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Nanoparticles as a source for the treatment of fish diseases

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ABSTRACT

Objective: The present study was aimed to investigate the antibacterial activity of 5 different nanoparticles against fish bacterial pathogens viz., *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *serratia* sp. **Methods:** The antibacterial activity of the chosen nanoparticles was assessed by well diffusion method. Different concentrations of the nanoparticles were analyzed by MIC and MBC techniques. Finally the potential nanoparticle CeO_2 which showed maximum antibacterial activity was also subjected for the time kill assay method. **Results:** Among the five nanoparticles, CeO_2 showed maximum activity against *Bacillus subtilis* (13±0.35 mm dia.) followed by *Vibrio harveyi* (11±0.25 mm dia.). The MIC test was also carried out by the liquid dilution method. The results suggested that, the CeO_2 nanoparticles showed maximum inhibition at a concentration of 20 $\mu g.ml^{-1}$ against *Bacillus subtilis* and 30 $\mu g.ml^{-1}$ against *Vibrio harveyi* than the other nanoparticles. It is also noted that, 10 $\mu g.ml^{-1}$ concentrations of the CeO_2 nanoparticles showed the maximum reduction of bacterial growth from 2nd h up to 12th h. **Conclusion:**It is concluded from the present study, the CeO_2 nanoparticles could be used as an effective antibacterial agents for disease free fish management.

1. Introduction

The era of nanotechnology has allowed new research strategies to flourish in the field of drug delivery [1]. Now a days nanoparticle based drug delivery systems are suitable for targeting chronic intracellular infections in human as well as animals. The nanomedicine has the potential to revolutionize the various disease treatments in animal systems worldwide. Existing research has clearly demonstrated the feasibility of introducing nanoshells and nanotubes into animal systems which destroy the targeted cells. 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 co-enzymatic systems. The interaction of these nanoparticles with biologically 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 of bacterial resistance, new 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] and fish pathogens [5]. Moreover, the other metal nanoparticles particularly, the ZnO nanoparticles showed antibacterial activity against various bacterial pathogens includes *E.coli*, *Staphylococcus aureus* and *Bacillus Subtilis* respectively [6-8] .However, studies related with antimicrobial property of metal oxide nanoparticle against bacterial fish diseases are too limited. To fill up this gap, the present study made an attempt to find out effective antibacterial agents from various metal oxide nanoparticles and to evaluate the antimicrobial effects.

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2. Materials and Methods

Commercial nanoparticles of Al_2O_3, Fe_3O_4, CeO_2, ZrO_2, and MgO were procured from Sigma Aldrich Company, India. The characteristics of the nanoparticles are represented in Table 1.

2.1. Test Organisms

Five fish pathogens Viz., *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *serratia* sp. were obtained from Central Institute for Brackish water Aquaculture (CIBA), Chennai, Tamil Nadu, India.

2.2. Antibacterial assay

The antibacterial activity of the chosen nanoparticles was performed by using well diffusion method. About 20 ml of sterile molten Mueller Hinton agar (HiMedia Laboratories Pvt. Limited, Mumbai, India) was poured into the sterile petriplates. Triplicate plates were swabbed with the overnight culture (10^8 cells/ml) of pathogenic bacteria viz., *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *serratia* sp. The solid medium was gently punctured with the help of cork borer to make a well. Finally the nanoparticle samples (50 $\mu g.ml^{-1}$) were added from the stock into each well and incubated for 24 h at 37±2℃. After 24 h the zone of inhibition was measured and expressed as millimeter in diameter.

