International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Short Communication

MORAXELLA OSLOENSIS MEDIATED SYNTHESIS OF TIO_2 NANOPARTICLES

VALLI NACHIYAR C.*, VIJAYALAKSHMI R., NIVEDHA KUMAR, BAVANILATHA M., SWETHA SUNKAR

Department of Biotechnology, Sathyabama University
Email: vnachiyar@gmail.com

Received: 01 Jan 2016 Revised and Accepted: 15 Mar 2016

ABSTRACT

Objective: The main idea behind the present investigation is to explore the ability of *Moraxella osloensis* to reduce Titanium dioxide to nanosizes in a facile manner.

Methods: The bacterium was grown in nutrient broth for 24 hr. The culture supernant was used for the bioreduction process. The synthesized nanoparticles were characterised by instrumental analyses namely FTIR, SEM and XRD. Toxicity of the nanoparticles was tested against HaCaT and Hep2 cell lines.

Results: *Moraxella osloensis* with the dye degrading ability was found to be capable of reducing bulk Titanium dioxide to nanostructure. These biogenic TiO_2 nanoparticles (TiO_2 NPs) were in the size range of 60–150 nm with the average size of 72 nm. The particles were found to irregularly shaped and uniformly dispersed with less aggregation. In addition, XRD analysis indicated the presence of titanium beta. FTIR confirmed the involvement of proteins in the bioreduction and stabilization. Nano TiO_2 was found to be more toxic against HaCaT and Hep2 cell lines than its bulk counterpart.

Conclusion: *Moraxella osloensis* successfully synthesised TiO_2 NPs in environmentally safe and cost effective method in an extracellular fashion.

Keywords: *Moraxella osloensis*, Biogenesis, TiO_2 NP, Characterization, Cytotoxicity

Nano biotechnology, an emerging area of research, interlaces various disciplines of science such as physics, chemistry, biology and material science. As the size of material decreases to nanoscale the percentage of surface atoms increases, thus increasing their high surface-to-volume ratio, surface energy, spatial confinement and reduced imperfections, which in turn impart characteristic physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties to nanoparticles as opposed to bulk materials [1, 2]. As the biological processes also occur at the nanoscale and due to their amenability to biological functionalization, the nanoparticles are finding important applications in the field of medicine [3].

Titanium dioxide (TiO_2) is a white pigment and because of its brightness and very high refractive index it is most widely used. TiO_2 can be used in paints, coatings, plastics, papers, inks, medicines, pharmaceuticals, food products, cosmetics, and toothpaste. TiO_2 nanoparticles (TiO_2 NPs) are also used in sunscreens [4]. The non-toxic and biocompatible properties of titania, make it find its applications in biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries [5]. Even though, number of physical and chemical methods are available for the synthesis of TiO_2 nanoparticles, biological synthesis is gaining importance as it is relatively simple, cheap and environmentally friendly [6].

Biological methods involve either plants or microorganisms or sometimes biomolecules derived from them that facilitate both intracellular and extracellular synthesis. This method limits the use of expensive chemicals and advocates the more acceptable "green" route. Biological synthesis of the TiO_2 is still in its nascent stage and there are few reports confirming the ability of the various bioentities like bacteria [7-9], fungi [10-12] and plants [13, 14] successfully synthesizing TiO_2 nanoparticles. Hence an attempt has been made to isolate, identify the microorganisms and biologically synthesize TiO_2 nanoparticles, characterize them using various instrumental analyses and check for their cytotoxic activities.

The organism used in this study, *Moraxella osloensis* was isolated from a textile industry effluent in an attempt to isolate an organism with an excellent dye degrading ability [15]. The bacterium was grown in 100 ml nutrient broth for 24 h. The culture supernatant was obtained after centrifugation of the broth at 10,000 rpm for 15 min followed byfiltration using Whatman filter paper No 1. This filtrate was challenged with 0.025 M TiO_2 and stirred for 1 h.

This was followed by heating the solution at 60 °C for 30 min. The white deposit formed at the bottom of the flask, indicating the formation of TiO_2 NPs, was separated by centrifugation and dried. A control containing only TiO_2 solution was maintained under similar conditions [9].

