

Biosynthesis of Zinc Nanoparticles Using Actinomycetes for Antibacterial Food Packaging

Usha Rajamanickam⁺, PriyangaMylsamy, Subadhra Viswanathan and Palaniswamy Muthusamy
Department of Microbiology, School of Life Sciences, Karpagam University, Coimbatore, 641 021, Tamil Nadu, India

Abstract. There have been impressive developments in the field of nanotechnology in the recent years, Recently, there has been tremendous excitement in the study of nanoparticle synthesis by using natural biological system. In the present study, we have demonstrated the synthesis of metal oxide nanoparticles by a Streptomyces sps isolated from the rhizosphere soil in India. Extracellular production of metal oxide nanoparticles by Streptomyces sps was carried out. It was found that zinc nitrate when exposed to Streptomyces sps are reduced in solution, thereby leading to the formation of nanoparticle. The possibility of the reduction of metal ions may be by reductase enzyme. The antibacterial activity of nanoparticle was evaluated by well diffusion method. The inhibition of viable S. aureus, E. coli, and Salmonella sps was observed after 24h and 48 h of incubation. This antibacterial activity as a key factor in making the suitable for long life application in food packaging.

Keywords: Zinc nanoparticle, Streptomyces and antibacterial activity

1. Introduction

Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) materials for the development of science. Though chemical methods continue to be investigated for the shape and size controlled synthesis of metal nanoparticles, they can be hazardous to the environment due to the use of toxic chemicals. Research over the past few years has demonstrated that utilization of biological organisms has been emerging as a novel method for the synthesis metal nanoparticles, which can be preferred over the existing chemical and physical methods.

Nanotechnology in the food industry can take a number of forms. These include the use of nanotechnology in packaging materials. Nanotech to develop antimicrobial packaging for food products. The nanoparticles are dispersed throughout the plastic and are able to block oxygen, carbon dioxide and moisture from reaching fresh meats or other foods. Packaging can contribute to the control of microbial growth in foodstuffs [1], which can lead to spoiling or in the case of pathogenic microorganisms, disease and illness. Most activities to combat this have centred around nanocoatings or nanocomposites using nanoparticulates of silver and zinc oxide, however there is also research into the antimicrobial effects of natural biological compounds [2]. Nanomaterials are being actively researched for specific functions such as microbial growth inhibition, [3] as carriers of antibiotics and as killing agents [4]. Zinc oxide exhibits antibacterial activity that increases with decreasing particle size [5].

Zinc oxide nanoparticles have been incorporated in a number of different polymers including polypropylene [6]. Zinc nanoparticles used as an additive for numerous materials and products⁺ including plastics, ceramics, glass, pigments, foods, etc. ZnO is nontoxic and it is a strong antibacterial agent. ZnO nanoparticles is used in industrial sectors including environmental, synthetic textiles and food packaging. With the above back ground information the present study was carried out with the main objective of

⁺ Corresponding author. Tel: +91-422-6471113; Fax: +91-422-2980022, 2980023
E-mail address: ushaanbu09@gmail.com

evolving a simple method for the biosynthesis of Zn nanoparticles and assessed the nano particle in terms of antibacterial action and characterized the nanoparticles for further food preservation.

2. Materials and Methods

2.1. Isolation and Screening of Potential Actinomycetes

In the present study isolation of actinomycetes were performed by soil dilution plate technique using glycerol yeast extract agar complemented with nystatin (50µg/ml) at 27°C [7]. Rhizosphere soil sample from Coimbatore, Tamilnadu, South India were collected and processed. Actinomycete isolates were distinguished from other microbial colonies by characteristics such as rough, leathery colonies. Then isolated colonies were preserved in glycerol based media and stored at 20°C. Antimicrobial activities of pure actinomycete cultures performed by using spectra-plak method. Muller hinton agar plates were prepared and inoculated with actinomycetes cultures by a single streak of inoculum in the center of the petridish and incubated at 27°C for four days. Later the plates were seeded with test organism by a single streak at a 90° angle to actinomycetes strains. Antagonism was measured by the determination of the inhibition zone.

2.2. Identification of Potential Actinomycete Isolates

The most potent actinomycete isolate which showed activity against bacteria was characterized by macroscopic, microscopic and morphological methods. Morphological observation carried out by the methods and media proposed by Shirling and Gottlieb [8]. The chemical compositions of cell wall and whole-cell hydrolysates were analyzed by the methods of Lechevallier and Lechevalier [9].

2.3. Synthesis of Zinc Nanoparticles and Assessment of Antibacterial Activity:

The Erlenmeyer flask with the *Streptomyces* sps supernatant were a yellow color before the addition of zinc and this changed to a brownish color on completion of the reaction. The appearance of a brown color in solution suggested the formation of zinc nanoparticles. The solution was extremely stable, with no evidence of flocculation of the particles even several weeks after reaction. Aliquots of the reaction solution were taken after 24 and 48 hrs and the antibacterial activity was observed by well diffusion method.