2.3. Minimum Inhibitory Concentration (MIC)

Different concentrations (10, 20, 30, 40, 50 and 60 $\mu g{\cdot}ml^{-1}$) of chosen nanoparticles were prepared with Dimethyl sulphoxide (DMSO) and mixed with 450 $\mu l.ml^{-1}$ of nutrient broth and 50 μl of 24 h old bacterial inoculum and allowed to grow overnight at 37℃ for 48 h. Nutrient broth alone served as negative control. Whole setup in triplicate was incubated at 37℃ for 24 h. The MIC was the lowest concentration of the nanoparticles that did not permit any visible growth of bacteria during 24 h of incubation on the basis of turbidity [9]

2.4. Minimum Bactericidal Concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any, the MBC was determined by sub-culturing the above (MIC) serial dilutions after 24 h in nutrient agar plates using 0.01 ml loop and incubated at 37°C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media [9]

2.5. Time kill assay

The potential nanoparticle (CeO_2) which showed maximum antibacterial activity against *Bacillus subtilis* was also subjected 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 $\mu g.ml^{-1}$) of CeO_2 nanoparticles and the total volume was made up to 5 ml by using minimal medium ($g.l^{-1}$) [Sucrose-10; K_2HPO_4-2.5;KH_2PO_4-2.5; $(NH_4)_2HPO_4$-1; $MgSO_4.7H_2O$-0.20; $FeSO_4.7H_2O$-0.01; $MnSO_4.H_2O$-0.007 and H_2O-1000 ml]. The negative control was maintained without the nanoparticle. The growth of the bacterial species was assessed at every 1 h interval by measuring the optical density at 600 nm by using spectrophotometer (Cyber UV-1, Mecasys Co Ltd) [10]

3. Results

The results of the present study reveal that, the CeO_2

Table 1.
Properties of nanoparticles

Formula	Molecular weight	Form	Particle size in TEM (nm)
Al_2O_3	101.96	Powder	<50
Fe_3O_4	231.53	Powder	9-11
CeO_2	172.11	Powder	<25
ZrO_2	123.22	Powder	<100
MgO	40.30	Powder	<30

Table 2.
Antibacterial activity of 5 metal oxides nanoparticles against fish pathogens

	Aeromonas hydrophila	*Bacillus subtilis*	*Vibrio harveyi*	*Vibrio parahaemolyticus*	*Serratia* sp.
	Zone of inhibition (mm dia)				
Al_2O_3	-	12±0.11	8±0.05	-	-
Fe_3O_4	-	11±0.28	8±0.40	-	-
Ceo_2	-	13±0.35	11±0.25	-	-
ZrO_2	-	12±0.30	9±0.30	-	-
MgO	9±0.40	8±0.30	6±0.49	6±0.43	6±0.20

- no sensitivity; mean±SD

Table 3.
MIC and MBC of 5 metal oxide nanoparticles against fish pathogens

	Aeromonas hydrophila		*Bacillus subtilis*		*Vibrio harveyi*		*Vibrio parahaemolyticus*		*Serratia* sp.	
	Concentration (μg.ml^{-1})									
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Al_2O_3	–	–	30	40	50	50	–	–	–	–
Fe_3O_4	–	–	40	40	60	50	–	–	–	–
Ceo_2	–	–	20	20	30	40	–	–	–	–
ZrO_2	–	–	30	50	50	40	–	–	–	–
MgO	50	50	60	60	–	–	–	–	–	–

