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# Green Synthesis of $TiO_2$ Nanoparticles using Mesophilic Bacterial Strain *Bacillus subtilis* SP2 and its Characterization

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**A B S T R A C T**

Nanotechnology has recently materialized as a fundamental scientific branch that sightsees the interface of synthetic and biotic materials. Nanotechnology is presently employed as a tool to combat frightfulailments caused by drug-resistant microbes. $TiO_2$ nanoparticles have been well known for their inhibitory and bactericidal effects. $TiO_2$ nanoparticles were synthesized by a biogenic approach mediated by using the culture supernatant of *Bacillus subtilise* SP2. The biogenic $TiO_2$ nanoparticles were characterized by UV-visible spectroscopy, X-ray diffraction (XRD), FTIR, scanning electron microscopy (SEM), and Transmission electron microscopy (TEM). $TiO_2$ nanoparticles exhibited maximum antagonistic activity on *Escherichia coli* (17mm).

## Introduction

Nanostructures have attracted huge interest as a rapidly growing class of materials for many applications. Several techniques have been used to characterize the size, crystal structure, elemental composition, and a variety of other physical properties of nanoparticles (Mourdikoudis *et al.,* 2018). Nanoparticles are clusters of atoms in the size range of 1–100 nm. “Nano” is a Greek word synonymous to dwarf meaning extremely small. The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 (Karthika *et al.,* 2015). Nanoparticles have fascinated scientific devotion due to their fascinating properties, commercial and biotechnological applications advantageous over their bulk counterparts (Tharanya *et al.,* 2015).

Methods employed for the synthesis of nanoparticles are broadly classified under two processes as “Top-down” process and the “Bottom-up” process. Top-down approach: Bulk material is broken down into particles at the nanoscale with various lithographic techniques e.g.: grinding, milling, etc. Bottom-up approach: Atoms self-assemble to new nuclei which grow into a particle of nanoscale (Kavitha *et al.,* 2013).

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, the development of safe, eco-friendly, reliable, and non-toxic methods for the synthesis of nanoparticles is of utmost importance to expand their biomedical applications (Li *et al.,* 2011). The biological procedure for the fabrication of nanoparticles has been proven to be far superior, to those nanoparticles produced by physical and chemical methods. Presently a diverse variety of nanoparticles has been synthesized by using different microbial sources. Among the various applications, nanoparticles have extensive applications in environmental remediation due to their potential oxidation strength, high photostability, and non-toxicity (Tharanya *et al.,* 2015; Bavani *et al.,* 2021; Bavani *et al.,* 2022).

The present study deals with the green synthesis of $TiO_2$ nanoparticles using the culture supernatant of *Bacillus subtilis* SP2 and its characterization by UV– Vis spectra, XRD, SEM and TEM analysis and to evaluate its antagonistic activity against selected bacterial pathogens.

## Materials and Methods

### Biogenic approach for the synthesis of $TiO_2$ nanoparticles

### Bacterial strain and Chemicals used

The bacterial strain used in this study was isolated from environmental samples including sludge and effluents were collected from textile and tannery industries located in and around Ranipet, Tamil Nadu. Based on the morphological, cultural, and biochemical characteristics and 16s rDNA sequencing, the isolate was identified as *Bacillus subtilis* SP2. $TiO(OH)_2$ (99.9 %) was procured from Sigma Aldrich Chemicals, Bangalore, India. All other reagents used in the reaction were of analytical grade with maximum purity.

### Synthesis of $TiO_2$ nanoparticles

24 h old culture of *Bacillus subtilis* SP2 was incubated to grow as suspension culture for 2 days at 37°C in shaking conditions at 150 rpm and was considered as source culture. 50 ml of the broth culture was taken and centrifuged at 8000 rpm for 10 min. Following centrifugation,20 ml of the culture supernatant was transferred to a sterile tube and mixed with 20 ml of 0.025M $TiO(OH)_2$ to form a ratio of 1:1. The mixture was heated using a water bath at 80°C for 10–20 min until the appearance of white deposition at the extremity of the flask, demonstrating the development of Titanium nanoparticles (Tharanya *et al.,* 2015).

