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Biogenic Approach for the Synthesis of Titanium Dioxide Nanoparticles Using a Halophilic Bacterial Isolate - *Chromohalobacter salexigens* Strain PMT-1

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A B S T R A C T

Green nanotechnology in the development of material synthesis is of considerable importance to expand their biological applications. Currently, a wide array of inorganic nanoparticles has been synthesized by using biogenic enzymatic methods and their applications in many cutting-edge technological areas have been explored. In the present work, Titanium dioxide nanoparticles were synthesized by using the culture supernatant of *Chromohalobacter salexigens* strain PMT-1 cells. Further, the sample was characterized by UV-Visible spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The antibacterial activity of the biosynthesized TiO_2 nanoparticles was tested against *Staphylococcus aureus*, *Escherichia coli and Serratia marcescens*. Maximum zone of inhibition was observed against *Escherichia coli*. Thus the biogenic TiO_2 nanoparticles can be explored in biomedical and nanotechnology applications without any adverse side effects.

Introduction

Ever since inception of life on the earth, the biological entities and inorganic materials have been in constant touch with each other. Due to this regular interaction, life could sustain on this planet with a well-organized deposit of minerals. Recently the global research focus is aimed towards the interaction between inorganic molecules and biological species (Li *et al.*, 2011).

Nanoparticles have attracted scientific attention due to their fascinating properties, commercial and biotechnological applications advantageous over their bulk counterparts (Daniel and Astruc, 2004). Different types of nanoparticles are synthesized by a large number of physical, chemical, biological, and hybrid methods (Luechinger *et al.*, 2010; Tiwari *et al.*, 2008). Although physical and chemical methods are routinely employed in the synthesis of nanoparticles, the utility of harsh and potentially hazardous chemicals, requirement of high energy, capping agents for size stabilization and involvement of difficult separation techniques greatly suppress their role in clinical and biomedical applications (Sundrarajan and Gowri, 2011). Therefore, development of safe, eco-friendly, reliable, and nontoxic methods for synthesis of nanoparticles is of utmost importance to expand their biomedical applications (Li *et al.,* 2011).

Biogenic enzymatic process for the production of nanoparticles has been proven to be far superior, in several ways, to those particles produced by physical and chemical methods. Biogenic approach involving a wide range of microorganisms including bacteria, yeast, fungi and actinomycetes can be categorized into intracellular and extracellular synthesis according to the location where nanoparticles are formed (Li *et al.,* 2011). Currently different variety of nanoparticles with well-defined chemical composition, structure and particle size has been synthesized by using different ambient microorganisms and their applications in many cutting-edge technological areas have been explored. Among the various metal oxide nanoparticles, titanium dioxide nanoparticles have wide applications in environmental remediation due to their potential oxidation strength, high photo stability and non-toxicity (Ochiai and Fujishima, 2012). Moreover titanium nanoparticles possess interesting optical, dielectric, antimicrobial, antibacterial, chemical stability and catalytic properties which leads to industrial applications such as cosmetics, pharmaceuticals, pigment, fillers, whitening and brightening foods, daily used hygiene products such as tooth paste and orally - administered drugs, catalyst supports and photocatalyst (Powell *et al.*, 2010; Kirthi *et al.,* 2011). In addition, the non- toxic and biocompatible properties of TiO_2 find its applications in biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries (Malarkodi *et al.*, 2013).

The present study deals with the ecofriendly biogenic approach for the synthesis of TiO_2 nanoparticles using the culture supernatant of *Chromohalobacter salexigens* strain PMT-1 cells and its characterization by UV–Vis spectra, XRD, SEM and TEM analysis. Further the antibacterial activity of the biosynthesized TiO_2 nanoparticles was tested against *Staphylococcus aureus, Escherichia coli and Serratia marcescens.*

Materials and Methods

Biogenic approach for the synthesis of TiO_2 nanoparticles

Chemicals used

$TiO(OH)_2$ (99.9 %) was procured from Sigma Aldrich Chemicals, Bangalore, India. All other regents used in the reaction were of analytical grade with maximum purity. Deionized water was used throughout the experiment. The glass wares were washed in dilute nitric acid and thoroughly washed with double distilled water and dried in hot air oven.

