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Biosynthesis of Titanium Dioxide Nanoparticles and Their Antibacterial Property

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***Abstract*—**This paper presents a low-cost, eco-friendly and reproducible microbe mediated biosynthesis of TiO_2 nanoparticles. TiO_2 nanoparticles synthesized using the bacterium, *Bacillus subtilis*, from titanium as a precursor, were confirmed by TEM analysis. The morphological characteristics state spherical shape, with the size of individual or aggregate nanoparticles, around 30-40 nm. Microbial resistance represents a challenge for the scientific community to develop new bioactive compounds. Here, the antibacterial effect of TiO_2 nanoparticles on *Escherichia coli* was investigated, which was confirmed by CFU (Colony-forming unit). Further, growth curve study of *E. coli* Hb101 in the presence and absence of TiO_2 nanoparticles was done. Optical density decrease was observed with the increase in the concentration of TiO_2. It could be attributed to the inactivation of cellular enzymes and DNA by binding to electron-donating groups such as carboxylates, amides, indoles, hydroxyls, thiols, etc. which cause little pores in bacterial cell walls, leading to increased permeability and cell death. This justifies that TiO_2 nanoparticles have efficient antibacterial effect and have potential to be used as an antibacterial agent for different purposes.

***Keywords*—**Antibacterial effect, CFU, *Escherichia coli* Hb101, growth curve, TEM, TiO_2 nanoparticle, toxicity, UV-Vis.

I. Introduction

NANOTECHNOLOGY has become one of the most practical technologies, because of the unique physical and chemical properties of nanomaterials. Nanoparticles have high surface area and they have been studied extensively because of their unique physicochemical characteristics like catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties [1]-[4].

Titanium dioxide (TiO_2) nanoparticles are an important material for photocatalysts [5], cosmetics, and pharmaceuticals [6]. TiO_2 nanoparticles like other nanoparticles are extremely energetic, adaptable and promising due to their potential metabolic fluxes. *Bacillus subtilis* is an aerobic, spore forming bacteria. Its ability to survive even in the harsh unfavourable conditions makes it a suitable candidate for the biosynthesis of the metal nanoparticles like TiO_2. In this work, it is used in order to assess its potentiality as a putative candidate bacterium for the synthesis of TiO_2 nanoparticles. It is a cost effective, eco-friendly and amenably reproducible approach for the purpose of scaling up.

The recent discovery of the biosynthesis of metal nanoparticles points towards new biotechnological methods in materials science [7], [8]. The scope of development of novel applications and the antimicrobial effect of nanoparticles makes them attractive as an alternative to antibiotics. Nanoparticles demonstrate wide-ranging therapeutic application properties [9]. Antibiotic resistant bacterial strains with different mechanisms are found continually and thus new drugs are required [10]. Therefore, the finding of new antimicrobial agents with novel mechanisms of action is essential and extensively pursued in antibacterial drug discovery [11]. The effect of nanoparticles on bacteria is very important, since they constitute the lowest level and hence enter the food chain of ecosystem [12], [13]. TiO_2 has an important role in our environmental purification due to its nontoxicity [14]. Here, the antibacterial effect of these nanoparticles was examined on *E. coli* and the TiO_2 nanoparticles were found to be effective.

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II. Methodology

Luria Agar and Luria broth were purchased from HiMedia. All other reagents used were of analytical grade.

A. Synthesis of TiO_2 Nanoparticles Using B. subtilis

B. subtilis cells were allowed to grow in sterile medium with suitable carbon and nitrogen at 37°C for 36 h. This suspension culture was treated as source culture. 20 ml of culture was taken and diluted four times, and it was again allowed to grow for next 24 h. $1/5^{th}$ $TiO(OH)_2$ (0.025 M) of the total volume of culture was added to the culture and heated at ~ 55-60 °C (on steam bath) until white deposition starts to appear at the bottom of the flask. This marks the initiation of transformation. The culture solution was then cooled and allowed to incubate at ambient room temperature. After 1-2 days, the culture solution showed distinct coalescent white clusters deposited at the bottom of the flask [15].

1. Characterization of TiO_2 Particles

The UV absorbance of the synthesized TiO_2 nanoparticles was measured in Hitachi U-2900 UV-Vis spectrophotometer operated at a resolution of 1 nm.

B. Toxicity of TiO_2 Nanoparticles

2. TiO_2 Stock Preparation

TiO_2 nanoparticles with a size approximately <25nm were procured from Sigma. It was suspended in distilled water and sonicated for 15 minutes before use [16] and different concentrations (100, 50, 25 mg/l) were prepared.

