TiO₂ Nanoparticles from Baker's Yeast: A Potent Antimicrobial^S

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Titanium dioxide (TiO₂) has wide applications in food, cosmetics, pharmaceuticals and manufacturing due to its many properties such as photocatalytic activity and stability. In this study, the biosynthesis of TiO₂ nanoparticles (NPs) was achieved by using Baker's yeast. TiO₂ NPs were characterized by X-ray Diffraction (XRD), UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray analysis (EDX) studies. The XRD pattern confirmed the formation of pure anatase TiO₂ NPs. According to EDX data Ti, O, P and N were the key elements present in the sample. SEM and TEM revealed that the nanoparticles produced were spherical in shape with an average size of 6.7 ± 2.2 nm. The photocatalytic activity of TiO₂ NPs was studied by monitoring the degradation of methylene blue dye when treated with TiO₂ NPs. TiO₂ NPs were found to be highly photocatalytic comparable to commercially available 21 nm TiO₂ NPs. This study is the first report on antimicrobial study of yeast-mediated TiO₂ NPs synthesized using TiCl₃. Antimicrobial activity of TiO₂ NPs was greater against selected Gram-positive bacteria and *Candida albicans* when compared to Gram-negative bacteria both in the presence or absence of sunlight exposure. TiO₂ NPs expressed a significant effect on microbial growth. The results indicate the significant physical properties and the impact of yeast-mediated TiO₂ NPs as a novel antimicrobial.

Keywords: Titanium dioxide, Yeast, X-ray diffraction, photocatalytic activity

Introduction

Titanium dioxide (TiO₂) nanoparticles (NPs) have a wide range of applications in industry and medicine. TiO₂ NPs have unique optical, electronic, photocatalytic and antimicrobial properties [1] which can be exploited towards novel drug delivery systems and also for water purification [2], dye-sensitized solar cells, photonics and food preservation [3]. Three main phases of TiO₂ are anatase, brookite and rutile [4].

Synthesis of TiO₂ NPs with controlled particle size can be reached using energy-consuming, sophisticated, conventional chemical and physical synthesis methods. However, this approach has intrinsic drawbacks due to high cost and risk to the environment. Comparatively, eco-friendly, cost-effective and non-toxic NP synthesis techniques using microorganisms and plant sources are in demand [5]. This green synthesis has the ability to produce higher yields of

NPs under mild laboratory conditions facilitating large-scale production. Microbial TiO₂ NP synthesis has been reported using several microorganisms including *Bacillus cereus* [6], *Fusarium oxysporum* [7], *B. subtilis* [8, 9], *Aspergillus* species [10, 11], *B. mycoides* [12] and *Lactobacilli* [13]. Since these are renewable sources, it is environmentally less harmful and generates less amount of toxic waste.

TiO₂ NP biosynthesis is thought to arise as a result of the reduction of the metal salts to nanosized metal particles mediated by the enzymatic and chemical interactions. Jha *et al.* [14] suggested that the negative electro-kinetic potential of microbes can attract cations triggering the series of events leading to biosynthesis of NPs. The presence of reducing agents such as glucose reduces the redox potential thereby enhancing NP formation.

TiO₂ NPs have good antimicrobial potential, where they deactivate cellular enzymes and DNA and also form pores in bacterial membranes leading to fluid loss and cell

death [15]. This potential is further supported by their photocatalytic activity. When TiO₂ NPs are treated with visible light [16] or UV light [12], reactive oxygen species are produced resulting in enhanced microbial killing [17].

Yeast cells (Class Ascomycetes) can act as a template inducing biomineralization [16]. The present investigation was undertaken to synthesize anatase TiO₂ NPs by using Baker's yeast (Y-TiO₂) and to characterize their antimicrobial and photocatalytic properties.

