USES OF CERIUM OXIDE AS ANTIBACTERIAL AGENT FOR MANAGEMENT OF DISEASE FREE FISH

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Abstract

An experiment was designed to investigate the antibacterial activity of two nanoparticles against fish bacterial pathogens Bacillus subtilis, Vibrio harveyi species. Different concentration of nanoparticles was assessed by well diffusion method against an antibacterial activity. The nanoparticles was analysed by MIC and MBC technique. The potential nanoparticle CeO_2 which showed maximum antibacterial was also subjected for the time killed assay method. Among the two nanoparticles CeO_2 shows maximum activity against Bacillus subtilis (14±0.45 mm diameter) MIC test carried by the liquid dilution method. The result suggested that the CeO_2 nanoparticles showed maximum inhibition at the concentration of 19 µg/ml against Bacillus subtilis and 29 µg/ml against vibrio. It is also noted that 9 µg/ml concentration of CeO_2 nanoparticles showed the maximum reduction of bacteria growth 2nd hour up to 12th hours. It is concluded from the present study the CeO_2 could be used as an affective antibacterial agent for disease free fish management.

Keywords: *Nanoparticles, MBC, MIC, Time Kill Assay*

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INTRODUCTION

Nanotechnology has been defined by U.S. National Nanotechnology Initiative (NNI) as understanding and control of matter at dimension of roughly 1 to 100 nm (1 nano meters being equal to 1 X 10^{-9} of a meter). CeO_2 Cerium oxide adopts a Fluorite crystalline structure that has unique antioxidant properties. Nowadays nanotechnology has a tremendous potential to revolutionize agriculture and allied fields including aquaculture and fisheries it can provide new tools for aquaculture neon particles are used as smart drug delivery for the treatment of fish diseases [1]. The nanoparticles have been used to deliver the drugs into the cells with negligible side effects
[2]. The Synthesis of nanoparticles from metals possesses various biological processes through to enzymatic systems. The interaction of these nanoparticles with biological active ligand in the animal system through chelation
[3] Due to the increase in the outbreak of bacterial diseases in the aquaculture industry and the development at bacterial resistance, now antibacterial agents are required. Silver nanoparticles have proved to be one of the most effective metallic nanoparticles and good antibacterial activity against some bacterial pathogens [4] moreover the other metal nanoparticles the Zno nanoparticles showed antibacterial activity against various bacterial pathogen includes *E. coli,* staphylococcus aurous and *Bacillus* respectively [5–7].

MATERIAL AND METHODS

Commercial nanoparticles of Al_2O_3 and CeO_2 were procured from Sigma Aldrich company, India. The Characteristics of the nanoparticles are represented in Table 1.

Test Models

Two fish pathogens *Bacillus subtiles* and *Vibrio harveyi* were taken.

Antibacterial Assay

The two nanoparticles was chosen for antibacterial activity. This is performedby well diffusion method. About 20 ml of sterile molten Mueller Hinton agar (Himedia Laboratories Pvt. Limited Mumbai India) was filled into the sterile petriplates.

Triplicate plates were filled with overnight culture (10^8 cells/ml) of pathogen bacteria *Bacillus subtilis,* and *Vibrio harveyi* sp. And make a well or punctured on solid medium culture with the help of cork. Finally the nanoparticle samples (50 g/ml) were added from the stock into the each well and incubated for 24 hours at 27±2°C and some of inhibition was measured and expressed as millimetre in diameter.

MIC (Minimum Inhibitory Concentration) Different concentration of 10, 20, 30, 40, 50 g/ml of Chosen nanoparticles were prepared with dimethylsulphoxide (DMSO) and mixed with 450 g/ml of nutrient broth and 50 l of 24 hours old bacterial inoculum and allow to grow overnight 37°C 48 hours nutrient broth alone served as negative control.

Minimum Bactericidal Concentration (MBC) The minimum bactericidal concentration (MBC) was conducted by sub-culturing the above serial dilution after 24 hours in nutrient agar plates using 0.01 ml drop and incubated at 37°C for 24 hours MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media.

Time Kill Assay

The potential nanoparticles (CeO_2) which showed maximum antibacterial activity against *Bacillus subtilis* for time kill assay the inoculum of *Bacillus subtilis* (50 µl) at a concentration of (10^8 cells ml^{-1}) was mixed with 50 µl (contains 10 g/ml) of CeO_2 nanoparticles and the total Vol. was made up to 5 ml by using minimal medium (g/l) [Sucrose 5 g/l, potassium hydrogen phosphate Manganese sulphate mono hydrate 1.5 g/l, $(NH_4)2$ Hydrogen Phosphat·5 g/l, $MgSO_4$ $7H_2O$ 0.10 g/l,Manganese sulphate monohydrate H_2O 0.0035 g/l] and H_2O 1000.0 ml). The negative control was maintained without nanoparticle.

