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Biosynthesis of TiO2 Nanoparticles and Evaluation of Their Antibacterial Activities

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Abstract

The production of TiO2 NPs using *Staphylococcus aureus* was simplified and made more efficient. Titanium sulfate concentration, reaction time, temperature, and pH were among the variables optimized. The ideal conditions were a titanium sulfate concentration of 0.025M, a reaction period of 30 minutes, a temperature of 60°C, and a pH of 7. Bacteria isolated from otitis media infections were shown to be susceptible to the in vitro activity of TiO2 NPs. AFM, SEM, and FTIR spectroscopy were among the tools and techniques used to characterize the produced nanoparticles. The TiO2 NPs measured 40 to 50 nm in size and had a spherical shape. Based on the results of the antibiotic susceptibility test, it was found that the bacterial isolates tested were sensitive, resistant, or moderately resistant to four different antibiotics. To test the sensitivity of several G^+ and G^- bacteria to 5 different doses of TiO2 nanoparticles, 25, 50, 100, 200, and 400 µg/ml were used. These bacteria included multidrug-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. The pathogenic bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae* necessitated a concentration of 100 µg/ml of TiO2 nanoparticles to inhibit their growth, unlike *Klebsiella pneumoniae*.

Keywords: TiO_2 · Nanoparticles · Antimicrobial · Biological synthesis.

Introduction

Research in nanoscience has opened many new directions, and creative, practical, and occasionally unexpected applications have resulted [1]. Nanotechnology's development has significantly increased the use of nanoparticles in antibacterial materials and medicine delivery systems [2]. Chemical, physical, and biological techniques can create and stabilize metallic NPs. Physical procedures like arc discharge and physical vapor condensation, as well as chemical approaches like chemical reduction, electrochemical methods, photochemical reduction, and pyrolysis, are frequently used. Microorganisms have enormous

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potential for producing nanoparticles and Nano devices with a variety of uses [3]. A critical domain of investigation in contemporary nanoscience and nanotechnology is the interaction between inorganic NPs and biological structures [4,5]. Overall, because biomaterial-based synthesis does not require harsh or poisonous chemicals, biological materials offer an environmentally benign or green chemical technique to make valuable products [6]. One of the most produced and utilized materials in the world is TiO_2 nanoparticles [7]. They are provided with biomedical applications, implant biomaterials, sunscreen goods, and antimicrobial plastic packaging. Recently, techniques for producing TiO_2 NPs biologically using extracts from bacteria, yeast, fungi, and plants were disclosed [8].

Materials and methods:

Samples collection of fifty clinically diagnosed cases by ENT specialists at private clinics of 25 isolates of *Streptococcus pneumonia*, 15 from *staphylococcus areas* and ten isolates of *Klebsiella pneumonia* were collected randomly from children and adults with otitis media infections. Every sample was collected under sterile conditions and conveyed to the laboratory within 1-2 hours.

Isolation of bacteria:

All of the isolates were using Gram stain. After injecting the samples into different culture media, such as blood agar and MacConkey agar, they were cultured in an aerobic environment at 37 °C for 24 hours. The colonies' shape, colour, appearance, and positive cultures were investigated. These isolates were subjected to various biochemical tests, including catalase, oxidase, and IMViC. The Biomatrix Vitec 2 compact autoanalyzer instrument validated the isolates' identity in additional biochemical tests.

Antibiotic Susceptibility tests of bacterial isolates:

Employed a modified Kirby-Bauer disk diffusion technique following the standards established by the Clinical and Laboratory Standards Institute (CLSI).

Bacterial isolates were cultivated on Müller-Hinton agar, resulting in one to three colonies after 24 hours at the optimal incubation temperature of 37°C. A sterile swab was employed to inoculate the bacterial suspension onto Müller Hinton agar via the streaking technique after the bacterial cultures were calibrated to 0.5 McFarland standards. According to the CLSI's suggestion, the isolates were categorized as either susceptible (S) or resistant (R) [9,10].