The synthesized nanoparticles were subjected to various instrumental analyses like FESEM-EDAX (Carl Zeiss, Germany) and XRD (Rigaku, Japan) and FTIR (Perkin Elmer FTIR spectro-photometer) to identify the size, size distribution, shape, the crystalline structure and the functional groups of the stabilizing molecules. While FESEM provides information about the size and size distribution of nanoparticles, EDAX gives information about the elemental composition. XRD provides the crystalline structure, size and size distribution of the nanoparticles and the FTIR provides information about the functional groups of the stabilizing molecules. EDAX analysis indicated the chemical composition and purity of TiO_2 NPs which is evident from the peaks corresponding to Ti element and oxygen.

FESEM analysis of TiO_2 NPs produced by *Moraxella osloensis* has confirmed the bio reduction of bulk TiO_2 to nanoscale with the size in the range of 60–150 nm. The particles were found to be irregularly shaped and uniformly dispersed with less aggregation (fig. 1). While the TiO_2 NPs synthesized by *Aeromonas hydrophila* [16] was reported to be spherical and uneven, whereas the nanoparticles synthesized by *Bacillus subtilis* [17] and *Propionibacterium* species [18] were found to be spherical.

The XRD pattern of the TiO_2 NPs produced by *Moraxella osloensis* has shown broad peaks indicating the small size of the particles (fig. 2). The broad peaks indicate either particles of very small crystallite size, or particles are semicrystalline in nature [5, 9, 18].

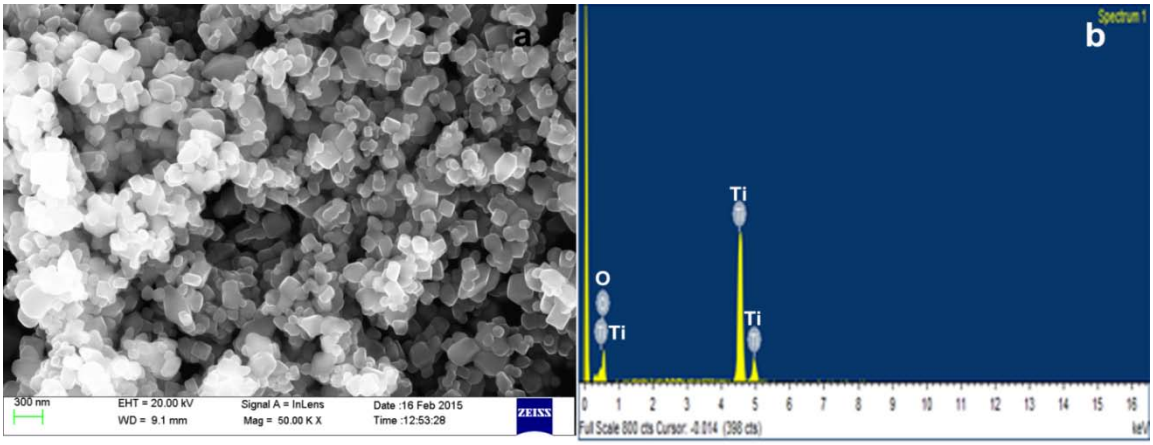

Fig. 1: (a) FESEM graph and (b) EDAX picture of TiO_2 nanoparticles synthesized by *Moraxella osloensis*

The formation of anatase crystal phase was confirmed from the peak signals at 25.38 °, 37.86 °, 48.09 °, 53.96 °, 55.11 °, 62.73 ° and 75.07 ° corresponding to planes (1 0 1), (0 0 4), (2 0 0), (1 0 5), (2 1 1), (2 0 4) and (2 1 5) respectively, which coincide with Crystallography Open Database (COD) DB Card number 9008213. While most of the biologically synthesized TiO_2 NPs were found to be predominantly in anatase crystal phase [8, 9, 18] rutile crystal phase were also reported [5, 16, 17]. In addition to these peaks, three prominent peaks at 38.63, 55.11 and 68.80 corresponding to planes (1 1 0), (2 0 0), (2 1 1) respectively indicating the presence of Titanium beta. The peaks are comparable with the standard peaks in COD DB Card number 9008554. The average particle diameter of the nanoparticles was calculated from XRD pattern using the Scherrer's equation

$$d = \frac{k\lambda}{\beta \cos\theta}$$

Where d is the crystal size; k is the wavelength of the X-ray radiation (k = 0.15406 nm) for Cuka; k is usually taken as 0.89; and β is the angular FWHM of the XRD peak at the diffraction angle θ [17]. The TiO_2 NPs synthesized by *Moraxella osloensis* were found to have their size in the range of 62 to 90 nm with the average being 72 nm.

