I J R B A T, Issue (Special-17), June 2021: 500-507
A Double-Blind Peer Reviewed & Refereed Journal
OPEN ACCES
e-ISSN 2347 – 517X
Original Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© www.ijrbat.in

A COMPARATIVE STUDY OF THE EFFECT OF TIO_2 NANOPARTICLE ON *BACILLUS COAGULANS* BOTH IN LIGHT AND DARK CONDITION – AN APPROACH TOWARDS SAFETY ON BODY'S IMMUNITY

Nabanita Mukherjee[1, 2]*, Arunava Goswami [1]
1. Agricultural & Ecological Research Unit,Biological Science Division, Indian Statistical Institute,203 B. T. Road,Kolkata 700108, India
2. Centre for Research in Nanoscience and Nanotechnology, University of Calcutta
Email: nabanitamukherjee7@gmail.com

ABSTRACT:
Anthropogenic activities against nature and interaction with social, environmental determinants create profound health risks for human beings. Combine the response of humans and their ecosystem with the shift of climate results in changes in productivity, species interactions, and the emergence of invasive species. The outbreak of new viral, bacterial, and other zoonotic strains raised questions on new infection, the influence of climate, and ultimately the potential risk on human health. During the era of multiple drug resistance, probiotic treatment becomes a major concern for human society. Along with digestion and absorption, it produces the highest number of antibodies than any other organ of our body, thus, plays a key role to boost up our body's immune system. Along with that, in the new era of nanotechnology, we get new dimensions in medical and pharmaceutical, and agricultural fields such as vaccine design, drug delivery, cancer therapy, crop yields, etc. Among oxide nanoparticles (as they play a crucial role in various fields because of their size, density, physical and chemical properties), titanium dioxides nanoparticles (TiO2 NPs) are used mostly in the agri-food sector. The safety of TiO2 NPs was tested on various human cell lines and its effect on human beneficial gut flora has tested in dark conditions. This research article focused on the comparative study of titanium dioxide nanoparticles on *Bacillus coagulans* (the dominant, spore-forming, lactic acid bacillus used as probiotic) both in light and dark conditions for safe use of drugs. Here, we report TiO2 nanoparticle (40 nm) inhibits the growth of bacteria in presence of light, whereas, in the dark condition it shows increased growth, electron microscopy images also reveals changes in bacterial cell wall integrity due to stressful condition in presence of light.

Keywords: Titanium dioxide nanoparticle, gut bacteria

INTRODUCTION:

