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SEMI-SOLVO THERMAL SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOSTRUCTURES AGAINST FOOD BORNE PATHOGENS

JAYANT PAWAR[1], MANISH SHINDE[2], RAVINDRA CHAUDHARI[3], AND E. A. SINGH[1]*

[1] Department of Plant Biotechnology, RajivGandhi Institute of Information Technology and Biotechnology, Pune – 411043, India
[2]Centres for Materials for Electronics Technology (C-MET), Pune – 411008, India
[3]P. G. Department of Zoology and Research Centre, Shri Shiv Chhatrapati College of Arts, Commerce and Science, Junnar- 410 502, India

ABSTRACT

A global threat posed by foodborne pathogens can be rectified by the use of antimicrobial nanostructures. In this trajectory, ZnO nanomaterials are very important as they are biocompatible, cheap and easy to produce. The synthesis of ZnO nanostructures was carried out by facile semi-solvo thermal route using zinc acetate and urea as precursors mixed in 20 % ethylene glycol solvent at 160^0 C for 24 hours. The structural and morphological analysis reveals formation of hexagonal ZnO nanostructures having flower like morphology. The antimicrobial activity against *Bacillus cereus* NCIM 5293 gram positive and *Salmonella typhimurium* NCIM 2501 gram negative model bacteria reveals their effective potential in food packaging applications. The MIC and MBC of ZnO nanomaterial was found to be 125µg/mL and 250µg/mL for *Bacillus cereus* and 250µg/mL as well as 500µg/mL for *Salmonella typhimurium,* respectively. Therefore, ZnO nanostructures can be used for the protection of assorted food products from the food pathogens.

KEYWORDS: *Zinc oxide nanostructures, antimicrobial activity, foodborne pathogens, semi-solvo thermal method*

E. A. SINGH[1]*

Department of Plant Biotechnology, RajivGandhi Institute of Information Technology and Biotechnology, Pune – 411043, India

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INTRODUCTION

The contamination of food stuffs by different micro-organisms is one of the serious concerns for consumers and food industries, since the growth and metabolism of microorganisms can cause serious foodborne diseases and rapid spoilage of the food products. Although potential spoilage organisms in liquid food stuffs are heat sensitive and eradicated by pasteurization process. Food industries faces potential economic losses caused by heat resistant microorganisms which enable them to survive current pasteurization process and post pasteurization contamination of food stuffs by such microorganisms.[1] In addition, it also produces some undesirable effects on food stuff such as loss of nutrition and freshness.[2] Since, consumers continue to demand ready to eat, fresh, minimally processed, preservative free and safe food products, it is mandatory to protect food in their natural forms without affecting their nutritional value.[2] Hence, variety of substances have been investigated such as bacteriocins, organic acids, phenolic compounds, lysozyme, chitosan, poly-lysine, essential oils, isothiocyanates isolated from white mustard seeds, limoidglucosides, flavonoids, vanillinet, calcium lactate, etc to replace chemical preservatives.[3-10] Although these substances are effective antimicrobial agents, their use in liquid food stuffs is limited because they either alter the sensory attributes of food or they are costly. Thus, food processors need alternative antimicrobial substances which are functionally effective without altering physicochemical and organoleptic properties of the food, in addition to being cost effective. Therefore, developing novel antibacterial agents against major food pathogens, such as *Bacillus cereus* and *Salmonella typhimurium* etc. has become supreme mandate. *Bacillus cereus* is gram positive rod frequently isolated from foods which has emerged as one of the more important causes of food poisoning in the canned food stuffs, meat, dairy products, cooked rice and pasta.[11-12] It produces an emetic toxin and three different enterotoxins. *B. cereus* causes two different types of food poisoning, one emetic (intoxication) due to a preformed small cyclic peptide, and other diarrheal (infection) due to enterotoxins.[11] *Salmonella typhimurium,* a gram negative rod, is one of the more important causes of food poisoning in the poultry, meat, pork, beef, unpasteurized milk or juice, cheese,contaminated raw produce andpeanut butter.[13, 14] Here, we proposed to synthesis of zinc oxide (ZnO) nanostructures by semi-solvo thermal method to inhibit the growth of leading food borne pathogenic bacteria such as *Bacillus cereus* and *Salmonella typhimurium.* Since, zinc oxide is non-toxic to human beings and noxious to microorganisms, it can be used in food packaging systems for food protection from various food borne pathogens. Moreover, zinc is a mineral element necessary to human health and ZnO is a form in the daily supplement for zinc. ZnO nanoparticles are also reported to possess good biocompatibility towards human cells.[15-16]

