IN VITRO ECO FRIENDLY SYNTHESIS OF CADMIUM SULFIDE NANOPARTICLES USING HETEROTROPHIC BACILLUS CEREUS

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Biological application of nanoparticles is very much interesting and increasingly recognized for their utility especially in bio-nanomedicine. The present investigation was biological synthesis of cadmium nanoparticles using *Bacillus cereus* and toxic effect on prokaryotic pathogens. UV spectrum, XRD and SEM analysis of the cadmium sulfide nanoparticles indicated that they are in varying size from 30 to 100 nm size. Antibacterial activity of Cds nanoparticles against three clinical isolates of Gram positive *Staphylococcus aureus* bacterial isolates and two *Pseudomonas aeruginosa*, isolates analyzed. Gram positive *Staphylococcus aureus* was sensitive than Gram negative *Pseudomonas aeruginosa*. *Pseudomonas* species showed higher inhibitory effect than *Staphylococcal* isolates.

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1. Introduction

Nanotechnology is concerned with materials whose structures showed important novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nano size [1, 2]. Nanoparticles are having much attraction and attention for their unique characters that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface-to-volume ratios and an increased percentage of atoms at the grain boundaries [3]. The worldwide revolution in nanotechnology is predicted impact on several areas of biotechnology, biomedical research and scientific and engineering applications [4, 5]. Nanoparticles are being used in drug delivery, cell imaging, and cancer therapy are important biomedical applications of nanotechnology and nanobiotechnology.

There are a number of ways to synthesis different types of nanoparticles using physical, chemical, biological, and other hybrid methods. The popularity in synthesis of nanoparticles using physical and chemical process greatly regretted in biomedical applications especially in clinical due to use of toxic chemicals. Hence, the most importance has been given towards the reliable, nontoxic methods with exploitation of microorganisms in synthesis of nanoparticles [6]. Biological approaches to nanoparticles and nanocrystal synthesis have been extended to intact biological particles. The biological process is more acceptable green rout and is not energy intensive is also ecofriendly. This biogenic approach is greatly indented with bacteria by providing ambient conditions such as temperature, pH, pressure and etc., the nanoparticles synthesized by biological process are higher catalytic reactivity, greater specific surface area and also improve the enzyme and metal salt [6].

Cadmium sulfide nanoparticles synthesis of with different sizes and shapes makes great importance for their applications in the field of optical devices, electronics, biomedical and biotechnology [7, 8]. Microorganisms has the ability to regulate and synthesis of inorganic materials such as amorphous silica (diatoms), magnetite (magnetotactic bacteria), gypsum, and calcium carbonate layers (S-layer bacteria) and minerals such as calcite into functional superstructures endogenously [9]. So, it increases keen interest to exploit microorganisms such as bacteria and fungi for nanomaterial synthesis. Earlier investigations showed that number of organisms used for synthesis of cadmium nanoparticles such as *Clostridium thermoaceticum,* in the presence of cysteine hydrochloride *Klebsiella pneumonia* used Cd2++ ions [10, 11]. *Escherichia coli* also used for formation of Cds nanocrystals similarly *S. pombe* and *C. glabrata* produced Cds with cadmium salt solutions [12]. The present investigation was on eco-friendly synthesis of cadmium nanoparticles using *B. cereus* and their antibacterial activity reported.

2. Experimental

2.1 Isolation and maintenance

The *B. cereus* strain was isolated from coalmine soil by enrichment of the soil sample for one week with and Cadmium sulfate followed by standard method of dilution plating on Triptic soy agar (TSA) medium. The colonies obtained were purified, characterized authenticated in Kings Institute, Chennai. The pure isolate was routinely cultured on TSA plates containing Cadmium sulfate (0.01 mM). Cultures maintained in TSA slant medium at 4˚C.