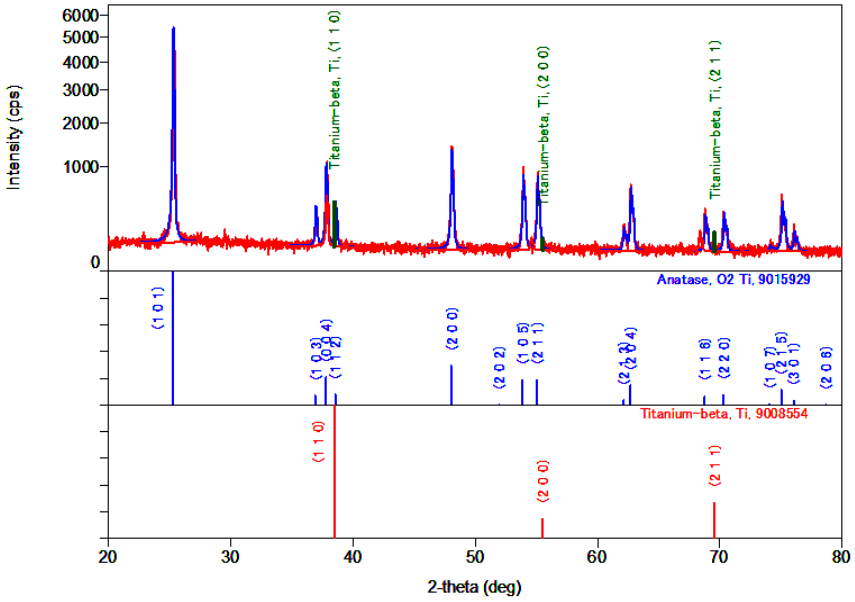

Fig. 2: X-ray diffractogram of TiO_2 nanoparticles synthesized by *Moraxella osloensis*

Fig. 3 Shows the FTIR spectra of bulk and nano TiO_2 (reduced by *Moraxella Osloensis*) respectively. While peaks at 3333 cm-1, 1651 cm-1, 773 cm-1 (C-H rock of alkanes) can be seen in both bulk and nano TiO_2, peaks at 2333 cm-1, 1117 cm-1 (C-N stretch of aliphatic amine), 678 cm-1 (N-H wag of 1° and 2° amines) and 516 cm-1 could be been only in TiO_2. The peaks found in bulk TiO_2 were comparable with the peaks for pure TiO_2 obtained by Wan *et al.* [19] and Wu *et al.* [20]. A shift in peak wavelength from 3333 cm-1 to 3412 cm-1 was observed after reduction of bulk TiO_2 to nano TiO_2. This may be due to the overlapping of 3333 cm-1 with the N-H stretching vibrations of 1° and 2° amines and amides of bacterial proteins that are involved in the bio-reduction.

In recent years, TiO_2 NPs have been widely used in industrial and consumer products due to their stronger catalytic activity when compared to TiO_2 Fine Particles. Their application in cosmetic products such as sunscreen creams, whitening creams, morning and night creams and in skim milk [21, 22] (web ref) makes the toxicity analysis mandatory.

Further, TiO_2 NPs are reported to be more toxic than FPs [4]. Hence, the biologically synthesized TiO_2 NPs and the bulk TiO_2 were checked for their cytotoxicity using MTT assay against normal (HaCaT) and cancer (Hep2) cell line. Cytotoxicity tests were carried in replicate as explained previously [23].

The cytotoxicity results given in fig. 4 clearly indicated that the NPs were more toxic than its bulk counterpart. The size dependent toxicity of TiO_2 was evident from the IC_{50} value of bulk TiO_2 for HaCaT which was found to be 150 µg/ml as against the IC_{50} value of 55 µg/ml for TiO_2 NPs. Similar trend was observed in case of Hep2 cell line except the fact that this cancer cell line was comparatively resistant than the normal cell line exhibiting the IC_{50} values of 550 µg/ml and 172 µg/ml of bulk and nano TiO_2 respectively. Swetha *et al.* [9] have reported that the biogenic TiO_2 NPs synthesized by an endophytic *Bacillus cereus* has an IC_{50} value of 465 µg/ml against Hep 2 cell line. Increased toxicity of the TiO_2 NPs synthesized by *Moraxella osloensis* may be due to the increased incubation period.