Amikacin (AK), Ciprofloxacin (CIP), imipenem (IPM), and colostrum (CT) were evaluated as antibiotics. All antibiotics utilized in the study were procured from Himedia in India.

Synthesis of TiO_2 nanoparticles:

Nanoparticles of TiO2 were synthesized using titanium sulfate manufactured in Germany by Merck. Titanium NPs were manufactured using the method described in [11], with a few adjustments. For the biogenesis of TiO2 nanoparticles, a 50 mL flask containing one colony of *staphylococcus aureus* was cultured in sterile nutrient broth. The flask was then placed in a shaker set at 37 °C and 150 rpm for 24 hours to produce TiSo4.

The TiO2 NPs were manufactured using bacterial cultures. The flasks containing 10 mL of bacterial cultures and 20 mL of TiSo4 solution (0.025 M) were subjected to heating at 60 °C for 30 minutes. Upon observation at the base of the flask following incubation, distinct coalescent white clusters were found to have been left behind by the culture fluid. To keep the pH of the precipitate at 7, it was first created by centrifugation and then rinsed with distilled water.

Optimization condition for TiO_2 synthesis:

Optimization of PH:

Titanium nanoparticles were synthesized at different pH values (5, 7and 9).

Optimization of temperature:

The impact of different temperature levels on the synthesis of TiO2 nanoparticles was examined. The temperatures recorded were 40°C, 60°C, and 80°C. The synthesis of titanium nanoparticles was conducted at an appropriate pH, as previously illustrated.

Effect of time on the synthesis of TiO_2NPs:

For 60 minutes, we looked at how reaction time affected the synthesis of titanium NPs. The reaction mixture's color changed every 30 minutes to signal the production of TiO2.Nebulae [12].

Effect of titanium sulfate concentration:

Various concentrations of titanium sulfate range from (0.025 to 0.075 m). The reactions were incubated at the optimal temperature and pH for the time determined by the previous steps [13].

Antibacterial activity of titanium nanoparticles:

One bacterium isolate is activated overnight in a 10 ml tube using an injection of Muller Hinton broth, as per the CLSI protocol. After 24 hours, the bacterial solution had grown to 1.5*108 CFU/ml, as determined by comparing it to the McFarland tube. A spectrophotometer (600 nm) with an appropriate absorbance of 0.08-0.1 further validated this. Titanium nanoparticles were produced in a volume-to-volume (v/v) ratio of 1:1 with one milliliter of bacterial inoculation [14-16]. In a separate tube, 1 mL of bacterial suspension and 1 mL of normal saline were mixed and agitated overnight.

Results and discussion:

Isolation of bacterial isolates

In this investigation, fifty isolates were categorized according to their cultural and microscopic characteristics. The purple bacterial isolates corresponded to Gram-positive bacteria, specifically *Staphylococcus aureus*, whereas the red isolates corresponded to Gram-negative bacteria, specifically *Klebsiella pneumonia*. The VITEC2 compact system was utilized to validate the categorization of bacterial isolates according to culture media and biochemical assays. After completing the identification process for all bacterial isolates, the findings indicated that the prevalence of persistent *Streptococcus pneumoniae* was 50%, *Staphylococcus aureus* was 30%, and *Klebsiella pneumoniae* was 20%.

Antibiotic susceptibility

A majority of the isolates exhibited resistance to multiple drugs; furthermore, all of the isolates were completely ineffective against amikacin. On the other hand, ciprofloxacin resistance was minimal at 14%, and imipenem resistance was 3.1%. Isolates of bacteria that showed resistance to imipenem and ciprofloxacin were considered appropriate for this study.

Biosynthesis of TiO_2 NPs using Bacteria *S.aures*

Within 24 hours of inoculation, the response color changes from brown to white, and the intensity of the color is increased throughout color incubation, indicating that *S. aureus* is synthesizing TiO2 NPs. Figure 1 (A and B) shows the time required for metal reduction. Nanoparticles of titanium oxide exhibit a characteristic color change due to the activation of surface Plasmon vibrations [17,18].