nanoparticle showed maximum sensitivity (13±0.35 mm) against *Bacillus subtilis* and showed minimum activity (11 ±0.25 mm) against *Vibrio harveyi*. Likewise, Al_2O_3 and ZrO_2 nanoparticles showed maximum sensitivity (12±0.11 mm) and (12±0.3 mm) against *Bacillus subtilis* and showed minimum sensitivity against *Vibrio harveyi* (8±.05 mm) and (9±0.3 mm) respectively. The Fe_3O_4 nanoparticle showed activity (11±0.28 mm) against *Bacillus subtilis* and (8±0.40 mm) against *Vibrio harveyi*. The MgO nanoparticle showed sensitivity against all the tested pathogens. It showed maximum sensitivity (9 ±0.40 mm) against *Aeromonas hydrophila* followed by 8± 0.30 mm against *Bacillus subtilis* and 6±0.49 mm against *Vibrio harveyi* 6±0.49 mm against *Vibrio parahaemolyticus* and 6±0.20 mm against *Serratia* sp. (Table 2). In MIC assay, the nanoparticle CeO_2 showed maximum sensitivity (20 μg.ml^{-1}) against *Bacillus subtilis* and 30 μg.ml^{-1} against *Vibrio harveyi* respectively. However, the nanoparticles Al_2O_3 and ZrO_2 showed high sensitivity (30 μg.ml^{-1}) against *Bacillus subtilis* and against *Vibrio harveyi* (50 μg.ml^{-1}). The Fe_3O_4 showed sensitivity against *Bacillus subtilis* (40 μg.ml^{-1}) and 60 μg/ml against *Vibrio harveyi*. Moreover, the MgO nanoparticle showed sensitivity (50 μg.ml^{-1}) against *Aeromonas hydrophila* and (60 μg.ml^{-1}) against *Bacillus subtilis*. None of the nanoparticles showed sensitivity against *Vibrio parahaemolyticus* and *Serratia* sp. (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 h up to 12th h when compared to the control (Fig. 1).

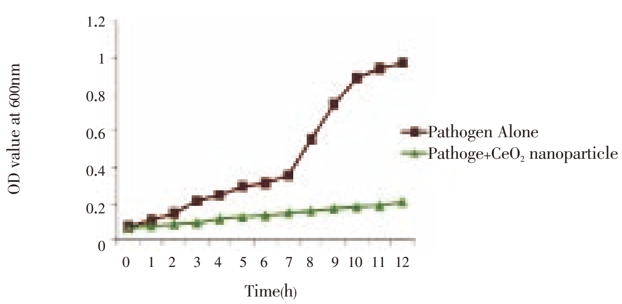

Figure 1. Time kill assay of CeO_2 nanoparticle against a fish pathogen *Bacillus subtilis*

4. Discussion

The fish sector contributes a major role in the aquaculture industry worldwide. These resources are expected to have high demand in national and international levels. Disease outbreaks in aquaculture as an important limiting factor in production and trade. Chemotherapeutics are drugs which are capable of affecting or killing microorganisms, especially bacteria in the fish culture [11]. Several chemicals viz., formalin, malachite green, methyl blue, copper sulphate and potassium permanganate have been used to cure the bacterial fish diseases [12-13]. But, these chemicals produced undesirable effects in the water as well as organisms [14]. Moreover, most of the biological resources such as mangroves, seaweeds, seagrasses and sponge etc. and silver nanoparticles showed antibacterial [15] and antifungal [16] activity. 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, five different metal nanoparticles have been used for the antibacterial property. Moreover, the advantages of inorganic antibacterial materials over organic antibacterial materials are that the superior durability, high surface area, less toxicity, heat resistance and more suitable for biological applications [17]. The antibacterial activity of 5 nanoparticles against fish pathogens viz., *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Serratia* sp. reveals that, all the nanoparticles showed activity against both gram positive as well as the gram negative bacterial strains. But the effect of the nanoparticles was found to be very high against gram positive bacteria than the gram negative bacteria. This might be due to the reactive oxygen species (ROS) mechanism. This mechanism can produce significant oxidative stress and altered the cell wall system into equally permeable levels[18]. Among the nanoparticles, CeO_2 nanoparticles showed maximum sensitivity against *Bacillus subtilis* and *Vibrio harveyi*. The remaining 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 [19]. 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 20 μg.ml^{-1} against *Bacillus subtilis* and 30 μg.ml^{-1} 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 [6]. 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 h up to 12th h. 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 [20]. It is concluded from the present study that, the CeO_2 nanoparticles could be used as an alternative antibacterial agents for the disease free fish culture systems.

Conflict of interest statement

We declare that we have no conflict of interest.

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