3. Preparation of Resting Cells

E. coli Hb101 was grown in a LB tube at 37 °C under shaking conditions overnight. 4% inoculum was added in LB medium and inoculated at 37 °C shaker. It was harvested (centrifuged at 10000 rpm for 5 min) when the ABS reached 0.4 at 600 nm. Then cells were washed twice by miliQ water and resting cells were obtained by centrifuging at 10000 rpm for 5 min. The cells were again suspended in miliQ water.

4. Exposure of *E. coli* to TiO_2 Nanoparticles

100, 50 and 25 mg/L TiO_2 was added to 1 ml culture and then incubated at 37°C in shaking conditions for 18-20 hr

5. CFU (Dropping Method)

Three dilutions were prepared and 10 µl from each and one control (culture without TiO_2) was plated (spot inoculated). The plates were incubated at 37°C for overnight.

C. Antimicrobial Effect of TiO_2 in Liquid Media

Stock suspension (homogenous) of TiO_2 nanoparticles (1 mg/mL) was kept in UV for 30 min for sterilization. Overnight culture of the *E. coli* was added to Luria broth with different concentrations of TiO_2 nanoparticles and incubated at 37°C for 16 hours. To study the bacterial concentration, the absorbance was taken at 600 nm.

1. Growth Curve of *E. coli*

Freshly grown bacterial inoculum was incubated in the presence of different concentrations of TiO_2 nanoparticles in the media and these were incubated at 37 °C temperature under shaking conditions (150 rpm). The growth rate was indexed by measuring absorbance (ABS) at 600nm after every two hours. The readings obtained were plotted and comparative studies were performed between control with and without TiO_2 nanoparticles.

III. Results & Discussion

The onset wavelength for TiO_2 appears at 366 nm in UV–vis spectroscopy (Fig. 1), which is blueshifted compared to the bulk anatase TiO_2, indicating the formation of nanoparticles solution.