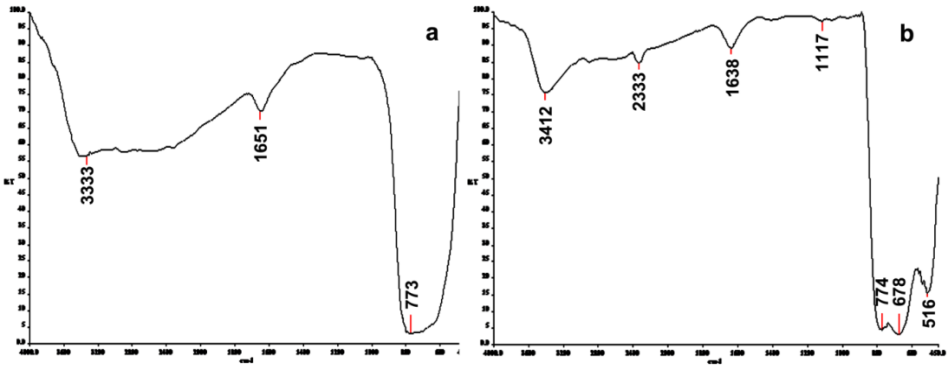

Fig. 3: FTIR spectrum of (a) bulk TiO_2 and (b) TiO_2 nanoparticles synthesized by *Moraxella osloensis*

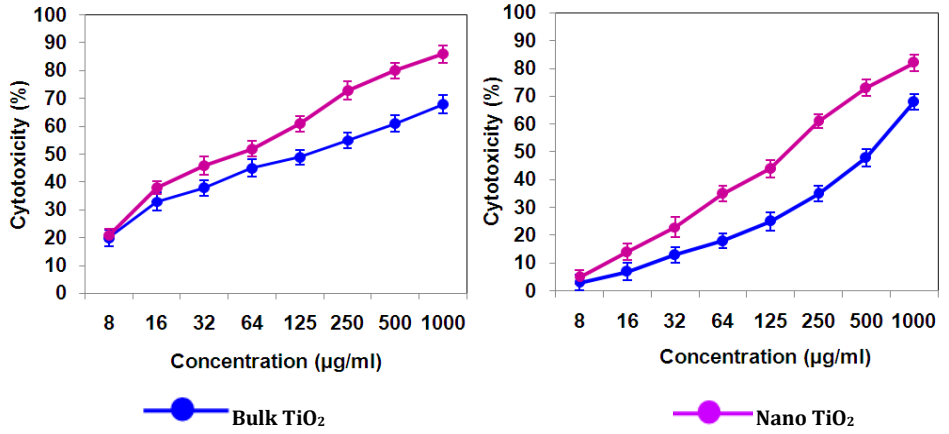

Fig. 4: Cytotoxicity analysis of Bulk TiO_2 and TiO_2 nanoparticles synthesized by *Moraxella osloensis* against (a) HaCat and (b) Hep2 cell lines

This study reports the environmentally safe and cost effective method of producing TiO_2 NPs by a dye degrading *Moraxella osloensis* in an extracellular fashion. In addition to TiO_2 NPs elemental Ti could also be identified from XRD. FTIR confirmed the presence of proteins which are involved in the bioreduction and stabilization of nanoparticles. Cytotoxicity studies against HaCat and Hep2 cell lines indicated that the nano TiO_2 is comparatively toxic than its bulk counterpart and HaCaT is more sensitive compared to Hep2 cell lines.

