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RESEARCH ARTICLE

BIOGENIC SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EFFECT OF TIO2 NANOPARTICLES

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ABSTRACT

In recent years, nanotechnology has been explosion. Nano-structured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications. Nanotechnology has become one of the most practical technologies, because of unique physical and chemical properties of nanomaterials. Our studies discuss recent advances in the synthesis, characterization, toxicity and antibacterial effect of TiO_2 nanoparticles obtained mainly through biogenic processes. The importance of biogenic synthesized nanoparticles has been increasing in recent years; however, more studies aimed at better characterizing the potent toxicity of these nanoparticles are still necessary for nanosafely considerations and environmental perspectives. 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 *Lactobacillus* sp. that was obtained from yoghurt. Lactobacilli have a negative electro kinetic potential, which is suitable for the attraction of cations, a step that is required for the biosynthesis of metallic nanoparticles. Ultrafine size and morphological properties of synthesized nanoparticles was determined by TEM, SEM and X- ray diffraction where the size of Nanoparticles were 5-20nm, found morphologically as aggregate form. The antibacterial activity of the biosynthesized TiO_2 nanoparticles was tested against *Staphylococcus aureus* and *Escherichia coli*. 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.

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INTRODUCTION

Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructures materials, play an ever increasing role in science, research and development as well as also in every day's life, as more and more products based on nanostructures materials are introduced to the market. The rapidly expanding field of nanotechnology is producing a wealth of new consumer products and pharmaceuticals (Rejeski 2009). One nanomaterial that has received considerable attention is titanium dioxide ($nTiO_2$).

$nTiO_2$ ranks among the most abundant of engineered nanomaterials (Borm *et al*. 2006; Mueller and Nowack 2008), and it is increasingly used in the production of solar cells, cosmetics, pharmaceuticals, paints, and food-products (Ju-Nam and Lead 2008; Sugibayashi *et al*. 2008).Titanium dioxide (TiO_2) is a photo catalyst and widely utilized as a self-cleaning and self disinfecting material for surface coating in many applications. It has a more helpful role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect Haghi *et al.* (2012). Titanium dioxide (TiO_2) is a photo catalyst and widely utilized as a self-cleaning and self disinfecting material for surface coating in many applications, titanium dioxide has a more helpful role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect.

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These properties have been applied in removing bacteria and harmful organic materials from water and air, as well as in self-cleaning or self sterilizing surfaces for places such as medical centers. Currently, there is considerable interest in the antimicrobial property of TiO_2 for applications in the food industry. TiO_2 is nontoxic and the American Food and Drug Administration (FDA) has approved TiO_2 for use in human food, drugs, cosmetics, and food contact materials. The photocatalytic reaction of TiO_2 has been used to inactivate a wide spectrum of microorganisms (Long *et al*. 2014; Altin and Sokmen, 2014; Gupta *et al.* 2013.). The bactericidal and fungicidal effects of TiO_2 on, for example, *Escherichia coli* (*E. coli)*, *Staphylococcus aureus*, and *Pseudomonas putida* have been widely reported (Bonetta *et al*. 2013; Yao and Lun Yeung 2011). The development of TiO_2-coated or incorporated food packaging has also received attention (Zhou *et al.* 2009; Luo *et al*. 2013; Gumiero *et al*. 2013; Chawengkijwanich *et al.*2008).

MATERIALS AND METHODS

Chemicals used

$TiO(OH)_2$ (99.9 %) was purchased from Hi-Media India. All other regents used in the reaction were of analytical grade with maximum purity. Deionized water was used throughout the experiment. All the glass wares were washed in dilute nitric acid, distilled water and dried in hot air oven.

Biological synthesis of TiO_2 nanoparticles

TiO_2 Nanoparticles by lactobacillus

Nanoparticles of TiO_2 were prepared using the procedure adopted by Nair and Pradeep (2002) with *lactobacillus* that was obtained from yogurt and probiotic tablets. Yogurt containing *lactobacillus* 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 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. In the presence of suitable carbon and nitrogen sources, lactobacillus or yeast cells interact with a $TiO(OH)_2$ solution to produce TiO_2 nanoparticles (8–35 nm) with few aggregates. Nanoparticles containing culture solution was filtered under the laminar flow through whatman filter paper, allowed drying under blow of hot air after which they were used for characterization by TEM SEM and X-ray diffraction.