Materials and Methods

Biosynthesis of TiO₂ NPs

Baker's yeast 0.8 g was cultured in 77 ml of sterile filtered 5% (w/v) glucose (SISCO, India) solution for 24 h [18]. Three milliliters of TiCl₃ solution (TiCl₃ 12% in HCl, Sigma Aldrich, USA) was added slowly to the 24 h old yeast culture, while stirring until a clear purple solution was observed. Then the solution was allowed to stand at room temperature for three days under dark conditions. The precipitate was centrifuged at 6,000 rpm for 20 min and washed several times using distilled water. Following washing the obtained precipitate was allowed to dry at room temperature. The precipitate was then heated at 250°C, 350°C, 450°C, 550°C, 650°C, and 750°C for 2 h time intervals each to obtain TiO₂ NPs as described by Bao *et al.* [18].

Characterization of Biosynthesized TiO₂ NPs

The obtained Y-TiO₂ powder was subjected to X-Ray Diffraction (XRD), Energy Dispersive X-ray analysis (EDX) and UV-Visible spectroscopy to confirm the formation of TiO₂ NPs. The size and morphology were determined using SEM and TEM.

X-Ray Diffraction (XRD)

XRD analysis was carried out using an X-ray diffractometer (RigakuUltima-IV). The air-dried nanoparticles were coated onto an XRD grid and diffracted intensities were documented from 20 to 90 of 2θ angles with a scan speed of 2°/min at 40 kV and 30 mA.

Energy Dispersive X-Ray Analysis

UV-Visible spectroscopy. Y-TiO₂ NP solution was prepared using distilled water. Then 1 ml of the NP solution was diluted with 9 ml of distilled water and UV-Visible spectrum was obtained in the range of 200–800 nm using a PerkinElmer Lambda 35 UV-Visible spectrophotometer. Data obtained were compared with 21 nm TiO₂ NPs (Sigma Aldrich, USA).

Scanning electron microscopy (SEM). NP powder was coated onto a coverslip and the morphology of the synthesized NP powder was studied using SEM imaging (ZEISS Sigma Scanning Electron Microscope, 10 kV accelerating voltage). EDX was also obtained (Ametek EDAX Z230, USA).

Transmission electron microscopy (TEM). The NP sample was coated onto a coverslip which was then placed onto a sample stub.

TEM imaging (JEOL JEM 2100, Japan) was performed using Drop casting method on a holey carbon-coated Cu grid (Accelerating voltage 200 kV).

Photocatalytic Activity

To study the photocatalytic activity, a modified method was used as described by Periyat *et al.* [19]. Two millilitres of 25 mg/ml TiO₂ NP solution was dispersed in 50 ml of 1 × 10⁻⁵ M methylene blue (HiMedia, India) solution. Before UV exposure, the suspension was stirred in the dark for 30 min. Then the suspension was exposed to 150 mJ/s of UV radiation (GS Gene Linker UV Chamber, Bio-Rad). Photodegradation was monitored at 5 min time intervals by withdrawing 3 ml aliquots of TiO₂ added methylene blue solution. The aliquots were filtered using 0.22 μm syringe filters and absorbance was taken using a UV-Visible spectrophotometer. Percentage of photodegradation was plotted against exposure time.

Antimicrobial Activity Using the Plate Coating Method

The selected microorganisms *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231, Methicillin resistant *Staphylococcus aureus* (MRSA) (confirmed clinical strain) and *Acinetobacter baumannii* (confirmed clinical strain) were obtained from the culture collection at the Department of Microbiology, University of Sri Jayewardenepura, Sri Lanka. According to the method described by Peiris *et al.* [20], sterile 6 cm petri dishes were coated with (A) Y-TiO₂ NPs (2 ml) and (B) bacterial/fungal culture supernatant (2 ml) as the negative control. NP concentration was 20 mg/ml. NP solution was prepared in distilled water and sonicated at 35 kHz for 1 h followed by autoclaving for 15 min at 121°C. The coated plates were allowed to dry completely for 24 h inside a class II biological safety cabinet. Subsequently 2 ml of standardized bacterial suspension (0.5 McFarland standard) was added to each petri dish and incubated for different time intervals (30 min, 45 min, 1 h, 2 h, 3 h, 4 h) at room temperature. To determine the photo-enhanced antimicrobial activity, one set of NP-coated plates containing bacterial cell suspension was exposed to sunlight (test and control) for 30 min and another set of NP-coated plates containing bacterial cell suspension (test and control) was kept in the dark for 30 min. Viable cell counts at different contact time points were obtained by spread plate method. The number of colony forming units per milliliter (CFU/ml) was calculated at each incubation time. All experiments were carried out in triplicate.