2.4. Characterization of Nanoparticles by UV-VIS and SEM

The zinc nanoparticles were observed with a Scanning Electron Microscope (SEM). The samples were analyzed for their variations in chemical groups using FTIR spectroscopy. The wavelength of light absorbed is characteristic of the chemical bond can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

3. Results and Discussion

3.1. Isolation and Screening of Antimicrobial Actinomycetes

In the present study, 31 different actinomycetes were isolated from the rhizosphere soil sample. The antibacterial activities of the isolates were varied. It was found that 15 strains suppressed the test microorganisms in different degree. The best actinomycete isolate has been selected for further study since it showed the greater activity against pathogens.

3.2. Characterization of the Most Potent Actinomycete Isolate

Macroscopic and microscopic methods were employed to describe the potent strain. According to waksman [7] such color form are exhibited by colonies of *Streptomyces*. (Table 1). However, the color and form of the colony could serve as bases for pointing out the genus to which actinomycete belongs to. Hence, its morphological properties must serve as the primary bases of characterization. After growing on the YMA, it was seen to exhibit a grey colony. The form of the colony was described to be compact raised. Hence it is suggested that the strain isolated from rhizosphere soil, possessing reduction activity to form the nanoparticles and could be assigned to *Streptomyces* sps and it is worth investigating actinomycetes future in detail for human welfare.

Table:1 Cultural and morphological characteristics of potent strain

Cultural and morphological characteristics	Observation
Temperature	27 °C
Ph	7.0
Color of the colony	Grey
Form of the colony	Powdery, raised
Pigmentation in the medium	No diffusible pigment
Substrate mycelium	Present
Aerial mycelium	Present
Melanin pigment	No pigment production
Spore surface	Rough
Diaminopimelic acid(DAP)	LL-DAP

3.3. Assessment of the Antibacterial Activity of Zinc Nanoparticle

After fermentation actinomycete biomass was obtained by filtration. The harvested supernatant and pellet were used for synthesis of nanoparticles. The aliquots of the reaction mixture were taken and antibacterial activities were determined by well diffusion method (Table 2) (Fig 1). Zinc nanoparticle showed greater activity against *S.aureus*. The antimicrobial activity of nanoparticles has been studied largely with human pathogenic bacteria, mainly *E.coli* and *S.aureus* [10].

Table 2: Assessment of the antibacterial activity of Streptomyces supernatant after addition with zinc nitrate

Antibacterial Components	<i>S. aureus</i>		<i>E. coli</i>		<i>Salmonella sps</i>	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Zinc nanoparticle	17mm	23mm	14mm	21mm	12mm	19mm
Positive control	10mm	12mm	2mm	6mm	5mm	8mm
Negative control	4mm	6mm	Nil	Nil	Nil	Nil



Nanoparticle against *S.aureus*

Nanoparticle against *E.coli*

Nanoparticle against *Salmonella sps*

Fig 1: Assessment of the antibacterial activity of supernatant containing zinc nitrate

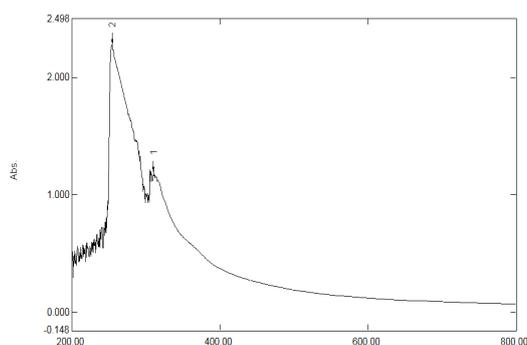


Fig.2: UV analysis of zinc nanoparticle

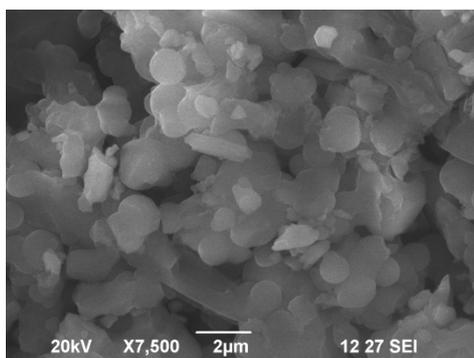


Fig.3: Scanning electron micrographs of the nano scaled zinc particles

3.4. Characterization of Zinc Nanoparticle

The formation of Zn-NPs by reduction of the aqueous Zn during exposure to the *Streptomyces* showed brown color, which suggested the formation of Zn-NPs in solution. The color arises due to excitation of surface Plasmon vibrations in the zinc nanoparticles. The reduction of zinc was subjected to analysis by using the UV-Vis Spectrophotometer. Absorption spectra of Zn-NPs formed in the reaction media has absorbance peak at 310nm, broadening of peak indicated that the particles are polydispersed (Figure 2). The frequency and width of the surface Plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [11].