### Characterization Techniques

The biosynthesized $TiO_2$NPs were characterized by using UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and TEM analysis (Transmission electron microscopy). The nanoparticles were characterized in a Shimadzu UV-VIS Spectrophotometer. The scanning range for the samples was 300-800 nm. The double-distilled water was used as a blank reference. The purified and dried pellet of synthesized $TiO_2$ NPs was subjected to X-ray diffraction (XRD) analysis. The XRD patterns were recorded at a scan speed of 2° $min^{-1}$. Meanwhile, the structures of the produced NPs were examined using Shimadzu PXRD–6000, powder X-ray diffraction. The particle size and morphology of the NPs were examined using SEM and TEM analysis. The size of the NPs was confirmed by using - Hitachi H-7100) using an accelerating voltage of 120 kV and methanol as solvent.

### Antibacterial Activity of $TiO_2$ Nanoparticles

The antibacterial effect of $TiO_2$ was examined by disc diffusion method against *Staphylococcus* *aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* 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 well and incubated for 24 h. Following incubation, the zone of inhibition against nanoparticles was observed and measured.

## Results and Discussion

Nanotechnology has recently arisen as a rudimentary branch of science and technology that inspects and legalizes the interaction at the cell level between synthetic and biotic constituents with the assistance of nanoparticles (Durairasu *et al.*, 2017). Among the various NPs, the metallic nanoparticles are considered to be the most promising ones, as they contain significant antibacterial and antifungal properties due to their large surface area to volume ratio, which is of great interest to researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Gong *et al.*, 2007).

### Biosynthesis of $TiO_2$ NPs

Biosynthesis of $TiO_2$ nanoparticles was carried out by using the broth culture of *Bacillus subtilis* SP2. On mixing the culture supernatant with $TiO(OH)_2$ for 12–48 h, the culture solution was observed to develop distinctly markable coalescent white clusters deposited at the bottom of the flask indicating the biosynthesis of $TiO_2$ Nanoparticles.

### Characterization of $TiO_2$ nanoparticles

The onset wavelength of the optical absorption for uncapped $TiO_2$ appears at 320 nm in UV–vis spectroscopy (Fig. 1). FTIR The synthesized $TiO_2$ NPs showed the presence of bands due to heterocyclic amine, O-H free bond (3315 $cm^{-1}$), alkanes, O-H bend (2951 $cm^{-1}$), Carboxylic acid, OH (very broad) (2839 $cm^{-1}$), arene, = C-H and Carboxylic acid derivative, C-O-H bending (1452 $cm^{-1}$) which was found to be characteristic of $TiO_2$ NPs.XRD analysis for the synthesized $TiO_2$ NPs showed distinct diffraction peaks indexed to the planes 110, 211, 220 respectively (Fig 2). The formation of titanium dioxide nanoparticles as well as their morphological dimensions was demonstrated using SEM analysis (Fig 3). The shapes were uniformly spherical and ellipsoidal. The average size of titanium dioxide nanoparticles synthesized using *Nyctanthes arbortristis* leaf extract was found to be between 100-150 nm, whereas the shapes were uniformly spherical (Sundrarajan and Gowri, 2011). From the TEM images, it is evident that the morphology of $TiO_2$NPs is nearly spherical and some are non-spherical in nature (Fig 4).

**Table.1** Antibacterial activity of Ag NPs against the selected bacterial pathogens

| S. No. | Bacterial strains | Zone of Inhibition |
|---|---|---|
| **1.** | *Staphylococcus aureus* | 11 ± 0.2 mm |
| **2.** | *Streptococcus pyogenes* | 12 ± 0.3 mm |
| **3.** | *Pseudomonas aeruginosa* | 15 ± 0.3 mm |
| **4.** | *Escherichia coli* | 17 ± 0.4 mm |

