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Original article

Antimicrobial Effects of TiO2 Nanoparticles against Drug-Resistant Bacteria and *Candida Albicans* [1]

Houneida Benbouzid [a, *], Zahra Obeizi [b] & Abd El Ghani Djahoudi [c]

[a] Laboratory of Cellular Toxicology, Department of Biology, Faculty of Sciences, University of Annaba, Algeria
[b] Laboratory of Biochemistry and Applied Microbiology, Faculty of Sciences, University of Annaba, Algeria
[c] General Microbiology Laboratory, Faculty of Medicine, University of Annaba, Algeria

Abstract

Nanotechnology is expected to open new avenues to fight and prevent disease using atomic scale tailoring of materials. The metallic nanoparticles is the most promising nanomaterials with antibacterial properties and which exhibit increased chemical activity due to their large surface to volume ratios and crystallographic surface structure. This work focuses on the antibacterial activity of TiO_2 against drug-resistant bacteria and *Candida albicans*. The antibacterial activity of TiO_2 was evaluated using the determination of minimum inhibitory concentration (MIC) by the dilution method on agar medium. The obtained results show a significant antibacterial activity on the strains tested: *Pseudomonas aeruginosa* ATCC27853: 0.5 µg/mL, *Staphylococcus aureus* ATCC29213: 64 µg/mL, *Escherichia coli* ATCC25922: 128 µg/mL and *Candida albicans* 0.5 µg/mL. The Titanium Dioxide nanoparticles appear to be attractive candidates of choice to be an effective alternative to antibiotics and pave the way for a promising new strategy antibacterial nanoscale.

Keywords: Nanoparticles, Titanium Dioxide, Antibacterial Activity, Minimal Inhibitory Concentration.

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* **Corresponding author:**

Houneida Benbouzid, Laboratory of Cellular Toxicology, Department of Biology, Faculty of Sciences, University of Annaba, Algeria.
Email: benbouzid_h@yahoo.com

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INTRODUCTION

Bacterial infections are a major cause of chronic infections and mortality. Antibiotics have been the preferred treatment method for bacterial infections because of their cost-effectiveness and powerful outcomes. However, several studies have provided direct evidence that the widespread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains (Wang et al., 2017).

Therefore, attention has been focused on new and exciting NP-based materials with antibacterial activity. The production of nanoparticles is increasing rapidly for applications in electronics, chemistry, and biology. This interest is due to the very small size of NPs which provides them with many interesting properties such as rapid diffusion, high specific surface areas, reactivity in liquid or gas phase, and a size close to biomacromolecules (Thill et al., 2006). Metallic nanoparticles and in particular nanoparticles of metal oxides have magnetic, electrical and optical properties that enable them to detect and to treat bacterial infections (Yuan et al., 2018). Among the metal nanoparticles TiO_2 NPs are known to be cost effective, stable and safe for humans and the environment. A unique property of TiO_2 NPs is the photocatalytic property resulting in enhanced microbicidal activity on exposure to light in the UV range (Ahmad & Sardar, 2013; Othman et al., 2014). TiO_2 NPs exist in three crystalline phases, where the anastase phase demonstrates high photocatalytic and antimicrobial properties (Ahmad and Sardar, 2013).

This study aimed to determine the antibacterial activity of TiO_2 nanoparticles against antibiotic-resistant bacteria and *Candida albicans*.

Materials and Methods

Chemicals and Reagents

Different chemicals and reagents used during this experiment include Mueller Hinton agar, nutrient broth and Nutrient agar are purchased from Sigma Aldrich. The nanomaterials tested in our study correspond to nanoparticles of TiO_2 (Sigma Aldrich), having a particle size of 50 nm and an anatase crystallography (Figure 1).

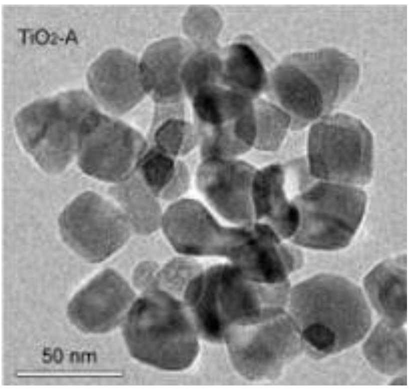

Figure 1. Anatase TiO_2NPs (TiO_2-A). (Yu et al., 2017).

Microorganisms and culture media

In this study, four microbial species were used: *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922 and *Candida albicans.* They were obtained from the laboratory of General Microbiology, Faculty of Medicine, Badji Mokhtar University of Annaba, Algeria and were cultured on Nutrient agar.

Antimicrobial sensitivity test

Antimicrobial activity of TiO_2 NPs was investigated against the chosen microorganisms by the determination of the minimum inhibitory concentration (MIC) using the diffusion technique on agar medium method in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI) (2009) (Wang et al., 2018). The MIC represents the concentration of an antibiotic totally inhibiting bacterial growth visible after 24 h of incubation at 37°C (Park et al., 2017).