Statistical Analysis

The percentage of photodegradation was calculated using the following equation:

$$\text{Percentage photodegradation} = \frac{A_0 - A_t}{A_0} \times 100$$

A₀ is the initial maximum absorbance of methylene blue; A_t is the absorbance at time t.

The viable counts obtained at different contact times were used to calculate the percentage reduction using the following equation:

$$\text{Average percentage reduction} = \frac{\text{CFU/ml in control} - \text{CFU/ml in TiO}_2 \text{ NPs}}{\text{CFU/ml in control}} \times 100$$

For graph preparation and statistical analysis, OriginPro 9.0 SR2 software was used.

Results and Discussion

In this study, the yeast *Saccharomyces cerevisiae* was used successfully to synthesize green anatase TiO₂ NPs of less than 12 nm that also showed strong antimicrobial activity. The milky white yeast culture obtained at 24 h was used for adding TiCl₃ which resulted in a purple-colored suspension with a pH of 5.4 due to the acidity of TiCl₃. A greyish white deposit was observed at the bottom of the flask after 72 h which was used for further purification of TiO₂ nanoparticles.

Biosynthesis of TiO₂ NPs occurs through redox reactions mediated by reducing agents such as glucose [14], alpha amylase [21] and urease [22] in the medium. In this study, we have synthesized Y-TiO₂ NPs using the surface of yeast cells as the site for Ti³⁺ nucleation [18]. The porous surface of yeast cells provides a template where the surface-attached macromolecules and the cells themselves induce TiO₂ NP nucleation [16]. Yeast cells stay attached together during nucleation and subsequent treatment at high temperature results in formation of a porous lamellar structure containing TiO₂ NPs and inorganic matter [18]. Heat treatment is a critical factor determining the phase of TiO₂ NPs. During this process, yeast cells are removed,

Fig. 1. X-ray diffraction pattern of Y-TiO₂ NPs.

Peaks appear when the X-ray beam has been diffracted by the crystal lattice. Characteristic peaks indicating crystalline structure of Y-TiO₂ NPs are shown here.

spilling over CO₂ gas and forming a unique lamellar mesophase structure [18].

Characterization of Y-TiO₂ NPs

The presence of Y-TiO₂ NPs was confirmed using XRD. XRD results indicated predominant formation of crystalline anatase TiO₂-NPs having characteristic peak positions at 25.30, 37.78, 48.03, 54.45, 62.80, 70.34, 74.91, and 82.81 degrees. Characteristic peaks for rutile phase were undetected. Narrow peaks indicate the crystalline structure. Prime orientation was the (101) plane as observed at the highest peak position at 25.3 degrees (Fig. 1).

Fig. 2 demonstrates the detailed EDX analysis of TiO₂

Fig. 2. EDX spectrum of Y-TiO₂ NPs.

For titanium, two K-shell lines are resolved, indicated as Kα and Kβ. This spectrum shows characteristic distribution of major elements in the Y-TiO₂ NP sample.

Fig. 3. UV absorption spectra for Y-TiO₂ NPs and the chemical TiO₂ NPs.

Both samples have shown a strong absorption below 400 nm.

NPs showing the surface element composition of Y-TiO₂ NPs. The measurements show that the Y-TiO₂ NP sample consists of Ti (20.89%), O (70.95), P (5.78%) and N (2.38%) by atomic percentage. According to EDX analysis, other than titanium and oxygen, nitrogen and phosphorous inherited from yeast cells were also detected in the spectrum. Carbon was undetected due to the oven drying process, where organic matter gets destroyed. Chlorine was also undetected showing that no residual chlorine is present in the sample after thorough washing prior to oven drying. Fig. 3 depicts the optical absorption of yeast-mediated TiO₂ NPs compared with chemically synthesized 21 nm TiO₂ NPs. The absorption spectra of both NPs exhibit strong absorption below 400 nm. The UV-Visible spectrum obtained was comparable to chemically synthesized TiO₂ NPs as previously reported [17].