In the twenty-first century, human civilization is facing a great challenge on "Climate change and global warming". The rise of average global temperature in terms of intensity and frequency in the last decades was caused by the increasing consumption of fossil fuels and increased emission of CO2. Cutting trees and forests are also one of the major causes of the rise of CO2 concentration in the air. The temperature rise accelerates with time and is expected to be faster in the coming year. The earth hits up. Life in terrestrial, marine and other aquatic ecosystems moves towards the pole to get out of the heat that causes contact of animals with the new ones and also the pathogen to harbour a new host body. Above all anthropogenic activities against nature and their response towards the ecosystem causes a shift of climate, that results in changes in productivity, species interactions, and the emergence of invasive species. Climate is a key determinant of health. Climate governed a range of infectious diseases and the intensity of disease outbreaks are controlled by weather (Dobson and Carper, 1993). Thus, climatic changes are the major cause for the appearance of new zoonotic diseases (caused by bacteria and viruses or other new strains of it) or the appearance of old diseases in new places. Antimicrobial resistance is one of the major health problems in the 21st century. Prevention and treatment of various infectious diseases caused by bacteria, parasites, fungi, and viruses showed resistance against common medicines. The number of drug-resistant bacteria increased substantially due to horizontal gene transfer. In this situation, probiotic treatment has become a major concern for human society. Because it controls the function of mucosa-associated immune cells to produce the highest number of antibodies than any other organ of our body, thus plays a key role to boost up our body's immunity (Helgeland and Brandtzaeg, 2000). Thus, beneficial gut floras maintain an ecological balance in our gut which harbours the largest collection of lymphoid cells. Two adaptive arms of defence were generated by mucosa-associated immune cells and a balanced microbial ecosystem helps those cells to maintain a beautiful balance between immune exclusion by secreted antibody and down regulatory mechanisms to avoid hypersensitivity. Along with that probiotic organisms also help to reduce the spread of antibiotic resistance (Ouwehand et al., 2016). Lactobacillus species is the dominant one for probiotic treatment. Among all lactobacillus, *Bacillus coagulans* is the dominant one and it's a spore-forming gram-positive bacteria that germinate in the acidic environment of the stomach, after that it establishes itself in the duodenum. In the new era of nanotechnology, we find various applications of engineered nanomaterials with less than 100 nm diameter in various fields such as drug delivery, disease diagnosis, agri-food sector, cancer therapy, food yields, etc (Van et al., 2002, Hirsch et al., 2005, Mousavi and Rezaei, 2011). Now a day's engineered nanomaterials are also used in vaccine design (Shin et al. 2020). The agricultural food sector also acts as a major field where this revolutionary technology has been used to yield increased growth of crops with high nutritional value (Mousavi and Rezaei, 2011). In-plant sciences, mainly two groups of nanoparticles were used: mainly metal-based nanomaterials and carbon nanomaterials (Arruda et al., 2015). Among all metal-based nanomaterials, TiO_2 stands top based on its use with various positive feedbacks of its effect on the plant (Rico et al., 2011, Zheng et al., 2005, Hong et al., 2005, Yang et al., 2007) along with that oxide nanoparticles are playing a crucial role in various fields because of its size, density, physical and chemical properties (Jeelani et al., 2020). The bio safety of these nanomaterials was tested in human cell lines (Rahmani et al., 2018, Brandão et al., 2020). Its effect on human beneficial gut flora was also tested in dark conditions (Mukherjee et al., 2020). This research compares the effect of TiO_2 anatase nanoparticles on gut bacteria both in light and dark conditions for the safe use of the drug.

MATERIALS AND METHODS:

Preparation of nanoparticle suspension

Titanium dioxide anatase (50nm, 98% pure, hydrophilic) nanoparticles were purchased from M K Implex Corp., division: MKnano, Missisauga, Canada, it was dispersed in filter-sterilized DI water (Millipore, resistivity = 18.3

MΩ/cm). A stock suspension of 1000 μg/ml was prepared by placing it in an ultra-sound water bath for 30 min to break aggregates (Jiang et al. 2009).

The size and the morphology of the titanium dioxide anatase nanoparticles were examined by scanning electron microscopy (SEM) transmission electron microscopy (TEM) and subjected to EDX and UV-VIS spectroscopy.

Bacillus coagulans- source, inoculum and culture condition

Each Vizylac capsule by Unichem contains 120 X 106 spores of the active product of *Bacillus coagulans*. The viable spore count of *Bacillus coagulans* was determined (Mukherjee et al., 2020). Viable cell count was enumerated by repeated serial dilutions of bacterial suspensions followed by plating in *Lactobacillus* MRS Agar plate (Chaudhari et al. 2012). The plate was incubated for 48-72 hours at 37˚C. After that a single colony was isolated and culture overnight in *Lactobacillus* MRS Broth (HiMedia, Mumbai, India) at 37˚C, 120 rpm.

Impacts of nanoparticle on bacterial cultures both in light and dark condition

TiO2 anatase nanoparticle of 1 μg/ml exposure concentration was made from 1000 μg/ml stock suspension. Adjusted bacterial inoculums were incubated in test tubes. Three sets of tubes each set with two controls and two treatments were kept in an incubator both in light and dark conditions respectively. After 16 hours of incubation at 37˚C, 120 rpm bacterial suspension was serially diluted and the viable count was enumerated by plating on a sterile *Lactobacillus* MRS Agar plate. The plate was incubated at 37˚C for 48-72 hours. Results were plotted with means ± standard error (S.E.M) to produce the histogram of bacterial growth.