The organism was cultured on Nutrient agar at 37 °C for 18 h. Suspensions of organisms were prepared in sterile nutrient broth to obtain a 0.5 Mac-Farland absorbance corresponding to 10^8 organisms/ ml.

A stock solution of TiO_2 NPs at a concentration of 2048 µg/ml is prepared, followed by sonication (Figure 2) and autoclaving at 121°C during 30 minutes. Semi-log dilutions of half-to-half in sterile distilled water are prepared (1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5) to a final concentration of 0.5µg/ml.

To prepare the dilution Petri dishes, we mixed 2 ml of each concentration and 18 ml of Muller Hinton agar in the corresponding Petri dish. After solidification of the medium, we deposited the bacterial spots. After that, the Petri dishes are left at room temperature until the moisture is absorbed (spot drying, no more than 30 minutes) and then the dishes are incubated at 37 ° C for 18-24 hours.

Figure 2. Installation of a sonicator type SONICS

Results and Discussion

Effect of TiO_2 nanoparticles on gram-positive cocci bacteria

Table 1 shows the obtained results of the effect of TiO_2 NPs against Gram positive Cocci. We report the average value of 64 µg/ml with the strain *S. aureus* 29213.

Table 1. Effect of TiO_2 NPs against Gram positive bacteria

Strain	MIC (µg / ml)
Staphylococcus aureus ATCC29213	**64**

Kiran et al. (2018) demonstrated that TiO_2 nanoparticles has antimicrobial inhibition against *S. aureus*. Also our results are in agreement with those of Aydın Aytekin et al. (2018) who have shown that the different strains of *S. aureus* present similar sensitivity to Ag-doped TiO_2 nanotubes.

Effect of TiO_2 nanoparticles on enterobacteria lactose positive

The MIC value obtained after treatment of enterobacteria lactose positive by TiO_2 nanoparticles are represented in the table 2.

Table 2. MIC value of enterobacteria lactose positive treated with TiO_2 NPs

Strain	MIC (µg / ml)
Escherichia coli ATCC25922	**128**

We report the average value of 128 µg/ml with the strain *E. coli* ATCC 25922. These results are in agreement with those of Brayner et al. (2006), who have shown that nanoparticles having a grain size between 10 and 15 nm have a bactericidal effect against this strain. Our results are similar to those obtained with Simon Deckers (2008), who have evaluated the cytotoxic effect of anatase-type TiO_2 against *E. coli* MG 1655 with a MIC of 100 µg / ml.

Effect of Lactose negative non-enterobacteria treated with TiO_2 NPs

Table 3 shows the obtained results of the effect of TiO_2 NPs against Lactose negative non-enterobacteria.

Table 3. MIC value of Gram negative bacilli not enterobacteria treated with TiO2 NPs

Strain	MIC (µg / ml)
Pseudomonas aeruginosa ATCC27853	**0.5**

The obtained results show that the MIC value obtained with the Lactose negative non-enterobacteria represented by *P. aeruginosa* ATCC27853 is 0.5 µg / ml. Our results are similar to those obtained with Priyanka et *al.* (2016) who showed that nanoparticles of TiO_2 synthesized by the sol-gel technique have antimicrobial potency in day light against *P. aeruginosa*.

Effect of TiO_2 NPs against Candida albicans

Table 4 shows the obtained results of the effect of TiO_2 NPs against yeast.

Table 4. MIC value of *Candida albicans* treated with TiO2 NPs

Yeast	MIC (µg / ml)
Candida albicans	**0.5**

The MIC value obtained with the yeast represented by *C. albicans* is 0.5 µg/ml. Our results are in agreement with those of Daou et al. (2017) who demonstrated that TiO_2-ZnO NPs allowed complete eradication of *C. albicans* with very low MIC.

Conclusion

Our results showed that MIC values in Gram-positive bacteria are higher than those in Gram-negative bacteria. This toxicity can be explained by the difference in the composition of the bacterial cell wall that facilitates or not access to NPs. It must be known that the wall of Gram-positive bacteria consists of a thick layer of peptidoglycan associated with a felting of teichoic acid. And the presence of porins in direct contact with the external environment facilitates the transmembrane diffusion of the NPs. On the other hand, the wall of Gram-negative bacteria consists only of a thin layer of peptidoglycans, phospholipids and polysaccharides. The presence of porins in the inner membrane induces the protection of bacteria against the excessive penetration of NPs.

At the end of this study, it should be concluded that the nanoparticles of TiO_2 has a high antibacterial potential on the bacteria tested and it can be possible to use them as an impregnating agent in a matrix to prevent microbial contamination by inhibiting microorganisms directly in contact with the receiving surface.

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