To study the surface morphology of Y-TiO₂ NPs, TEM and SEM techniques were used. Fig. 4 indicates that TiO₂ NPs were spherical in shape with rough surfaces and were embedded in a thin lamellar structure (Figs. 4A, 4B, and 4D). Dense dispersion of NPs was evident. A narrow particle size distribution was observed in the range of 3.6–12.0 nm with a mean particle size approximately 6.7 ± 2.2 nm (Fig. 4C). The TEM images of the Y-TiO₂ NPs demonstrated a unique lamellar structure as previously reported [18]. This lamellar mesophase occurs when inorganic layers are produced with semi conducting materials such as TiO₂ NPs placed in between them [23].

TiO₂ NP nucleation and growth depend on multiple factors relying on physical, chemical or biological conditions

Fig. 4. (A, B) TEM images of Y-TiO₂ NPs, (C) Particle size distribution of Y-TiO₂ NPs (D) SEM image of Y-TiO₂ NPs (Scale bars are given).

Particle size distribution was 3.6–12.0 nm with the average size of 6.7 ± 2.2 nm.

employed in the synthesis process [4]. The particle size, shape and photocatalytic activity are important determinants of potent antimicrobial action. This study reports a narrow particle size range of 3.6–12.0 nm as compared to previous reports of TiO₂ NPs by He *et al.* [16] which yielded 10–12 nm particles while 8–35 nm particles were reported from yeast by Jha *et al.* [14] (10–12 nm) with the smaller-sized particle suggesting strong antimicrobial potential.

The photocatalytic activity of synthesized Y-TiO₂ NPs was determined by monitoring the changes in methylene blue concentration using UV-Visible spectroscopy after TiO₂ NP treatment and UV exposure. Both the chemically synthesized 21 nm TiO₂ NPs and Y-TiO₂ NPs decolorized

Table 1. Percentage of degradation of methylene blue.

Time interval (min)	% Degradation	
	21-nm TiO ₂ NPs	Y-TiO ₂ NPs
0	22.7	1.8
5	38.1	24.0
10	71.9	40.2
15	81.6	50.0
20	84.8	64.6
25	97.2	93.3
30	99.0	99.0

Fig. 5. Photodegradation of methylene blue dye by (A) Y-TiO₂ NPs, (B) Chemical TiO₂ NPs, monitored by UV-Visible spectroscopy.

Reduction of the peak intensity indicated that both the chemically synthesized (Sigma Aldrich) 21-nm TiO₂ NPs and Y-TiO₂ NPs decolorized methylene blue dye within 30 min.

methylene blue dye within 30 min (Table 1). Fifty percent dye reduction was observed within 15 min of exposure for both TiO₂ NP samples. This was confirmed by the reduction of the intensity of the characteristic peak position of untreated methylene blue with time (Fig. 5). Initially at 0 min absorbance measurements, there was a slight reduction of absorbance in Y-TiO₂-treated methylene blue and a noticeable reduction in 21 nm TiO₂ NP-treated dye due to adsorption of NPs into the dye.

In the study on photocatalytic activity, the initial absorption of Y-TiO₂ NPs into methylene blue dye was negligible when compared to absorption of 21 nm TiO₂ NPs. The photocatalytic property of TiO₂ is dependent on the dopant concentration, optical absorption range, phase, size range and number of electrons produced [24–26]. The anatase form of TiO₂ reported in this study is known to have maximum photocatalytic activity compared to the rutile and brookite forms of TiO₂ NPs [27]. TiO₂ is efficient as a photocatalyst due to high rate of mass transfer, strong light absorption and high rate of H₂O₂ decomposition [16]. TiO₂ NPs act as indirect semiconductors and absorb light through direct and indirect electron transitions [28]. According to Diffey [29], on a typical summer day, 6% of UV consists of UVB (290–320 nm) which is responsible for more than 80% of photocatalytic damage and 94% has UVA which contributes to 20% of harmful effect. When exposed to sunlight, electrons are released through band gap forming holes in the valence band and move onto the surface of NPs. The holes react with oxygen forming oxygen radicals where they react

with water to produce reactive OH[•] radicals [19, 30, 31]. These reactive oxygen species exert potent antimicrobial activity during their very short life span.