2.2 Synthesis of Cds Nanoparticles

The cadmium stress culture grown in TS broth medium for 36 h at 37^{o}C. Grown cells were harvested by centrifugation (4000 X g) at 4^{o}C for 10 minutes. The bacterial cells were washed with sterile distilled water and phosphate buffer for three times. About 1g of wet bacterial cells suspended in saline phosphate buffer (0.8% NaCl and 10 mM) phosphate along with 1mM CdSO and incubated at 37^{o}C in orbital shaker (200 rpm) for 48 h [13].

2.3 Characterization of Cds Nanoparticles

UV-Vis spectroscopy measurement of the disrupted cell with reduced cadmium sulfate nanoparticle was carried out on JASCO dual-beam spectrophotometer (model V-570) operated at a resolution of 1 nm. To find the highest peak, a spectral scanning analysis was carried out by measuring optical density of the content from wavelength 250 to 700 nm. The powdered samples of cadmium sulfide nanoparticles were investigated with X-Ray diffraction method. XRD spectra were obtained using a XPERT-PRO diffractometer (0000000011014281) with the copper anode (40 KV and 30 mA) and scanning from 24o – 85o C at position 2 Θ. Crystalline deposits obtained from the surface of the treated mortar specimens were added to the sample holder. The XRD data was used to calculate Scherrer formula and stated the range of the nanoparticle size was determined. After incubation period the cells were harvested and washed three time physiological saline and fixation was carried out with Karnovsky fixative. Further dehydration process was done with alcohol, then freeze dried, then the samples were viewed under scanning electron microscope [14].

2.4 Antibacterial susceptibility test

The bacterial strains were clinical isolates of three *Staphylococcus aureus*, and two isolates of *Pseudomonas aeruginosa*, were obtained from Bose Laboratory, Madurai, India and maintained in nutrient medium. The antibacterial activity of cadmium nanoparticles against above human pathogenic bacteria were evaluated by using agar well diffusion method [15, 16]. The bacterial isolates were maintained on nutrient agar slants that contained peptone, 5.0; beef extract, 3.0; yeast extract 5.0; sodium chloride, 5.0; and agar 15.0 g per liter of distilled water. Muller

Hinton Agar (MHA) plates were inoculated with 100 of standardized inoculum ($1.5x10^8$ CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells or cups of 6 mm size were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. Freshly prepared Cds nanoparticles with different concentration of (100, 200, 400 and 800 µg) were added into the wells. Streptomycin (25 µg/ml) and sterilized distilled water were used as a positive and negative control respectively. The plates thus prepared were left at room temperature for 15minutes allowing the diffusion of the extract into the agar [14]. After incubation for 24 h at 37^{o}C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the Cds nanoparticles. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm [17]. The mean and standard deviation of the diameter of inhibition zones were calculated.

3. Results and discussion

3.1 Synthesis of cadmium nanoparticles

B. cereus was constantly exposed to cadmium in the medium during growth indicated that the organism consequently raising the tolerance in toxic condition. Further the culture was subjected to cadmium sulfide in saline phosphate buffer medium and incubated for 48h. UV – visible spectrum analysis is a very much important technique to assess the formation of nanoparticles in aqueous medium. The formation of nanoparticle indicated due to colour change in the medium, and thus, it is confirmed withUV spectrum peak for cadmium sulfide nanoparticles expressed 270 to 400 nm (Fig. 1). Previous reports also stated that the different culture times exhibit different optical properties and the absorption peaks for Cds obtained at 36 and 42 h are 282 and 332 nm using immobilized *Rhodobacter sphaeroides* [13]. Another report also exhibited the absorption for the cadmium nanoparticle was ~330 – 410 nm [18]. The change in the colour and UV visible pattern indication that the formation of nanoparticle in the medium with the progress of process similarly it was reported that growth progress of nanoparticle synthesis in the medium with *R.palustris* comparatively among the growth phases [19]. The diffraction peaks obtained in the XRD spectra (Fig. 2) showed the consequence of reduction of particle size phase of cadmium sulfide nanoparticle. The cubic peak planes at $2\theta = 20^o$ and they are in amorphous nature with varying size range from 30 to 100nm. It can be concludes as nanocomposite with the peak values calculated by Scherrer's equation [20, 21]. Reportedly, the exitation peak for the cadmium sulfide nanoparticles are time dependent and activity of cellular matrix. Scanning electron micrographic view confirmed the status of the nanoparticles displayed in (Fig. 3(a-d)). Dried powder of Cds scanned with Scanning electron microscope showed that the synthesized nanoparticles were various size and shape and had different morphology. The size of the particles ranged between 30 and 200 nm and the shape of the particles were found to be nanocomposite.