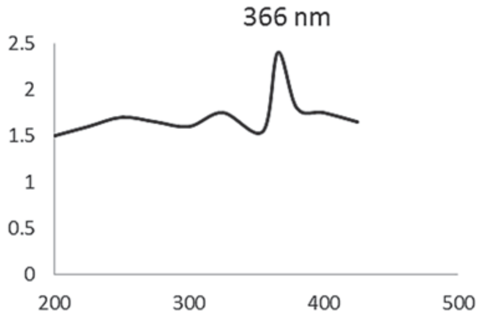
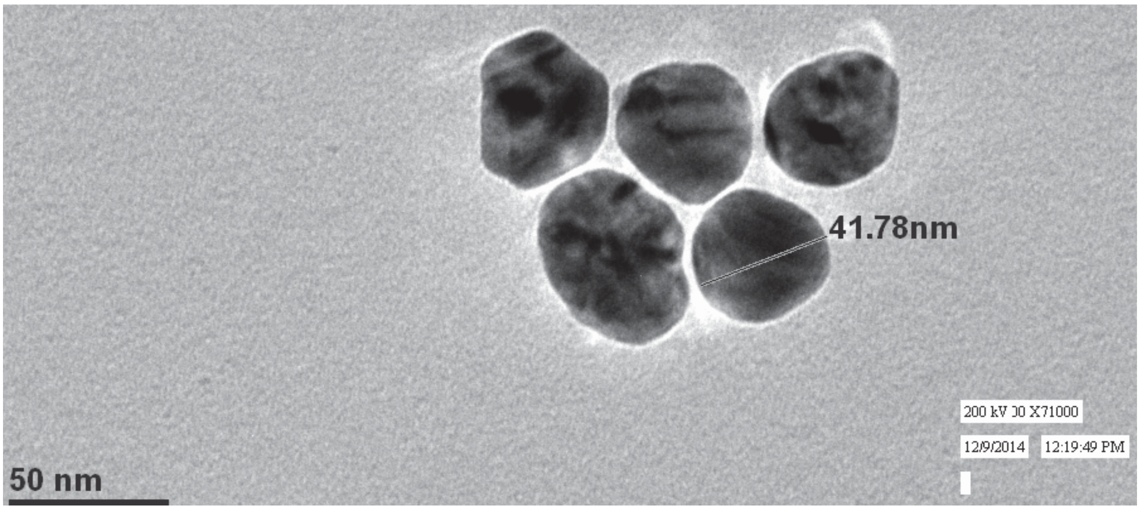
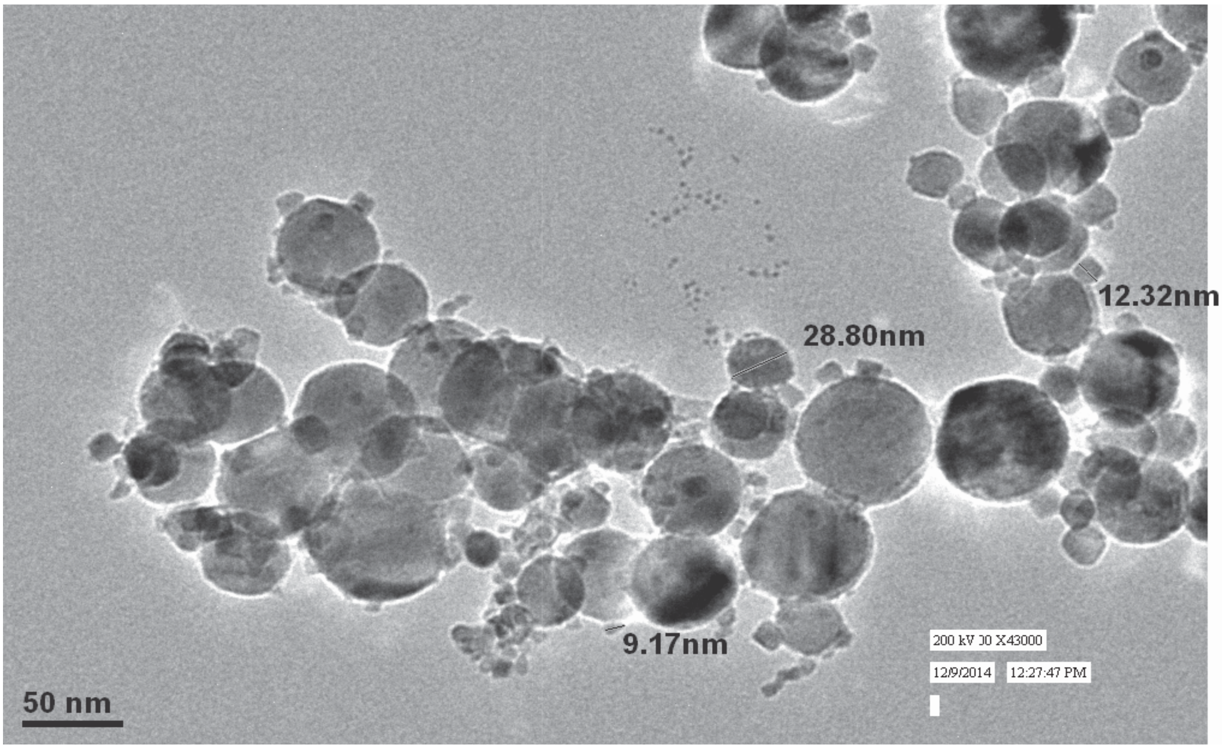


Fig. 1 UV absorption spectrum for the synthesized TiO_2 nanoparticles showing peak at 366 nm

The surface morphology of the particles is uneven due to the presence of some of the aggregates and individual particles of TiO_2. The TEM images of the *B. subtilis* synthesized TiO_2 nanoparticles shows spherical clusters of the nanoparticles (Fig. 2).



(a)



(b)

Fig. 2 TiO_2 images through Transmission electron microscope showing (a) individual and (b) aggregate form of nanoparticles

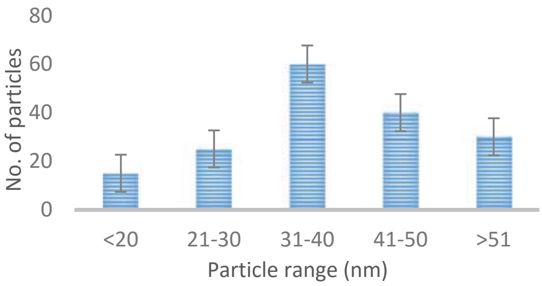


Fig. 3 Particle size distribution - Size range of synthesized TiO_2 nanoparticles

Nanoparticles were spherical with individual as well as a few aggregates size of 30–40 nm. The surface modification of nanocrystalline anatase TiO_2 particles with orthosubstituted hydroxylated enediols ligands improve the optical response in the visible region [17]. The synthesis of n- TiO_2 might have resulted due to pH-sensitive membrane bound oxidoreductases and carbon source dependent rH2 in the culture solution. Composition of nutrient media, thus plays a pivotal role in biosynthesis of metallic nanoparticles.