Table 1 Distribution and frequency of bacteria isolated from otitis media infection.

Bacteria	NO.	%
streptococcus pneumonia	25	50%
Staphylococcus aureus	15	30%
Klebsiella pneumonia	10	20%

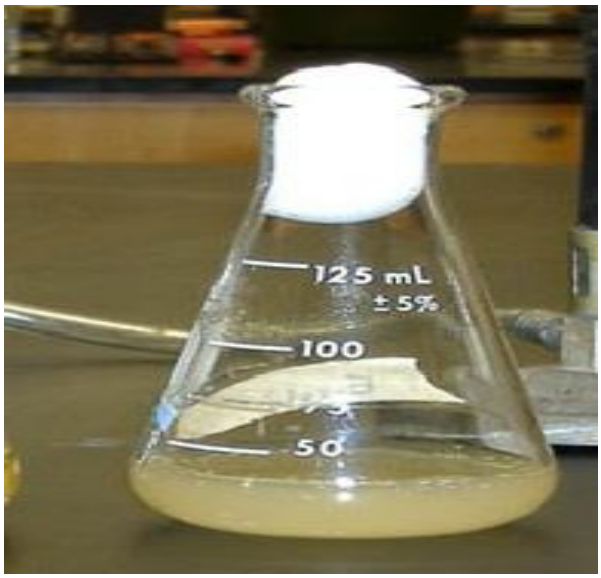

Figure 1 Synthesis of TiO_2 NPs

Optimum conditions for TiO_2NPs Synthesis

1- Optimization of Temperature:

Temperatures of 40°C, 60°C, and 80°C were chosen. Table 2 presents the AFM readings acquired following the incubation of TiO2 nanoparticles synthesized by *Staphylococcus aureus*. It is widely recognized that an increase in the mixture's temperature occurs. The dynamics of the reaction facilitate the nucleation process [19,20]. Previous studies indicated that increasing the reaction temperature influenced nanoparticle production [21].

2-Optimization of pH

With this optimization in mind, we chose three distinct pH levels: 5, 7, and 9. Table 3 shows that AFM readings were taken following incubation. When it came to making TiO2 NPs, *S.aures* found that pH 7 worked best. The synthesis of TiO2 NPs is enhanced at lower pH, according to multiple studies. The decrease in synthesis may be due to the inactivation of the enzyme reductase, which is responsible for catalyzing the process when the pH rises [22].

3- Optimization of time

An essential variable that influences nanoparticle biogenesis is time. The reduction process for the formation of nanoparticles started when reducing chemicals were quickly added to the titanium solution. The results show, however, that the particle size decays with time and then stabilizes when a certain amount of time has passed. Considering the influence of optimal conditions in the previous steps, the mixture was subjected to multiple time intervals for TiO2 NPs formation (15, 30, and 60 minutes), as indicated in Table 4 [23].

Table 2 The average size of TiO_2 NPs biosynthesized at different temperatures as measured by the AFM technique.

Exp No.	Temperature	AFM was used to measure the average size in nm.
1-	40 ℃	77.10
2-	60 ℃	55.10
3-	80 ℃	81.6

Table 3 The average size TiO_2 NPs biosynthesized in different pH as measured by the AFM technique

Exp No.	PH	AFM was used to measure the average size in nm.
1-	5	78.69
2-	7	51
3-	9	63.98

Table 4 The AFM technique was used to assess the average size of TiO2 NPs biosynthesized under varied pH conditions.