Bacterial strain used

The bacterial strain used in this study was isolated from fish pickle and fish sauce samples collected from the local markets. Based on the morphological, cultural, biochemical characteristics and 16s rDNA sequencing, the isolate was identified as *Chromohalobacter salexigens* strain PMT-1.

The pure cultures were maintained on halophilic agar slants at 4°C.

Synthesis of TiO_2 nanoparticles

Chromohalobacter salexigens strain PMT-1 cells were allowed to grow as suspension culture for 1 week at 37°C in shaking condition at 120 rpm and this was treated as source culture. 50 ml of the halophilic cultural broth was taken and centrifuged at 8000 rpm for 10 minutes. Following centrifugation, 20 ml of the culture supernatant was transferred to sterile tube, and mixed with 20 ml of 0.025M $TiO(OH)_2$ to form a ratio of 1:1. The mixture was subjected to heating using water bath at 80°C for 10–20 min until white deposition starts to appear at the bottom of the flask, indicating the initiation of transformation. The culture solution was cooled and allowed to incubate at room temperature in the laboratory ambience. After 12–48 h, the culture solution was observed to have distinctly markable coalescent white clusters deposited at the bottom of the flask (Kirthi *et al.*, 2011).

Characterization of synthesized TiO_2 NPs

The bioreduction of ions in the solution was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measured in the UV–Vis spectra. Samples were monitored as a function of time of reaction using Shimadzu 1601 spectrophotometer in the 100–700 nm range operated at a resolution of 1 nm. The reduced solution centrifuged at 8000 rpm for 40 min and resulting supernatant was discarded and pellet obtained was redispersed in deionized water. Centrifugation was repeated three to five times to wash off any adsorbed substances on the surface of the synthesized NPs. Thus obtained purified and dried pellet of synthesized TiO_2 NPs were subjected to X-ray diffraction (XRD) analysis. The particle size and morphology of the titanium dioxide nanoparticles were examined using Scanning electron microscopic observations. SEM measurements were performed on a JEOL JSM 6390 instrument operated at an accelerating voltage at 15kV. The size of the NPs was confirmed by using TEM analysis (Transmission electron microscopy – Hitachi H-7100) using an accelerating voltage of 120 kV and methanol as solvent.

Antibacterial activity of TiO_2 nanoparticles

The antibacterial effect of TiO_2 nanoparticles were examined by disc diffusion method against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli and Serratia marcescens* collected from lab stock. Muller Hinton agar was prepared and poured onto the sterile petriplates. After solidification, 2 wells were cut (for test and control) and each culture was swabbed individually on the respective plates.

The synthesized TiO_2 nanoparticles were diluted with distilled water (15µg/ml) and placed onto each wells and incubated for 24 hours. Following incubation the zone of inhibition against nanoparticle were observed and measured (Yokeshbabu *et al.*, 2013).

Results and Discussion

Nanotechnology has recently emerged as an elementary division of science and technology that investigates and regulates the interaction at cell level between synthetic and biological materials with the help of nanoparticles.

Characterization of TiO_2 nanoparticles

UV spectroscopic analysis

The onset wavelength of the optical absorption for uncapped TiO_2 appears at 366 nm in UV–vis spectroscopy, which is blue shifted compared to the bulk anatase TiO_2, indicating the formation of nanoparticles solution (Fig. 1). The surface modification of nanocrystalline anatase TiO_2 particles with orthosubstituted hydroxylated enediols ligands which improves the optical response in the visible region. Similar results were reported many researchers (Kirthi *et al.*, 2011; Malarkodi *et al.*, 2013).

X-ray diffraction analysis

The crystal structure of the TiO_2 nanoparticles was analyzed by X-ray diffractometer. The formation of titanium dioxide nanoparticles synthesized using the culture supernatant of *Chromohalobacter salexigens* was supported by X-ray diffraction measurements. XRD analysis for the synthesized TiO_2 NPs showed distinct diffraction peaks at 26.18°, 29.41°, 30.91°, 33.08°, 41.43°, 54.49°, 56.80° and 69.16° indexed to the planes 110, 101, 111, 211, 220 and 301, respectively (Fig. 2), indicating that nanoparticles structure dominantly correspond to anatase crystalline (Byranvand *et al.*, 2013), which is regarded as an attributive indicator of the biologically synthesized nanoparticles TiO_2 crystallites (Kirthi *et al.*, 2011). TiO_2 is preferred in anatase form because of its high photocatalytic activity (Macwan *et al.*, 2011).