ACKNOWLEDGMENT

The authors would like to thank Mr. Pradhaban, IRC-Sathyabama University for helping with the interpretation of XRD. The authors would also like to thank the management of Sathyabama University for providing the infrastructure and facilities to carry out this work.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Narayanan KB, Sakthivel N. Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Adv Colloid Interface Sci 2011;169:59-79.
2. Ahmad R, Sardar M. TiO_2 nanoparticles as an antibacterial agent against *E. coli*. Int J Innovative Res Sci Eng Technol 2013;2:3569-74.
3. Rai RV, Bai JA. Nanoparticles and their potential application as antimicrobials. Science against microbial pathogens: communicating current research and technological advances. A. Méndez-Vilas (Ed.) @ Formatex; 2011. p. 197-209.
4. Shi H, Magaye R, Castranova V, Zhao J. Titanium dioxide nanoparticls: a review of current toxicological data. Part Fiber Toxicol 2013;10:15.
5. Jha AK, Prasad K, Kulkarni AR. Synthesis of TiO_2 nanoparticle using microorganisms. Colloids Surf B 2009;71:226-9.
6. Kulkarni N, Muddapur U. Biosynthesis of metal nanoparticles: a review. J Nanotechnol 2014. Doi.Org/10.1155/2014/510246. [Article in Press]
7. Prasad K, Jha AK, Kulkarni AR. *Lactobacillus* assisted synthesis of titanium nanoparticles. Nanoscale Res Lett 2007;2:248-50.
8. Dhandapani P, Maruthamuthu S, Rajagopal G. Bio-mediated synthesis of TiO_2 nanoparticles and its photocatalytic effect on aquatic biofilm. J Photochem Photobiol B 2012;110:43-9.
9. Swetha S, Valli Nachiyar, Rashmi Lerensha, Renugadevi K. Biogenesis of TiO_2 nanoparticles using endophytic *Bacillus cereus*. J Nanopart Res 2014;16:2681.

10. Bansal V, Rautray D, Bharde A, Ahire K, Sanyal A, Ahmad A, *et al*. Fungus-mediated biosynthesis of silica and titania particles. J Mater Chem 2005;15:2583-9.
11. Khan SA, Ahmad A. Phase, size and shape transformation by fungal biotransformation of bulk TiO_2. Chem Eng J 2013;230:367-71.
12. Raliya R, Biswas P, Tarafdar JC. TiO_2 nanoparticle biosynthesis and its physiological effect on mung bean (*Vigna radiata* L). Biotechnol Rep 2015:5:22-6.
13. Sundarajan M, Gowri S. Green synthesis of Titanium dioxide nanoparticles by *Nyctanthes Arbor-tristis* leaves extract. Chalcogenide Lett 2011;8:447-51.
14. Rajakumar G, Rahuman AA, Priyamvada B, Khanna VG, Kumar DK, Sujin PJ. *Eclipta prostrata* leaf aqueous extract mediated synthesis of titanium dioxide nanoparticles. Mater Lett 2012;68:115-7.
15. Karunya A, Rose C, Valli Nachiyar C. Biodegradation of the textile dye Mordant Black 17 (Calcon) by *Moraxella osloensis* isolated from textile effluent-contaminated site. World J Microbiol Biotechnol 2014;30:915-24.
16. Jayaseelan C, Rahuman AA, Roopan SM, Kirthi A, Venkatesan J, Kim SK, *et al*. Biological approach to synthesize TiO_2 nanoparticles using *Aeromonas hydrophila* and its antibacterial activity. Spectrochim Acta A 2013;107:82-9.
17. Kirthi AV, Rahuman AA, Rajakumar G, Marimuthu S, Santhoshkumar T, Jayaseelan C, *et al*. Biosynthesis of titanium dioxide nanoparticles using bacterium *Bacillus subtilis*. Mater Lett 2011;65:2745-7.
18. Babitha S, Purna Sai K. Biosynthesis of titanium dioxide nanoparticles using a probiotic from coal fly ash effluent. Mater Res Bull 2013;48:4738-42.
19. Wan M, Li W, Long Y, Tua Y. Electrochemical determination of tryptophan based on Si-doped nano TiO_2 modified glassy carbonelectrode. Anal Methods 2012;4:2860-5.
20. Wu J, Yue G, Xiao Y, Lin J, Huang M, Lan Z, *et al*. An ultraviolet responsive hybrid solar cell based on titania/poly (3-hexylthiophene). Sci Rep 2013. Doi:10.1038/srep01283. [Article in Press]
21. Web ref. Titanium Oxide (Titania, TiO_2) Nanoparticles–Properties, Applications. Available from: http://www.azonano.com/article.aspx?ArticleID=3357. [Last accessed on 01 Dec 2015].
22. Web ref. About Titanium Dioxide. Available from: http://www.cinkarna.si/si/files/default/tio2/tdma_about_tio2__20120914.pdf. [Last accessed on 01 Dec 2015].
23. Bavanilatha M, Karthick Raja N, Bhattacharya A. Evaluation of toxic effect of biogenic silver nanoparticles on chick embryo. Int J Chem Pharm Res 2014;6:230-5.