The biosynthesized silver nanostructure by using *Strptomyces* was further demonstrated and confirmed by electron microscopy. The SEM micrographs of nanoparticle obtained in the prepared sample showed that Zn-NPs are spherical shaped, well distributed without aggregation in solution (Figure 3). The present study showed a rapid and economical route to synthesize Zn-NPs from actinomycetes. The zone of inhibition clearly showed that the bacterial strains tested were susceptible to Zinc nanoparticles. The present study proved that the biologically zinc nanoparticles seems to be promising and effective antibacterial coating material. This can be utilized to extend the shelf life of food materials. Further study is necessary for the commercialization.

4. Conclusion

Antibacterial nanopackaging can create a modified atmosphere in packaging with controlled gaseous exchange, so that; the shelf life of vegetables may be increased to weeks. The surface of an ordinary packaging material such as plastic or paper can be adapted to make it suitable for food by coating it with one or more sharply defined layers of tens of nanometers thickness. Developing coated paper with antibacterial properties of zinc nanoparticles could be an alternative to other food preservation method. Nano coatings for glass bottles are used for better preserving the quality of fruit and vegetable products by shielding them from light waves and thereby improve their shelf life. The simplicity of the process means it would be easy to scaled up to meet industry need. It also noted that its technology could be use as another option to preservation processes.

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6. Reference

- [1] J. W. RHIM, AND P. K. W. NG. NATURAL BIOPOLYMER-BASED NANOCOMPOSITE FILMS FOR PACKAGING APPLICATIONS. CRITICAL REVIEWS IN FOOD SCI. NUTR. 2007, 47(4): 411–433.
- [2] D. S. CHA, AND M. S. CHINNAN. BIOPOLYMER-BASED ANTIMICROBIAL PACKAGING: A REVIEW. CRITICAL REVIEWS IN FOOD SCI. NUTR. 2004, 44, 223-237.
- [3] N. CIOFFI, L. TORSI, N. DITARANTO, G. TANTILLO, L. GHIBELLI, AND L. SABBATINI. COPPER NANOPARTICLE/POLYMER COMPOSITES WITH ANTIFUNGAL AND BACTERIOSTATIC PROPERTIES. CHEM MATER. 2005, 17, 5255–5262.

- [4] L. HUANG, D. Q. LI, Y. J. LIN, M. WEI, D.G. EVANS AND X.DUAN. CONTROLLABLE PREPARATION OF NANO-MGO AND INVESTIGATION OF ITS BACTERICIDAL PROPERTIES. *J INORG BIOCHEM.* 2005, 99, 986–993.
- [5] [5] O. YAMMAMOTO. INFLUENCE OF PARTICLE SIZE ON THE ANTIBACTERIAL ACTIVITY OF ZINC OXIDE. *INT J INORG MATER.* 2001, 3, 643-646.
- [6] S. CHANDRAMOULEESWARAN, S.T.MHASKE, A.A. KATHE, P.V.VARADARAJAN V.PRASAD AND N.VIGNESHWARAN. FUNCTIONAL BEHAVIOUR OF POLYPROPYLENE/ZNO-SOLUBLE STARCH NANOCOMPOSITES. *NANOTECHNOL.* 2007, 18, 8.
- [7] S.A. WAKSMAN. *THE ACTINOMYCETES: CLASSIFICATION, IDENTIFICATION AND DESCRIPTIONS OF GENERA AND SPECIES.* BALTIMORE: THE WILLIAMS AND WILKINS COMPANY. 1961.
- [8] SHIRLING, E.B. AND D. GOTTLIEB, 1966. METHODS FOR CHARACTERIZATION OF STREPTOMYCES SPECIES. *INT. J. SYST. BACTERIOL.*, 16: 313-34.
- [9] M.P. LECHEVALLIER, AND H.A. LECHEVALIER. THE CHEMOTAXONOMY OF ACTINOMYCETES. IN *ACTINOMYCETES TAXONOMY.* ED., X. DIETZ AND Y. THAYER. SOCIETY FOR INDUSTRIAL MICROBIOLOGY, ARLINGTON, VA.1980.pp: 227-291.
- [10]P. GAJJAR, B. PETTEE, D.W. BRITT, W. HUANG, W.P. JOHNSON, AND A.J. ANDERSON. ANTIMICROBIAL ACIVITIES OF COMMERCIAL NANOPARTICLES AGAINST AN ENVIRONMENTAL SOIL MICROBE, PSEUDOMONAS PUTIDA KT2440. *J BIOL ENG.* 2009. 1-13.
- [11]J.GONZALO, R.SERNA, J.SOL, D. BABONNEAU, AND C.N.AFONSO. MORPHOLOGICAL AND INTERACTION EFFECTS ON THE SURFACE PLASMON RESONANCE OF METAL NANOPARTICLES. *J PHYS CONDENS MATTER.* 2003, 15(42):3001-3002.