SEM and EDAX analysis

The SEM images of titanium dioxide nanoparticles obtained with the culture supernatant of *Chromohalobacter Salexigens* strain PMT-1 is shown in the figure 3. The formation of titanium dioxide nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 96.8 -163.3 nm with interparticle distance, whereas the shapes were uniformed spherical and ellipsoidal. The average size of titanium dioxide nanoparticles synthesized using *Nyctanthes arbortristis* leaf extract was found to be in between 100-150 nm, whereas the shapes were uniformly spherical (Sundrarajan and Gowri, 2011). Analysis through EDAX confirmed the presence of elemental Titanium signal of the TiO_2 nanoparticles (Fig. 4). The weight percent of TiO_2 nanoparticle was found to be 68.97 %.

Transmission electron microscopy

The TEM images and their corresponding particle size distributions of TiO_2 NPs at different periods of time. The TEM images and their size distributions revealed that, the mean diameters of TiO_2 NPs were about 20, 20.1, 200 nm. These results approved that with increase in time of reaction at a moderate temperature, mean diameters and standard deviations of the TiO_2 NPs gradually increases. The TEM images and their size distributions of titanium dioxide nanoparticles obtained with the culture supernatant of *Chromohalobacter Salexigens* PMT-1is shown in figure 5.

Antibacterial activity of TiO_2 nanoparticles

Nanomedicine is a burgeoning field of research with tremendous prospects for the improvement of the diagnosis and treatment of human diseases. The antibacterial activity of TiO_2 nanoparticles was carried out by well diffusion method against *Staphylococcus aureus*, *Escherichia coli and Serratia marcescens*.