Compared to control, there was a decrease in CFU (i.e. decrease in the number of viable colonies) with the increase in TiO_2 concentration, which is due to the toxic effect of TiO_2 (Fig. 4).

With the increase in the TiO_2 concentration, a decrease in absorbance (ABS) was observed (Fig. 5).

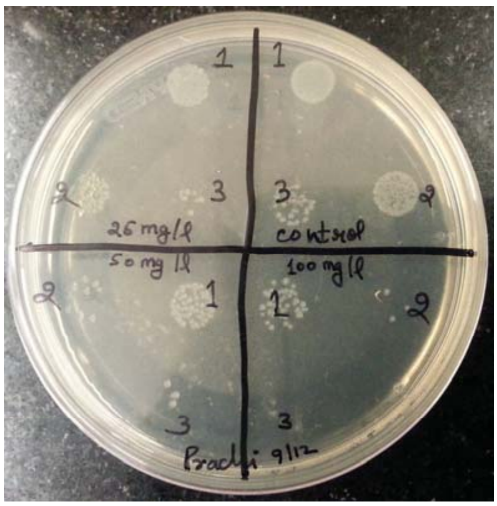


Fig. 4 CFU of control and culture suspension exposed to different TiO_2 concentrations in three different dilutions

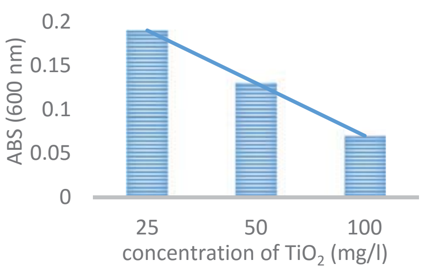


Fig. 5 *E. coli* Density in the presence of different TiO_2 concentrations

According to several studies, it is thought that the metal oxides carry the positive charge while the microorganisms carry negative charges; this causes electromagnetic attraction between microorganisms and the metal oxides which leads to oxidization and finally death of microorganisms [18]. Nano materials could also deactivate the cellular enzymes and DNA by coordinating to electron-donating groups, such as Thiols, Carbohydrates, Amides, Indoles, Hydroxyls etc. They cause pits in bacterial cell walls, leading to increased permeability and cell death [19].

In hospital environments, TiO_2 could be used as the suitable disinfectant, and in textile industry, cotton fabrics with antibacterial effect have been developed [20].

In control, the lag phase ends after 4 hours, then log phase starts which continues till 15 hours. In contrast, when cells were treated with TiO_2 nanoparticles, there occurred delay in the lag phase which further varies with the concentration of TiO_2 nanoparticles. Thus, a prominent decrease in growth of *E. coli* was observed.

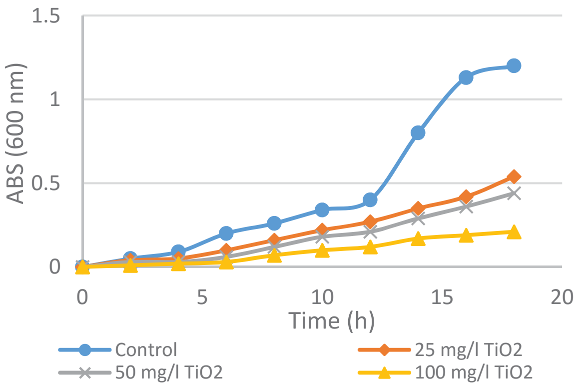


Fig. 6 Growth curve of *E. coli* in the absence of TiO_2 and in the presence of 25 mg/l, 50 mg/l, 100 mg/l TiO_2

IV. Conclusion

The work explains the use of inexpensive, non-toxic and eco-friendly easily available microorganisms for the rapid synthesis of TiO_2 nanoparticles. The synthesized TiO_2 nanoparticles were characterized by using UV–vis and TEM. The bacterial biosynthesis of the TiO_2 provides a fast, and purest form of producing nanoparticles than any other chemical method.

Therefore, this method is considered as a better approach for the synthesis of TiO_2 nanoparticles. TiO_2 nanoparticles shows inhibitory effect on the growth of *E. coli* strain. Hence TiO_2 nanoparticles can be considered as potent antibacterial compound and further can be used in various industrial and medical applications.

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