Antimicrobial Activity of TiO₂

TiO₂ NPs strongly inhibited Gram-positive bacteria and *C. albicans* when compared to Gram-negative bacteria (*E. coli*, *A. baumannii*, and *P. aeruginosa*). The percentage reduction of colony-forming units (CFU/ml) after exposure to Y-TiO₂ NPs following 30 min of sunlight exposure resulted in significant reduction of *S. aureus* (77%), MRSA (97%), and *C. albicans* (95%) compared to the control without TiO₂ indicating enhanced antimicrobial activity due to the photocatalytic properties of TiO₂ following sunlight exposure (Supplementary material). In contrast, the percentage reduction of CFU/ml for gram-negative bacteria *P. aeruginosa*, *E. coli* and *A. baumannii* were 58%, 46%, and 50%, respectively, after exposure to sunlight (Fig. 6A). Y-TiO₂ NPs themselves were seen to have antimicrobial activity in the absence of sunlight exposure when incubated under room conditions (Fig. 6B). After 30 min of contact with Y-TiO₂ NPs, percentage inhibition of *S. aureus* (20%), MRSA (25%), *C. albicans* (74%), *P. aeruginosa* (30%), *E. coli* (26%), and *A. baumannii* (23%) were seen but at a lower percentage than following exposure to sunlight (Supplementary material). TiO₂ NPs were also seen to have strong antimicrobial activity against *C. albicans* in the presence or absence of sunlight suggesting that they have much potential as an anti-Candida agent for future applications.

Fig. 6. Colony count after 30 min of contact time (A) with sunlight exposure (B) without sunlight exposure (*p* values are indicated as follows; “*” for *p* value between 0.01-0.05, “***” for 0.01-0.001 and “ns” for not significant).

Y-TiO₂ NPs were more sensitive to Gram-positive bacteria and *Candida* species (*C. albicans*) while Gram-negative bacteria were less susceptible to Y-TiO₂ NPs with or without sunlight exposure

In this study, Y-TiO₂ NPs were found to be more sensitive to Gram-positive bacteria (*S. aureus* and MRSA) and *Candida* species (*C. albicans*) while Gram-negative bacteria (*P. aeruginosa*, *A. baumannii*, and *E. coli*) were found to be less susceptible to Y-TiO₂ NPs with or without sunlight exposure. The phase of TiO₂ NPs also affects their biocidal properties. Anatase form is reported to show the highest antimicrobial activity [32]. Recent studies suggest several mechanisms of photo-enhanced antimicrobial activity. Reactive oxygen species (ROS) released by UV-irradiated TiO₂ NPs attack microbial phospholipids causing cell

damage [31]. Direct contact of bacterial cells with TiO₂ NPs facilitates ROS contact with microbial cell membranes. According to Singh [9], TiO₂ NPs have a positive charge on their surface and microbial cell membranes have a negative charge enhancing attachment. According to Carré *et al.* [33] TiO₂ NPs show high affinity to membrane proteins and alter their structures. Photocatalysis also influences TiO₂ NPs to bind with phospho-proteins and phosphor-lipids [34]. Moreover, Ahmad *et al.* [21] reported that TiO₂ NPs invade bacterial cells by damaging the cell membrane in both Gram-negative and positive bacteria causing cell leakage and death. The peptidoglycan layer of Gram-positive bacteria, which is cross-linked by a large number of short peptides, is found to be more sensitive to ROS which explains the higher antimicrobial activity against this group of bacteria.

In conclusion, Y-TiO₂ NP synthesis, as reported in this study, is a cost-effective approach yielding pure, highly stable, anatase TiO₂ NPs with small particle size. Due to their small size and photocatalytic activity, they have a significant antimicrobial effect on the selected pathogens. The results suggest the possibility of using green TiO₂ NPs for future antimicrobial investigation.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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