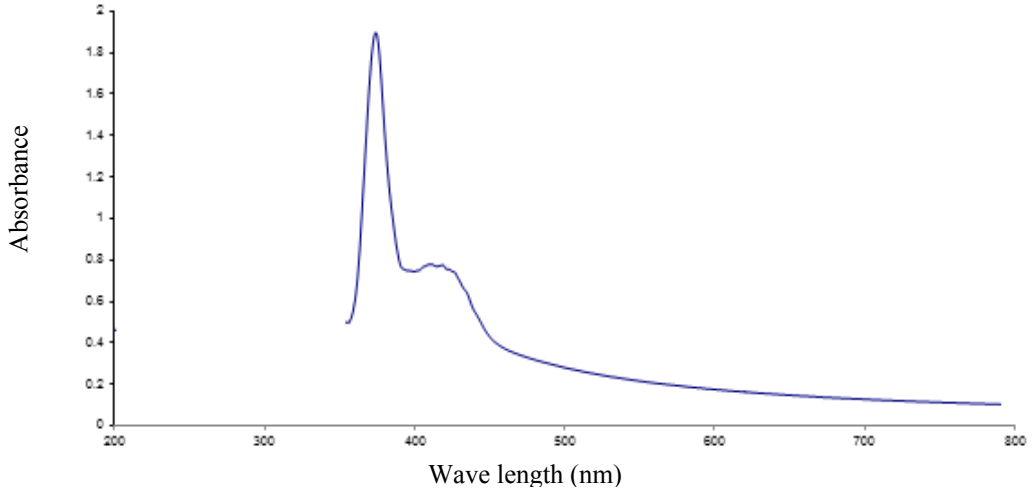

Fig.1. UV-Vis Spectra for Cadmium sulfide nanoparticles

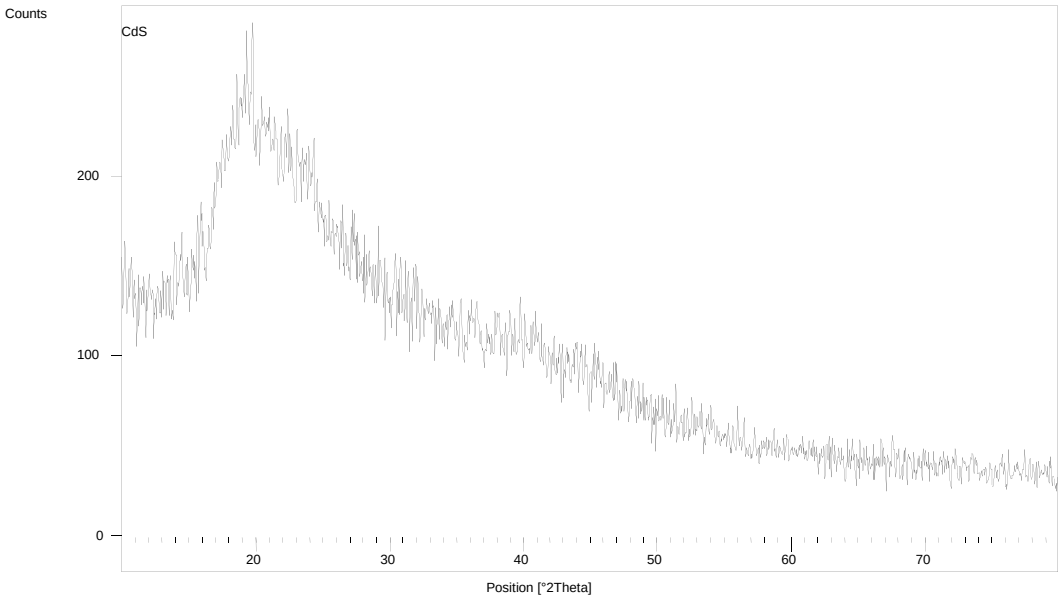

Fig.2. XRD for Cds Nanoparticles

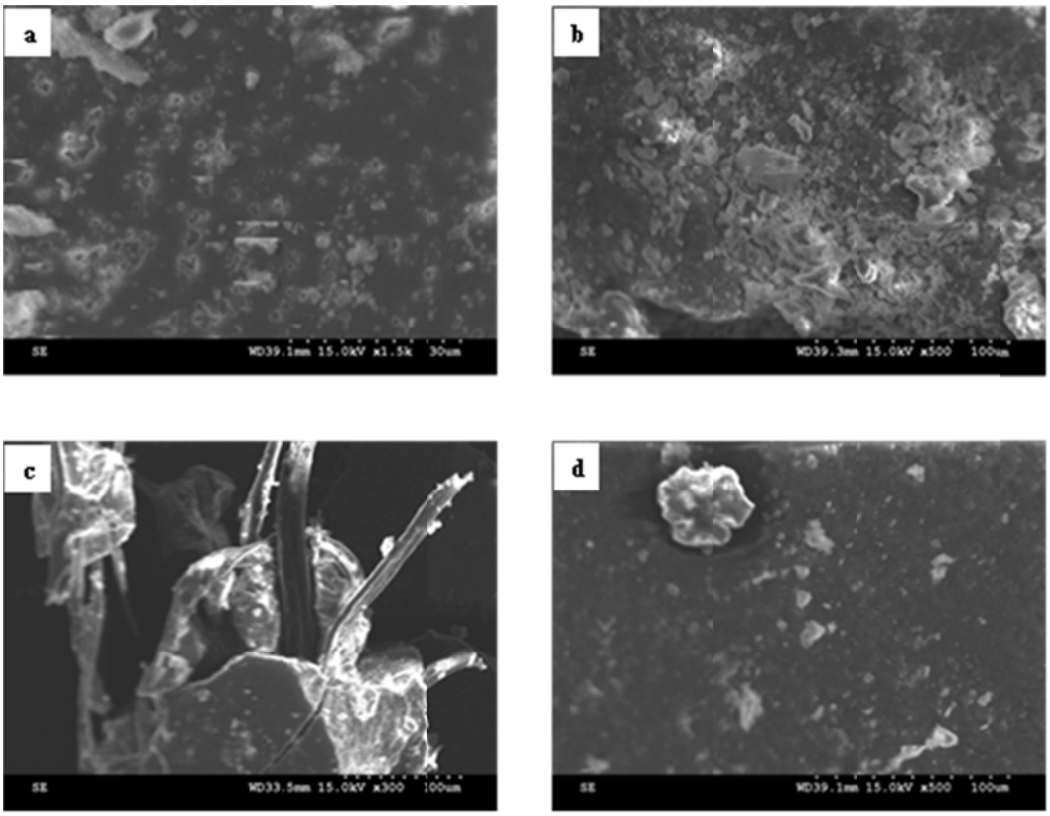

Fig. 3. Scanning electron microscopic view of Cds

3.2 Antibacterial senestivity test

The antibacterial activity of cadmium nanoparticles were tested against five different clinical isolates using the diameter of inhibition zone (DIZ) in agar well diffusion test. The DIZ reflects magnitude of susceptibility of the microorganism. The strains susceptible to Cadmium sulfide nanoparticles exhibited larger DIZ, whereas resistant strains exhibited smaller DIZ. Since DIZ was measured on agar plates using a ruler with 1 mm resolution, the possibility of measurement errors exist; however, the method illustrates the potential biocidal effect of nanoparticles to different clinical bacterial strains.