MATERIALS AND METHODS

Synthesis and Characterization of ZnO nanostructures

Synthesis of ZnO nanostructures was carried out by semi-solvo thermal route. All the analytical grade chemicals were used without any further purification. In typical experimental procedure, 0.1M zinc acetate Zn $[(CH_3\ COO)_2Zn.2H_2O]$was dissolved in 50 mL DI water. 0.6M urea $[NH_2.CO.NH_2]$ solution was prepared in 50 mL 40% ethylene glycol $[C_2H_6O_2]$. Both solutions were mixed under constant stirring for 5 minutes to get homogenous reaction mixture which was then transferred to Teflon-lined stainless steel autoclave. Reaction mixture was heated at 160^0 C for 24 hours in muffle furnace and then allowed to cool down at room temperature. The resultant settled precipitate was washed by repeated and alternative centrifugation of resultant product at 5000 rpm for 15 minutes each with DI water and ethanol. The final product was dried in hot air oven at 60^0 C for 8 hours to obtain white colored powder. Structural information of as synthesized powder was obtained by using X-ray diffraction (XRD, Bruker D8 Advance X-ray diffractometer, Germany). The morphological feature of the sample was studied using field emission scanning electron microcopy (FESEM, Hitachi S-4800). For performing the FESEM analysis the powder was dispersed in ethanol using ultra- sonication and a drop of resultant solution was dried on aluminium film. Subsequently, a thin gold palladium layer was sputter deposited to avoid the effects due to charging.

Qualitative Estimation of Antimicrobial Activity of ZnO

The antibacterial activity of ZnO nanomaterial was tested on *Bacillus cereus* NCIM 5293 and *Salmonella typhimurium* NCIM 2501 obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. The proposed assays were performed in qualitative manner. The antibacterial activity of ZnO was carried out by agar well diffusion assay (AWDA).[17- 18.] The Muller-Hilton (MH) agar plates were spread inoculated with overnight grown cultures of *B. cereus* and *S. typhimurium.* The synthesized ZnO nanomaterial were dispersed in sterile DI water by ultra-sonication to make colloidal solution.On the surface of agar plates, wells of 5 mm in diameter and of 18 µL in capacity were formed by using sterile gel borer. The 15 µL of ZnO solution were placed in each well and incubate all plates at 37^0 C for 24 hours.

Determination of Minimum inhibitory concentration and Minimum bactericidal concentration (MIC & MBC)

MIC and MBC are the lowest concentration of ZnO inhibits growth or kills more than 3 logs (99.9 %) of bacterial cells. All the in vitro bactericidal activities of ZnO were performed by plate count method on MH agar plates (in the concentration range of 15 µg - 1000 µg/mL).The test cultures of final cell density of 1×10^5 CFU/ mL were used for spread inoculation. The plates were incubated at 37^0 C for 24 hours and subsequent growth inhibition of bacterial cultures was determined. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Characterization of ZnO powder

The crystalline nature of as synthesized ZnO powder was analysed by X-ray diffraction technique

(Figure 1). The diffractogram reveals formation of ZnO possessing hexagonal crystal structure (JCPDS card # 36-1451) based on thediffraction angle peak positions and relative intensities of the peaks. Peaks corresponding to (100), (002), (101), (102), (110), (103), (112), (207) and (202) planes have been identified. The preferred orientation corresponding to the (101) plane is observed for this sample.