Fig.1 UV-Vis absorption spectrum of TiO_2 Nanoparticles

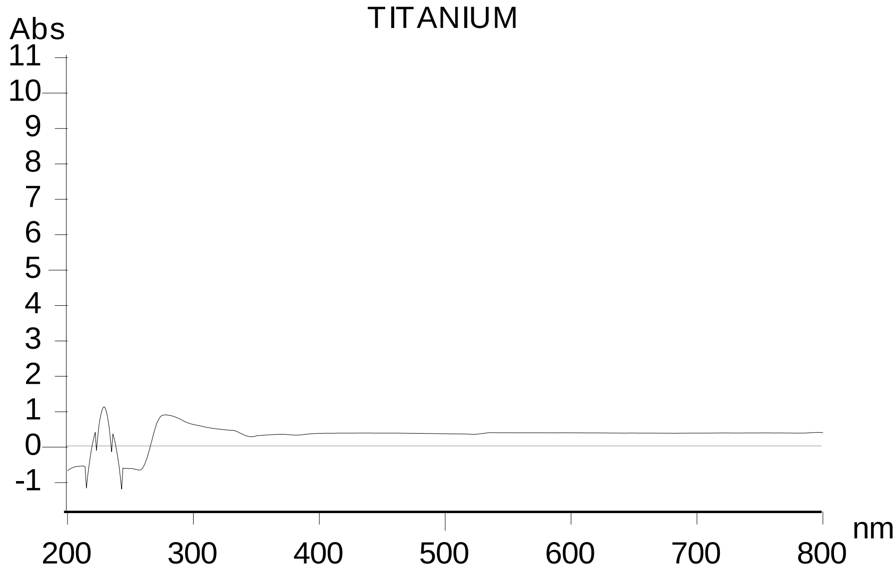


Fig.2 XRD Pattern of biosynthesized TiO_2 nanoparticles

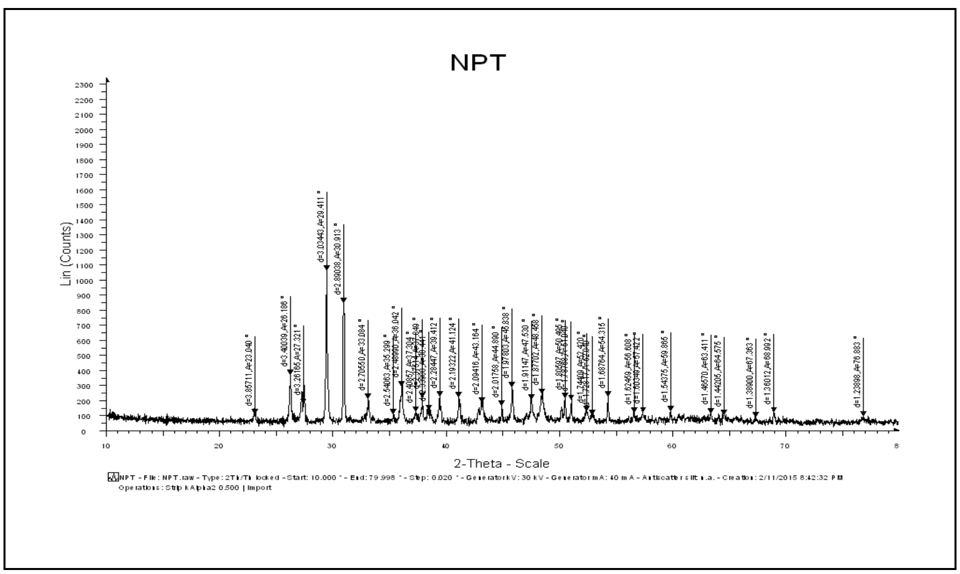


Fig.3 SEM Images of TiO2 Nanoparticles synthesized using *Chromohalobacter salexigens*