Exp No.	time	AFM was used to measure the average size in nm.
1-	15mim	75.24
2-	30min	67.5
3-	60min	52.6

4- Effects of titanium sulfate concentration on the synthesis of TiO_2NPs

Table 5 The average size TiO2 NPs biosynthesized in different concentrations as measured by the AFM technique

Exp No.	Concentration M	AFM was used to measure the average size in nm.
1-	0.025M	52.3
2-	0.50 M	61.14
3-	0.75 M	73.8

Characterization of Titanium Oxide NPs by *S.aures* UV–Vis Analysis:

The nanoparticles' optical properties were investigated with the use of a UV-A visible spectrometer. Figure (2) shows the sample's absorbance at room temperature in the nanometer range. There is a noticeable peak at 374 nm, which suggests that electrons in the conduction band are directly recombining with holes in the valence band [24].

Atomic Force Microscopy (AFM) analysis:

The biosynthesized TiO2 NPs were found to have a nearly spherical surface topography and a particle size of around 61 nm, as illustrated in Figure 3 of the AFM micrographic pictures **Figure 3**

(FTIR) analysis

The FTIR measurements of TiO_2 NPs revealed the characteristics of high-purity product manufacture These nanoparticles' FTIR spectra (Fig.4) showed just TiO_2 peaks. The vibration of the Ti-O-O bond causes the 590-cm1 peak. The FTIR spectrum demonstrates the presence of Ti -O bonds in the final product and the absence of peroxo and OH groups [25]. This approach gives maximum TiO_2 NPs that may be employed in a range of applications, The N H stretching frequency coming from the peptide links found in bacteria biosynthetic proteins utilizing TiO_2 may be ascribed to a large intensity band at 3430 cm1 in the spectra, A high absorption peak at 1779 cm1 characterizes the anhydride group's asymmetrical C- O coupled vibration. The carbonyl area (1600-1800 cm1) changed, and typical bands at 1779 cm1 and 1639 cm1 attributed to amide I and amide II appeared, as well as a large drop in the relative strength of the bands (diagnostic of the symmetric C- O stretching vibrations[26].

FESEM of TiO_2 NPs.

The structural morphology of TiO2 NPs was investigated using FESEM, as shown in Fig (5). FESEM analysis showed that the particles were spherical and ranged from 40 to 51 nm in size.

The antibacterial activity of TiO_2 nanoparticle

An evaluation was conducted to determine the antibacterial efficiency of titanium dioxide nanoparticles. The bacteria tested were Gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae*, as well as multi-drug resistant Gram-negative *Klebsiella pneumoniae*. Numbers of 25, 50, 100, 200, and 400 µg/ml of TiO2 were measured. Based on the findings, after 24 hours of incubation in an aerobic environment at 37ºC, turbidity was observed in every test tube that contained *S. aureus* and *S. pneumonia* along with TiO2 nanoparticles at dosages of 50, 100, 200, and 400 µg/ml. Furthermore, the outcomes were produced by combining *Klebsiella pneumonia* with TiO2 at doses of 25, 50, 100, 200, and 400 µg/ml. According to Table 6, the MIC50 values for Gram-negative bacterial pathogens ranged from 25 to 400 µg/ml, while for Gram-positive bacteria, the range was 400 µg/ml.

One explanation for TiO2's antibacterial properties is that it can produce free hydroxyl radicals (OH) [27]. Some studies have demonstrated that TiO2 nanoparticles are effective against bacteria and fungi [28]. Adenosine triphosphate (ATP) synthesis, DNA replication, and the induction of ROS, which directly damage cellular structures, were all negatively affected by TiO2NPs, which not only adhered to but also penetrated the bacterial cell surface [29].