Characterization of TiO_2 nanoparticles

The X-ray diffraction (XRD) patterns of nanoparticles were carried out with a diffractometer (PANalytical X'Pert Pro) using Cu Kα radiation at 45 kV and 40 mA. Size characterization of the 0.1 % w/v TiO_2 Nanopartilcles sample dispersed in 10 mM NaCl was made on a Zeta Sizer Nano ZS at 25°C. Particle size distribution obtained from Zeta sizer Nano ZS. Scanning Electron Microscopic (SEM) analysis was carried out using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes. Further characterization of the nanoparticles involved the use transmission electron microscope (TEM) to comprehend the morphology, size and the distribution of nanoparticles.

Antibacterial activity of TiO_2 nanoparticles

The antibacterial effect of TiO_2 nanoparticles were examined by agar well diffusion method on Nutrient agar medium and the test organisms used were *Staphylococcus aureus (*gram positive) and *Escherichia coli* (gram negative). The respective test organisms were prepared by spreading 500 µL of revived culture on the nutrient agar plate. 6 wells were cut with the help of a sterilized stainless steel cork borer into which different concentrations of TiO_2 nanoparticles solution (10, 20, 30, 40, 50 and 100 µL) was loaded and incubated at 37° C. The plates were examined for presence of zones of inhibition indicative of the antibacterial activity.

RESULTS AND DISCUSSION

Characterization of TiO_2 nanoparticles

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 lactobacillus was supported by X-ray diffraction measurements. The wide-angle region of the X ray diffraction (XRD, PANalytical X'pertPRO) patterns exhibited a high-intensity diffraction peak at $2\theta = 25.47$ and three additional peaks at $2\theta = 38.03$, 48.17 and 68.8 with the respective size 15.3, 9.2, 7.3 and 10.4 nm. XRD analysis for the synthesized TiO_2 NPs showed distinct diffraction peaks (Fig. 1), 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).

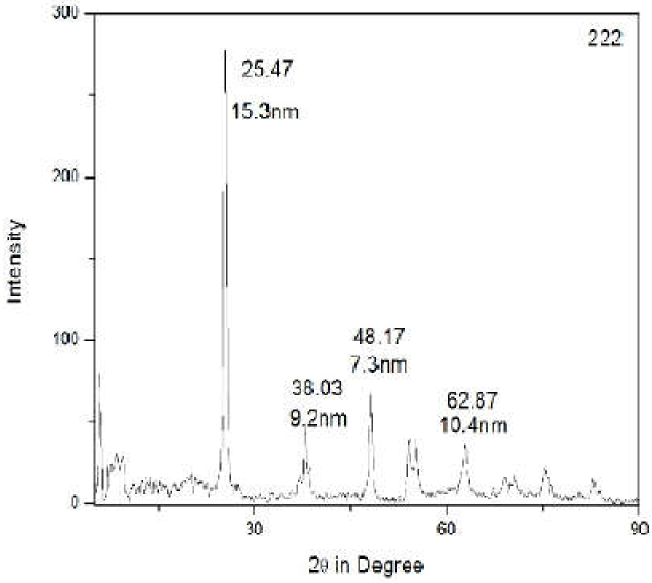

Figure 1. Characterization of TiO_2 Powder X-ray diffraction patterns of anatase TiO_2 with Miller indices (h k l) showing crystal family of planes for each diffraction peak