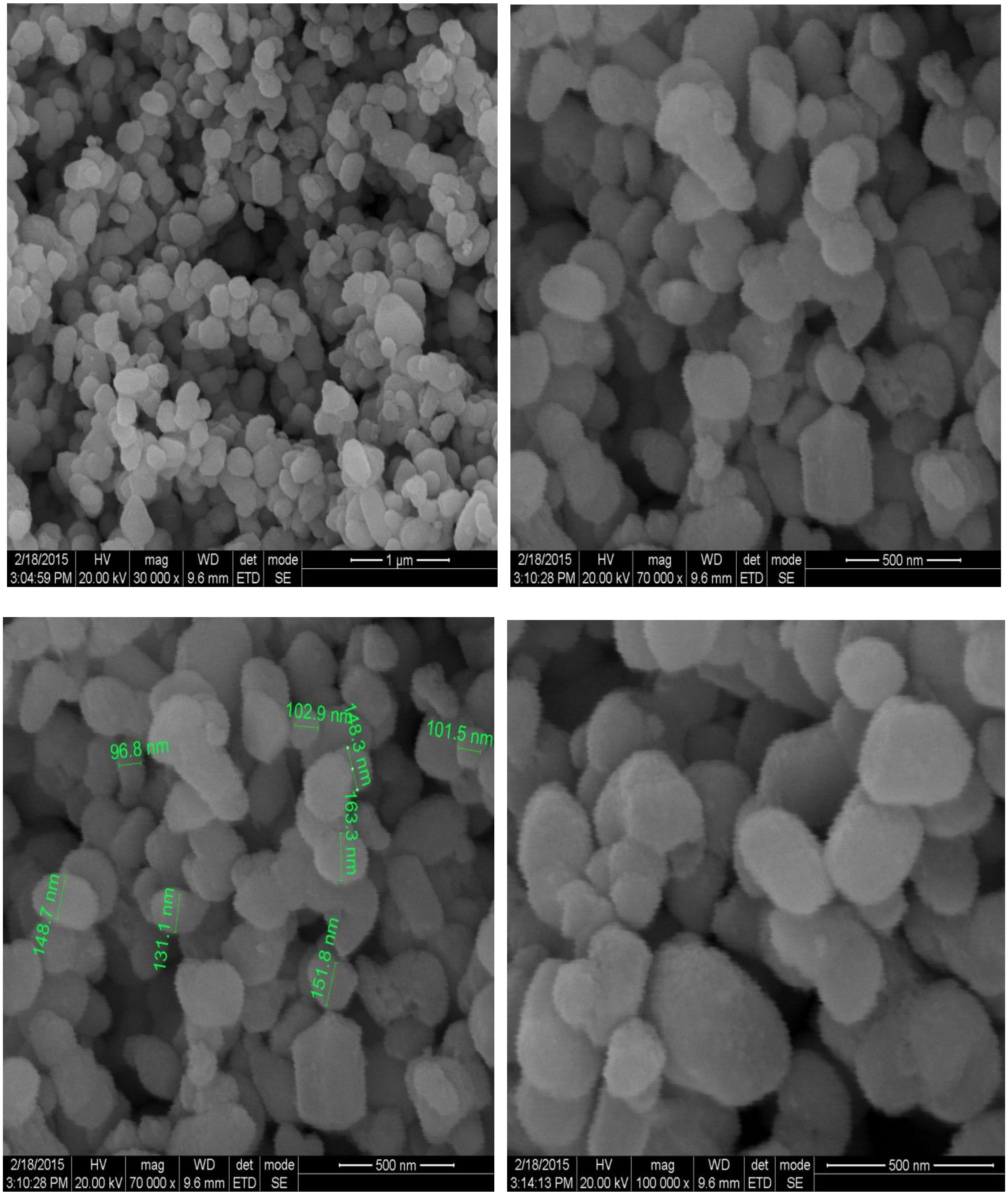


Fig.4 EDAX of TiO2 Nanoparticles synthesized using *Chromohalobacter salexigens*

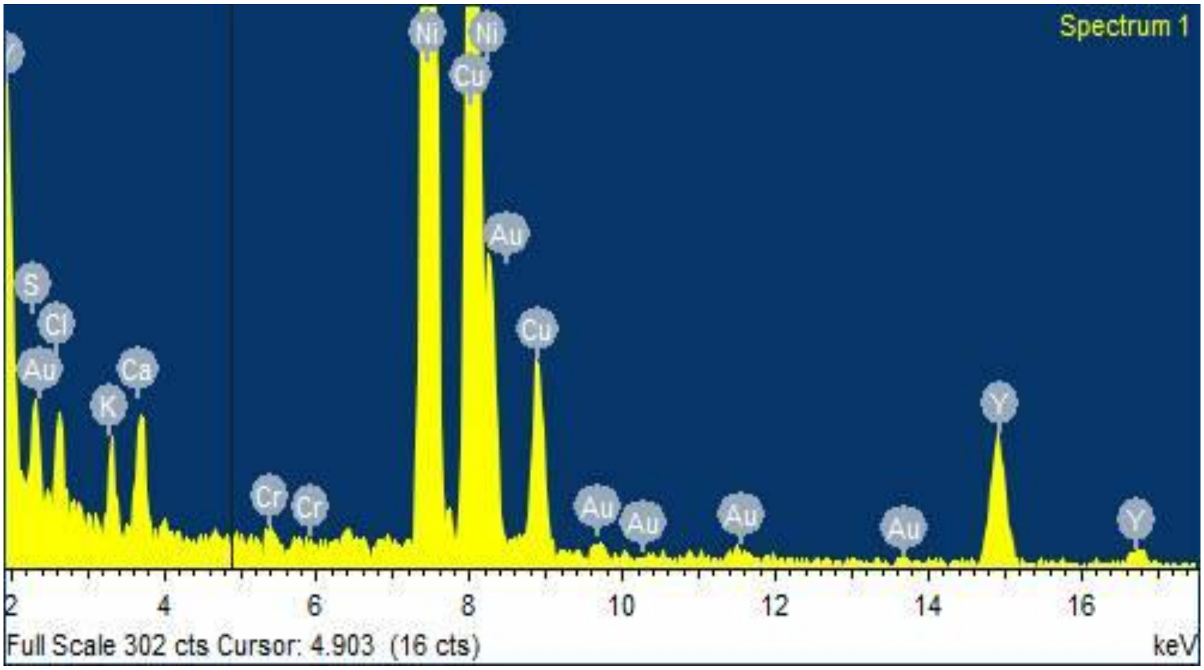
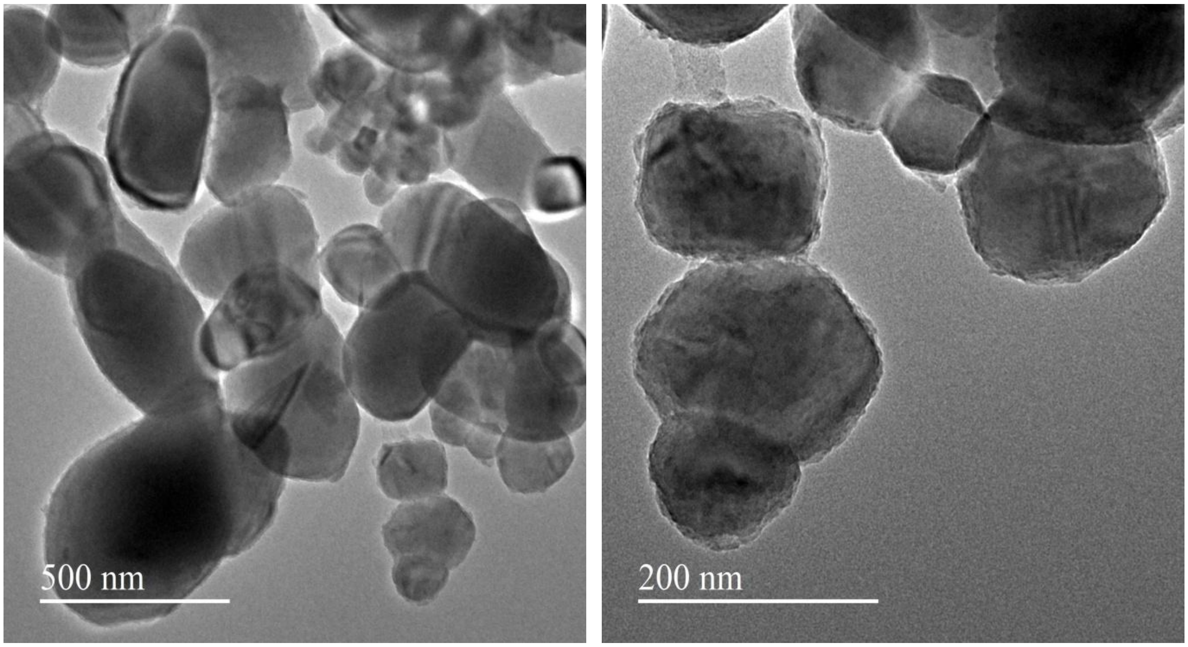