Fig. 4 showed the presence of antibacterial effect of the nanoparticles on the corresponding clinical strains. The cadmium nanoparticles displayed zone inhibitions toward the tested clinical strains were *P. aeruginosa* (8-20 mm) and *S. aureus* (10-18 mm), with respect to different concentration nanoparticles viz., 100 to 800 µg. Among five clinical isolates *P. aeruginosa* strain 1 depicted the highest sensitivity to nanoparticles compared to the other strains and was more adversely affected by the cadmium nanoparticles that were observed between DIZ observed in disk diffusion test. The clinical isolates also tested against streptomycin (25 µg/ml) and values reported for *P. Aeruginosa* and *S. aureus*were 22 to 24 mm respectively (22-28).

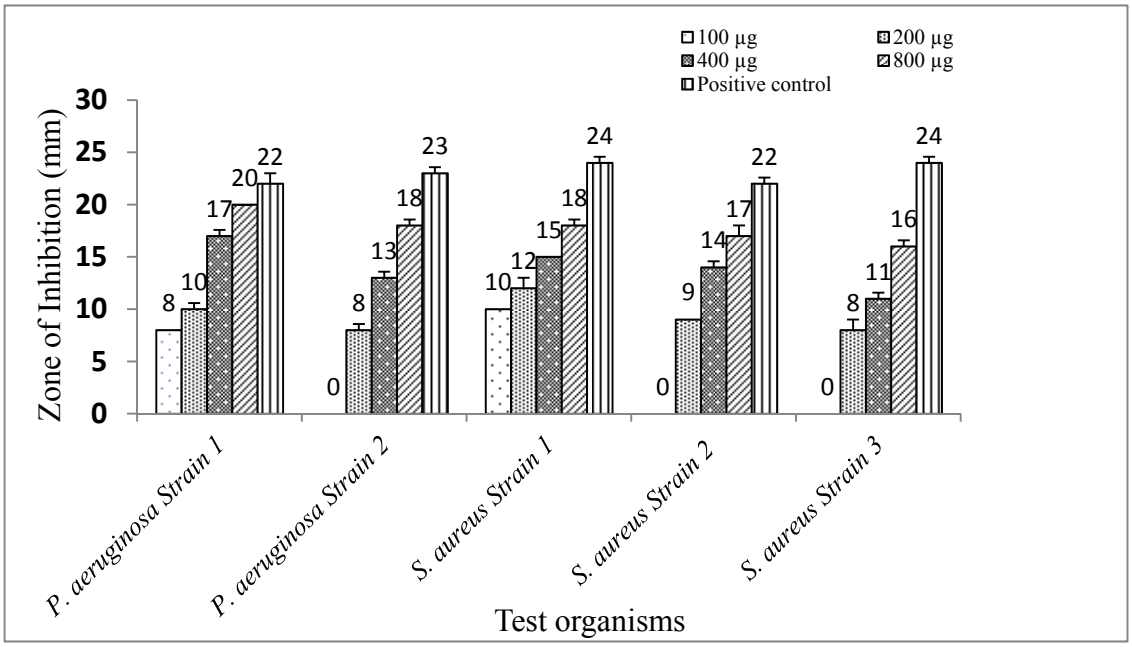

Fig. 4. Antimicrobial activity of Cds nanoparticles

4. Conclusions

The Bacillus cereus species isolated from mining are has the ability to resuce to produce cadmium sulfide nanoparticles. It has been confirmed with UV, XRD and Scanning electron microscopy. And the nanoparticles has potential antibacterial actitiy against clinical isolates. This is an inexpensive procdure and ecofriendly process to produce this nanoparticles. Hence, this kind of microorganisms can be used for synthesis of nanoparticles and heavy metal absorption for detoxification of environment.

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