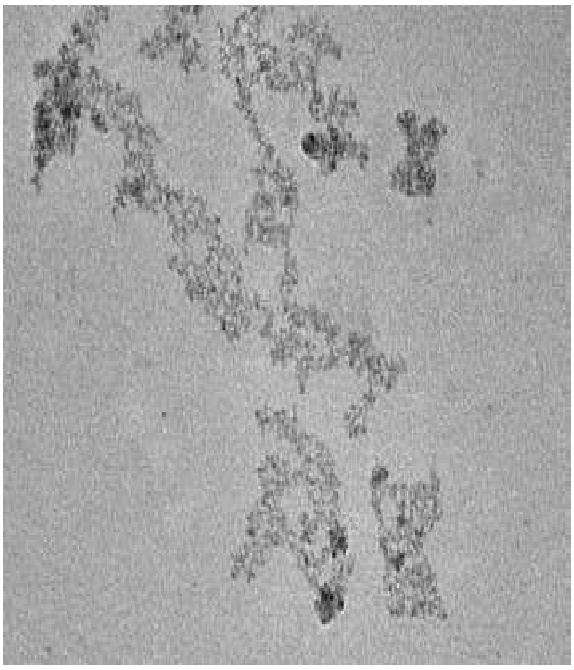

Figure 2. TEM image of TiO2 NPs size is calculated with TEM and Range from 5-20 nm. Analysis was done at 200kv

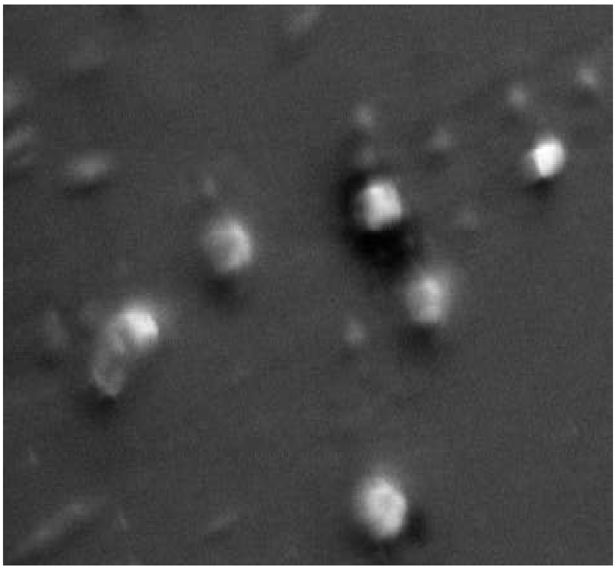

Figure 3. Morphology and microstructure characterization by SEM

TEM

The size of the TiO_2 nanoparticles (TNPs) was determined by transmission electron microscopy (TEM, JEOL-JEM-2100F).Figure 2 shows the typical TEM images of TNPs, from which it was observed that the TNPs had an average diameter of 5-20 nm. Clustering of NPs was also observed from the images.

SEM

SEM is the scanning electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material. Magnification is of order 10,000 X and resolution 10 nm (Figure. 3).

Antibacterial activity of TiO_2 nanoparticles against *Staphylococcus aureus* and *Escherichia coli*

The antibacterial activity of TiO_2 nanoparticles was carried out by Agar well diffusion method against *Staphylococcus aureus* and *Escherichia coli* by zone inhibition. The results of zone inhibition method has been described from the Figure 4 and 5, it is seen that TiO_2 NPs shows good inhibition zone in both of gram positive and gram negative bacteria but it has more antibacterial properties for *E. coli* compared to *S. aureus.* For testing the susceptibilities of *S.aureus* and *E. coli,* we used Disc diffusion assay, and it was seen that as concentration of TiO_2 nanoparticle has been increased, simultaneous increase of diameter of zone of inhibition of both was observed in Table-1. It is evident from the zone of inhibition that TiO_2 nanoparticles possess potent bactericidal activity.

Figure 4. Inhibition zone in *S.aureus*

Figure 5. Inhibition zone in *E.coli*

Table 1. Agar well diffusion method of TiO_2 nanoparticles against *Staphylococcus aureus* and E. *coli*

Concentration of TiO_2(µL)	Diameter of zone of inhibition (mm)in S.aureus	Diameter of zone of inhibition (mm) in E. coli
10	8	11
20	9	13
30	11	16
40	14	17
50	16	22
100	19	26

Nanomaterials exhibit strong inhibiting effects towards a broadened spectrum of bacterial strains. According to several studies, it's believed that the metal oxides carry the positive charge while the microorganism carry negative charge, this causes electromagnetic attraction between microorganism and the metal oxides which leads to oxidization and finally death of microorganism (zhang and chen 2009). In this study, Antibacterial effect of different concentrations of TiO_2 was studied. *E. coli* is one of the most important causative agents of nosocomial infections and resistant to most of the broad spectrum antibiotics.

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