Every one he internal the growth of the bacterial observed by measuring the optical density at 600 nm by using spectrophotometer. (CeO_2) nanoparticle showed maximum sensitivity (14±0.45 mm) against *Bacillus subtilis* and Showed minimum activity against *Vibrio harveyi* (10±0.35). The Al_2O_3 Showed maximum sensitivity 13±0.12 against *Bacillus subtilis* and showed minimums 9±0.15 against *Vibrio harveyi,* respectively (Table 2).

In MIC assay the nanoparticle CeO_2 showed maximum sensitivity (19 g/ml) against *Bacillus Subtilis* and 29 g/ml against *Vibrio harveyi* respectively. However, the nanoparticles Al_2O_3 showed high sensitivity 45 g/ml against *Bacillus subtilis* and against *Vibrio harveyi* 58 g/ml (Table 3).

The effect of CeO_2 nanoparticle against *Bacillus subtilis* was also performed with time kill assay. It reveals that the growth of the pathogen was inhibited gradually from the 2nd hours up to 12th hours when compared to the control.

Table 1: *Properties of Nanoparticles.*

Formula	Molecular weight	Form	Particle size in TEM (nm)
Al_2O_3	101.96	Power	<50
CEO_2	172.11	Power	<25

Table 2: *Antibacterial Activity of 5 Metal Oxides Nanoparticles against Fish Pathogens.*

	Bacillus subtilis	*Vibrio harveyi*
	Zone of inhibition (mm diameter)	
Al_2O_3	13+0.12	9+0.15
CeO_2	14+0.45	10+0.35

Table 3: *MIC and MBC of 2 Metal Oxide Nanoparticles against Fish Pathogen.*

Bacillus subtilis			*Vibrio harveyi*	
Concentration (g mg^{-1})				
	MIC	MBC	MIC	MBC
Al_2O_3	28	48	48	58
CeO_3	19	19	29	40

Nanotechnology undoubtedly presents a major opportunity for the economy and sustainable development of quatic resources in many countries. Although the application of nanotechnology is still at a very early stage in aquaculture [8–10].

In MIC assay, the nanoparticle CeO_2, showed maximum sensitivity (19 $g.ml^{-1}$) against *Bacillus subtilis* and 29 $.ml^{-1}$ against *Vibrio harveyi* respectively. However, the nanoparticles Al_2O_3 showed high sensitivity (29 $g.ml^{-1}$) against *Bacillus subtilis.*

However, the antimicrobial agents from metal nanoparticles against fish pathogens are poorly understood. Hence the present study has made attempt to find out the antimicrobial agents from nanoparticles. In the present study, different metal nanoparticles have been used for the antibacterial property. Moreover, the advantages of inorganic antibacterial material over organic antibacterial materials are the superior durability, high surface area les toxicity, heat resistance and more suitable for biological applications. The antibacterial activity of two nanoparticles against fish pathogens, viz., *Bacillus subtilis*, *Vibrio harveyi*.

RESULT

Among the nanoparticles, CeO_2 nanoparticles showed maximum sensitivity against *Bacillus subtilis* and *Vibrio harveyi*. The Al_2O_3 nanoparticles showed minimum activity when compared with CeO_2. This might be due to the size, surface morphology, particle morphology and structure of the nanoparticles. The material being tested is bactericidal or bacteriostatic; the MIC and MBC tests reveals that, the CeO_2 showed maximum inhibition at the concentration of 19 g/ml against *Bacillus subtilis* and 29 g/ml against *Vibrio harveyi* than the other nanoparticles. The reason behind that, CeO_2 nanoparticles tightly adsorbed on the surface and to control the further action of the bacterial cells. Moreover, the smaller size that enhanced the activity due to large surface area. The present study also attempts to find out the antibacterial activity of the CeO_2 nanoparticles against *Bacillus subtilis* at different time interval. It reveals that the bacterial growth was inhibited from the 2nd hour up to 12th hour. Generally, the toxic effects of the CeO_2 nanoparticles are dose dependent and time dependent. The oxidative stress increases the production of lactate dehydrogenase, which is an indicator of cell membrane damage. It is concluded from the present study that, the CeO_2 nanoparticles could be used as an alternative antibacterial agent for the disease free fish culture systems.

CONCLUSION

There are however many research gaps in the field of nanotechnology application in fish medicine. Different forms of nanoparticles in fish disease research the anti-fungal and anti-viral effect of nanoparticle against fish disease have yet to be explored. Demonstrated potential of nanoparticles there are needs for more targeted investigations of their application in may fish medicine research topics to promote more efficient fish disease diagnostics and therapy to meet the ever-growing aquatic animal health demand. It is concluded from the present study the CeO_2 nanoparticles could be used as the antibacterial agents for disease free fish management.

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