XRD pattern of ZnO powder

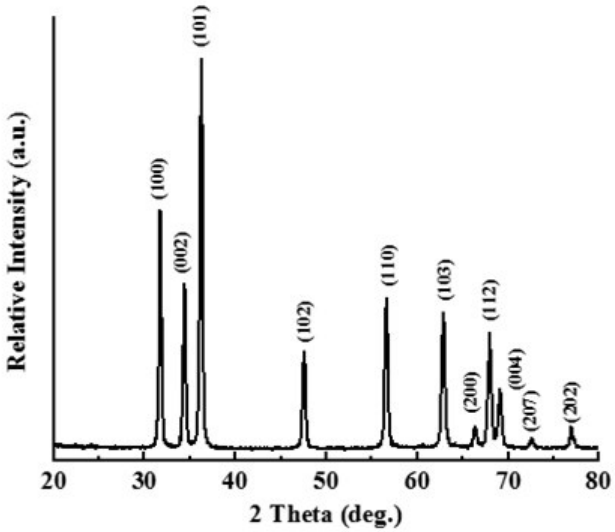

Figure 1
(a) XRD pattern of ZnO powder

The FESEM images of the synthesized nanosturctures are shown in figure 2. The low magnification image (Figure 2a) shows formation of flower like nanostructures. The size of the nanoflower is ~5µm. However, as shown in higher magnification image (Figure 2b), each flower is made up of petals having thickness of 10-40 nm. The length of these petals extends from 800nm to 1200nm. Due to such nano flower-like structures, the specific surface area of these nanostructures increases. Due to this they will be in better contact of bacterial species effectively killing them.

FESEM images of ZnO nanostructure

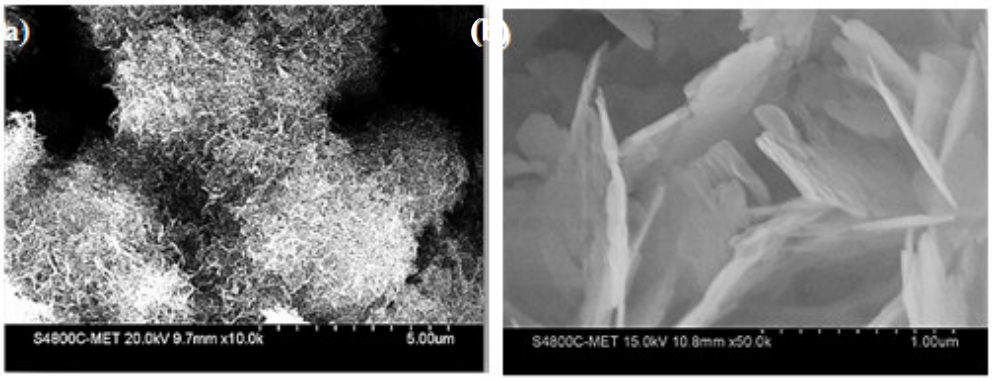

Figure 2
FESEM images of ZnO nanostructure at (a) low and (b) high magnification.

Qualitative estimation of antibacterial activity of ZnO
The antimicrobial activity of chemically synthesized ZnO nanomaterial suspension of 1000 µg/mL concentration were tested on test microorganisms and was found to possess antibacterial activity (Figure 3). As it was shown in the study of, [18] it has been found in this study that by increasing the concentration of ZnO in wells, the growth inhibition has also been increased consistently because of proper diffusion of nanoparticles in the agar medium. The release of Zn^{2+} ions is responsible for the antibacterial activity. [19] In our study, ZnO nanostructures showed a greater significant zone of inhibition against *B. cereus* and *S.typhimurium*.

Antimicrobial activity of ZnO

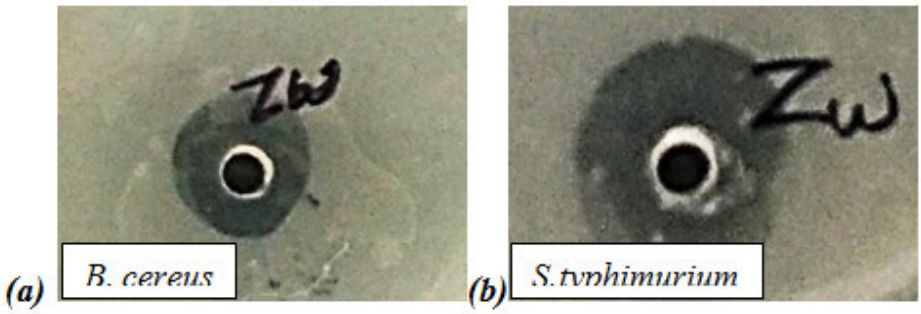

Figure 3
Antimicrobial activity of ZnO against (a) B. cereus (b) S.typhimurium

Determination of MIC & MBC

MIC and MBC of ZnO nanostructure suspension were determined by incubation of test bacteria with different concentrations of ZnO powder in the range of 15 µg - 1000 µg/mL (Figure 4).