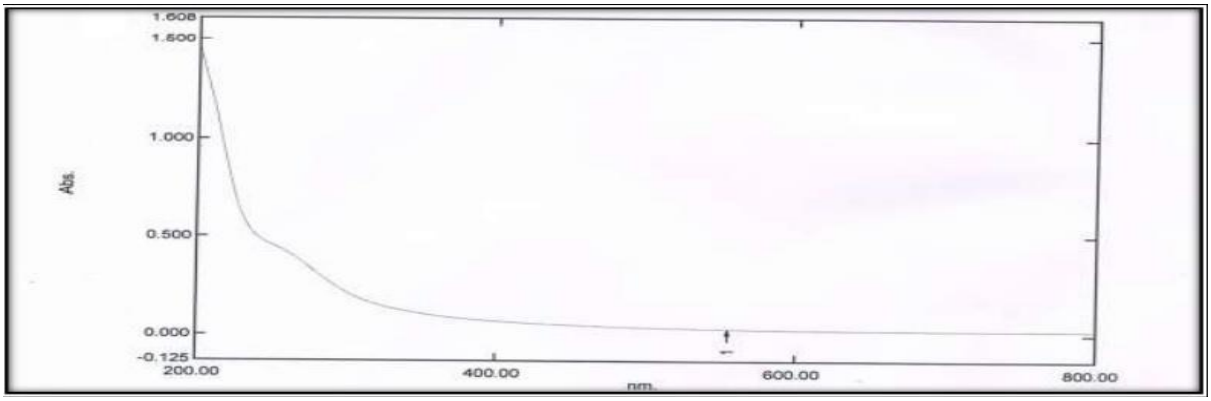

Figure 2 UV–VIS spectroscopy ofTiO_2 NPs with a peak at 374 nm.

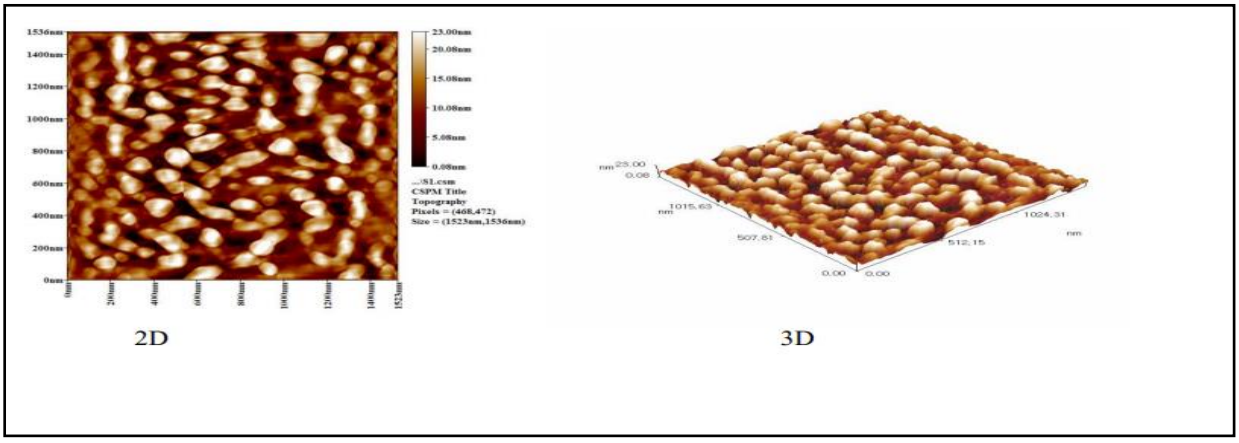

Figure 3 Atomic Force Microscopy analysis of titanium oxide nanoparticles biosynthesized by *S.aures*

