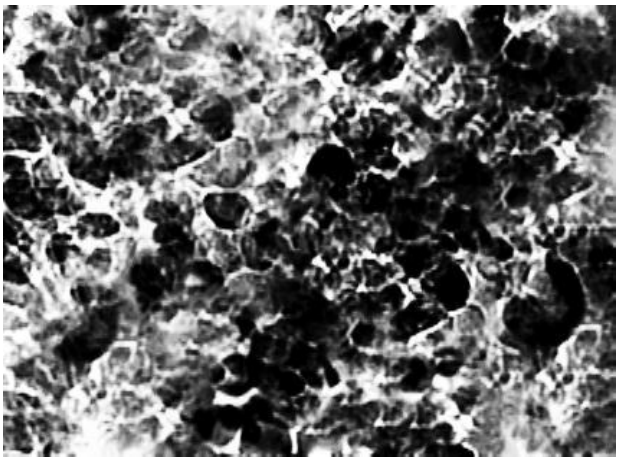


Figure 3: (a) The SEM images (b) TEM image of ZnO NPs synthesized by cell free extracts of S. Platensis

Antibacterial activity of Zinc Nanoparticles

The antimicrobial activity of biosynthesized ZnO NPs towards various bacterial strains were tested by the disc diffusion method and are represented in the Figure-3. The presence of zone of inhibition clearly indicates that the mechanism of the biocidal activity of ZnO NPs. Table-1 shows the four replicates experiments of zone of inhibition (mm) around the disc with synthesized ZnO NPs. The study revealed that ZnO NPs (50 µg/100 µL) had shown maximum inhibitory effect against *Proteus vulgaris*- MTCC-7299 i.e. 34.8±1.65 followed by *Bacillus cereus*- MTCC-9017 (25.3±0.48), *Staphylococcus aureus*- MTCC-9542 (25.0±0.41) *S. epidermidis*- MTCC- 2639 (25.0±0.71), *Klebsiella pneumonia*- MTCC-9751 (23.5±1.44), *Escherichia Coli*- MTCC-9721 (21.5±0.29) (Figure 4). This may be due to the destructive effect of ZnO NPs with the cells and increased production highly ROS such as OH, H_2O_2, and O_2^{2-} leads to the cell death. ZnO nanoparticles, after its attachment to the surface of the cell membrane, results in disruption in its respiration due to interact with enzymes of the respiration chains of bacteria and increased permeability through the bacterial cells that led to a loss of cellular transport mechanism (Padmavathy *et al* 2008). This could be due to the higher penetration potential of ZnO NPs (Veerapandian and Yun 2011).

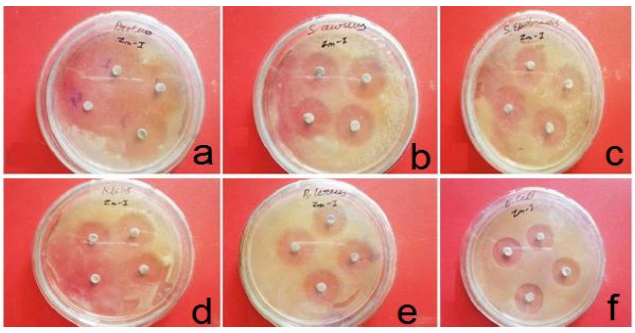


Figure 4(a-f) Zone of Inhibition against (a) *Proteus vulgaris* (b) *Staphylococcus aureus* (c) *S. epidermidis* (d) *Klebsiella pneumonia* (e) Bacillus cereus (f) Escherichia Coli with ZnO NPs (50 µg/100µL).

Table 1: Antibacterial activity of ZnO NPs (Zone of inhibition (ZOI) in mm)

Bacterial strain	ZOI-1	ZOI-2	ZOI-3	ZOI-4	Mean±SE
Escherichia Coli- MTCC-9721	22	21	22	21	21.5±0.29
Proteus vulgaris- MTCC-7299	30	35	37	37	34.8±1.65
Klebsiella pneumonia- MTCC-9751	27	24	20	23	23.5±1.44
Staphylococcus aureus- MTCC-9542	24	26	25	25	25.0±0.41
S. epidermidis-MTCC- 2639	23	26	25	26	25.0±0.71
Bacillus cereus- MTCC-9017	26	24	26	25	25.3±0.48

Conclusion

Overall, the nanomaterials, based on the metal oxide, exhibit broad-spectrum biocidal action towards different bacteria and have a distinct advantage over conventional chemical antimicrobial agents. This biological approach toward the synthesis of Zno NPs has numerous benefits i.e. non-toxic, cost effective, rapid reduction, economic viability. Experimental observations have explained significantly the antibacterial activity of ZnO NPs. In the present study, it can be concluded that the ZnO NPs can be used as an effective biocidal agent for Gram positive and Gram negative bacteria.

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