Determination of MIC & MBC of ZnO nanomaterial

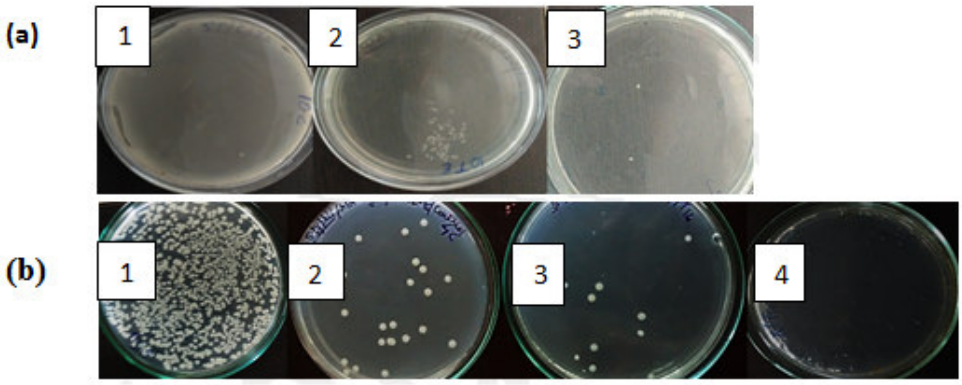

Figure 4
Determination of MIC & MBC of ZnO nanostructures against (a) B. cereus (b) S. typhimurium. In the given figure, plates of both bacterial cultures are shown as (1) control, (2) 125 µg/mL, (3) 250 µg/mL and (4) 500 µg/mLconcentration of ZnO nanostructures

No significant antibacterial activity was observed at concentrations less than 30 µg/mL for both the test organisms. However, the antibacterial efficacy at concentration 125 µg/mL is higher for *B. cereus* as compared to *S.typhimurium*. The MIC and MBC of ZnO nanostructures were found to be 125µg/mL and 250µg/mL, respectively, for *B. cereus* and 250 µg/mL and 500 µg/mL respectively for *S.typhimurium.* The antagonistic activity of ZnO nanostructures can be explained on the basis of the oxygen species released on the surface of ZnO, which cause fatal damage to microorganisms.[19]They react with hydrogen ions to produce hydrogen peroxide molecules (H_2O_2).The generated H_2O_2 can penetrate the cell membrane and kill the bacteria.[21] The generation of H_2O_2 depends strongly on the surface area of ZnO, which results in more oxygen species on the surface and the higher antibacterial activity of the smaller nanoparticles.[222]In the present case, the 2-dimensional petal-like morphology of ZnO nanoflowers provides more surface area which enables effective killing ofmicro-organisms. The results of this study may be applicable to food packaging materials that are coated with nanostructures against food pathogens.

CONCLUSION

A ZnO nanomaterial having flower like morphology with greater surface area was obtained by semi-solvo thermal method of synthesis. The nanomaterial synthesizes shows petal like structure at higher magnification by FESEM and having size of 10-40 nm. Due to such a smaller size, it has large surface are to volume ratio and hence found to be effective antibacterial agent against foodborne pathogens viz. *Bacillus cereus* and *Salmonella typhimurium*. The MIC and MBC of ZnO nanomaterial was found to be 125µg/mL and 250µg/mL for *Bacillus cereus* and 250µg/mL as well as 500µg/mL for *Salmonella typhimurium,* respectively. Therefore, ZnO nanostructures can be used for the protection of food stuffs against food pathogens by incorporating it into food packaging systems.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

INFORMED CONSENT

Not applicable

CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Rajendra patil, Ph.D

Faculty, Department of Biotechnology,
Savitribai Phule Pune University,
Ganeshkhind, Pune, India

Prof. Y. Prapurna Chandra Rao

Assistant Proffessor, KLE University,
Belgaum, Karnataka

Prof.Dr.K.Suriaprabha

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