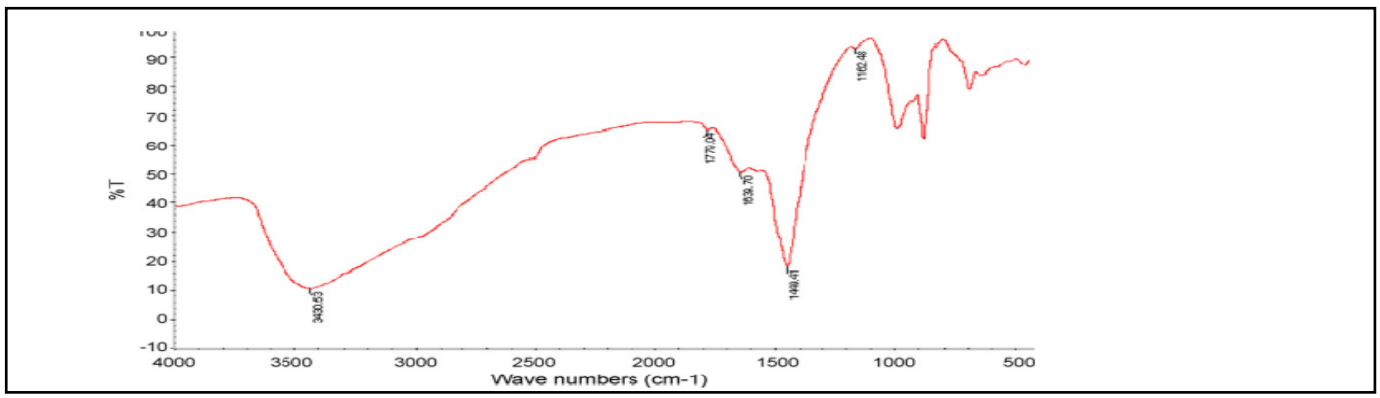

Figure 4 FTIR analysis of TiO_2 NPs biosynthesized by *S.aures*

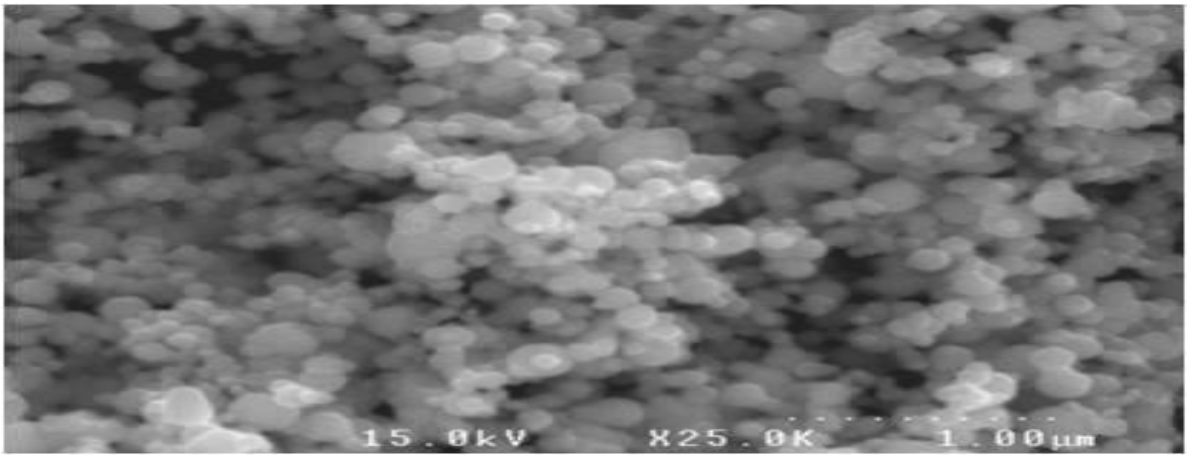

Figure 5 FTIR analysis of titanium oxide nanoparticles biosynthesized by *S.aures*

Table 6 Minimum inhibitory concentrations of TiO2 nanoparticles on bacteria after 24 hrs. Incubation at 37 C°

No.	Tio_2 concentration	*Staphylococcus aureus*	*Streptococcus pneumonia*	*Klebsiella pneumonia*
1-	400	+	+	+
2-	200	+	+	+
3-	100	+	+	+
4-	50	+	+	+
5-	25	-	-	+

Conclusion:

Eco-friendly synthesis TiO_2 NPs were synthesized by using bacteria *staphylococcus aureus* at size 41nm, at pH 7, temperature 60°C, reaction time 30min, with 0.025 M titanium oxide. The results demonstrated that TiO_2 NPs affected gram-negative and positive bacteria.

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Conflict of Interest:

The authors have not declared any conflicts of interest.

Authors contributions:

All authors equally contributed to this study.

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Ethical approval:

The project received approval from the local ethical commission at the University of Technology- Iraq.

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