**Fig.1** FT IR spectra of biosynthesized Silver Nanoparticles

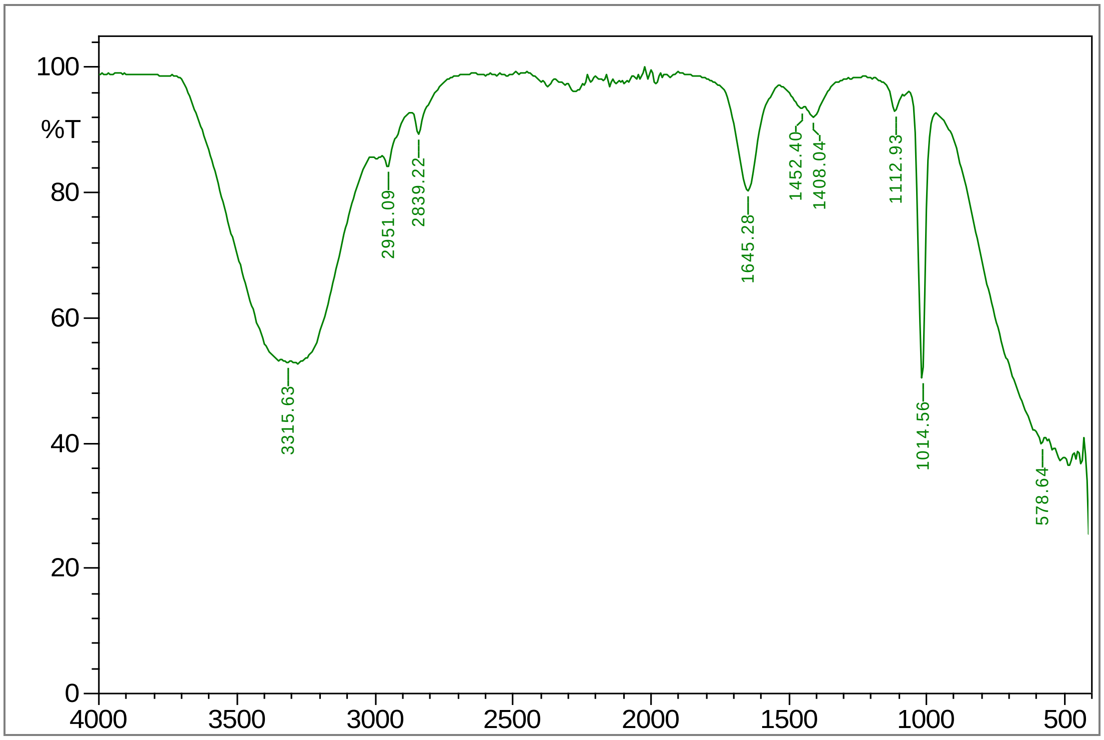



**Fig.2** XRD of biosynthesized Silver Nanoparticles

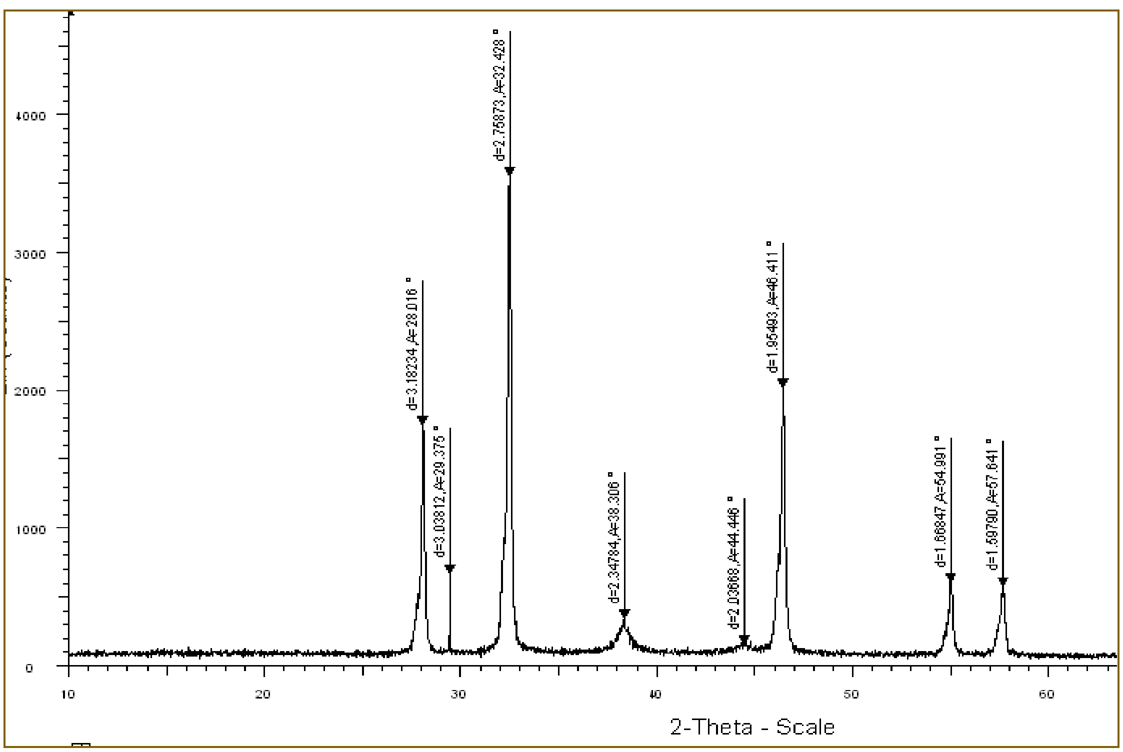


**Fig.3** SEM Images of biosynthesized Silver Nanoparticles

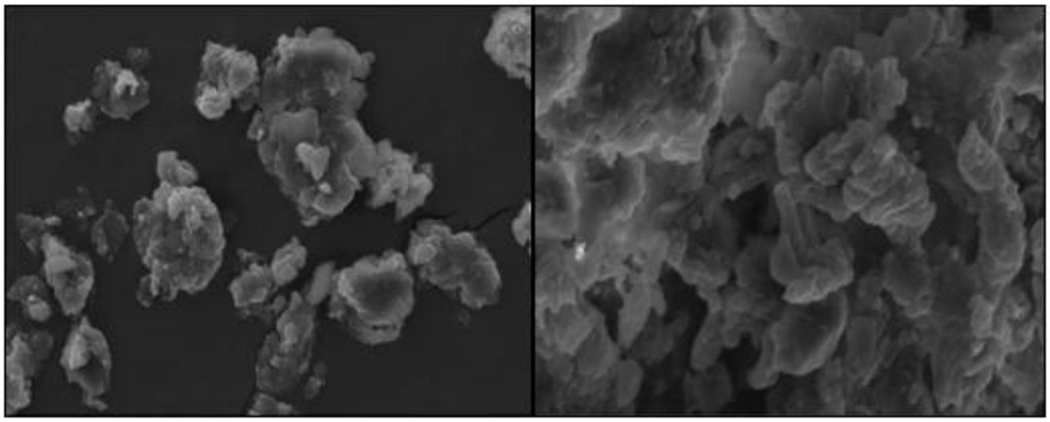
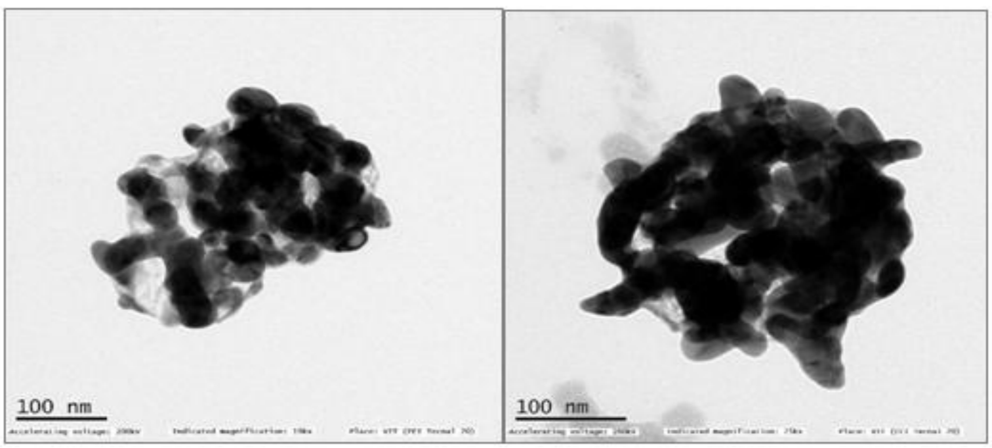


**Fig.4** TEM Images of biosynthesized Silver Nanoparticles




## Antibacterial activity of $TiO_2$ nanoparticles

The antibacterial activity of $TiO_2$ nanoparticles was carried out by a well diffusion method against *Staphylococcus aureus*, *Streptococcus pyogenes, Escherichia coli*, and *Pseudomonas aeruginosa.* $TiO_2$ nanoparticles exhibited maximum antagonistic activity on *Escherichia coli* (17mm), which was closely followed by *Pseudomonas aeruginosa* (15 mm) (Table 1). 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. Similarly, $TiO_2$ nanoparticles exhibited remarkable antagonistic activity against *Bacillus subtilis* and *Klebsiella planticola* respectively (Malarkodi *et al.,* 2013). The noble antibacterial effect (100% killing efficiency) of $TiO_2$ nanoparticles may be due to the small size, large surface area, large bandgap energy, and more active sites for carrying out photocatalytic reactions. $TiO_2$ nanoparticles mediated photocatalytic reactions lead 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).

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