Fig.5 TEM Images of TiO2 NP'S synthesized using *Chromohalobacter Salexigens*



TiO_2 nanoparticles exhibited maximum antagonistic activity on *Escherichia coli* (17mm). The differential sensitivity of Gram-negative and Gram-positive bacteria towards nanoparticles depends upon their cell outer layer attribute and their interaction with the charged TiO_2 nanoparticles. TiO_2 nanoparticles biosynthesized by using the culture supernatant of *Planomicrobium* sp. exhibited remarkable antagonistic activity against *Bacillus subtilis* and *Klebsiella planticola* respectively (Malarkodi *et al.*, 2013). The good antibacterial effect (100% killing efficiency) of TiO_2 nanoparticles may be due to small size, large surface area, large band gap energy, and more active sites of TiO_2 nanoparticles for carrying out photocatalytic reactions. TiO_2 nanoparticles mediated photocatalytic reactions leads to the production of highly reactive species such as hydroxyl radical, hydrogen peroxide, and superoxides that can cause great damage to microorganisms (Blake *et al.*, 1999). TiO_2 photocatalyst may destroy the outer membrane of the bacterial cell, directly leading to the leakage of minerals, proteins, and genetic materials, causing cell death (Sunada *et al.*, 1998).

Conclusion

A wide range of nanophasic and nanostructured particles are being fabricated globally with the aim of developing clean, nontoxic and eco-friendly technologies. Exploration of ambient biological resources in this area of science is rapidly gaining importance owing to its growing success and simplicity. TiO_2 NPs synthesized in the presence of *Chromohalobacter salexigens* showed its potential antibacterial activity against the opportunistic pathogen – *E. coli*, thereby proving its significance in biomedical applications.

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