Transmission Electron Microscopy (TEM) of bacteria:

For transmission electron microscopic studies samples were prepared in the following way: untreated bacterial sample and bacterial samples treated with 1 μg/ml of NP at 16 hours, both in light and dark condition were washed with cold 0.5 M phosphate buffer (pH 7.0). Then the samples were fixed in 4 % glutaraldehyde at 4˚C for 1 hour followed by staining with 1 % osmium tetroxide solution and dehydrated by graded ethanol (Hartmann et al., 2010). JEOL JEM-2010 high-resolution transmission electron microscope was used to obtain images.

RESULT AND DISCUSSION:

Characterization of TiO_2 anatase NP:

The FESEM image of nanoparticles (Fig.1.a) depicts a cluster of elliptical and spherical particles based on surface morphology. TEM (Fig.1.b) images gave detailed information on size of nanoparticle. It ranges between 50-100 nm.

The EDS data (Fig. 1.d) showed the purity and composition of the nanoparticles used. The main peaks were of Oxygen and Titanium. The lambda max is known to be around 250-450nm for Ti (Atif et al., 2015). The UV-Vis spectroscopy data showed the maximum absorbance in between 300-400 nm region with the lambda max at 352nm (Fig.1.c).

Bacterial growth measurement in presence of metal oxide nanoparticles

In this experiment, we gave a treatment of bacteria at 16 hours of incubation in different condition both in dark and in presence of light. Actually in previous experiment we found maximum growth of *Bacillus coagulans* is at 1 μg/ml in dark condition. At 16 hour of incubation the bacterial growth reached at 10^6 colony forming unit per millilitre and the treated bacteria in dark condition showed increased growth than the control one (Fig 2 a). Whereas, in presence of light bacterial growth suddenly falls down at 16 hours of incubation (Fig 2 b). Thus, TiO_2 anatase nanoparticle showed toxicity when the treatment occurs in presence of visible light or UV light. Actually, the nanoparticle showed photo catalytic activity in presence of visible light, UV- A, B, C, fluorescent light and X ray radiation. It causes photo activation of nano TiO_2 (mainly in case of anatase form) for the promotion of electron from the valence band to the conduction band, thus leaving behind a positive hole. Due to formation of highly reactive radicals, it reacts with most of the organic substances of its surroundings that seems to be toxic for the bacteria.

Interaction of TiO2 nanoparticle with bacteria as examined by TEM

Previous result in dark condition showed no aggregation of TiO2 anatase NPs (concentration 1 µg/ml) of 50 nmsize (Fig 3 b) and it does not damage bacterial cell wall like TEM images of bacteria without treatment (Fig 3 a, control). But, in presence of lightthe TEM image showed that bacterial membrane was highly disrupted. Active TiO2 nanoparticle interacts with bacterial cell wall at first and then with its membrane. This layer also becomes damaged with extrusion of cytoplasm. In Fig 3 c, we found presence of TiO2 nanoparticle within this protective layer of bacteria.

CONCLUSION:

In the end, we can conclude that TiO2 nanoparticles spatially in the anatase form showed a high level of toxicity in presence of light because of its photocatalytic activity. The reactive radicals in presence of light are capable of reacting with the cell wall of bacteria and then its membrane causing extrusion of cytoplasmic content of bacteria. Whereas, no such event occurred in dark conditions. Rather bacteria showed increased growth at a particular concentration of TiO2 anatase nanoparticle. Above all, this experiment showed a safety measure towards the protection of the spore-forming lactic acid bacteria in experimental conditions as well as for the safe use of drugs (probiotic capsule). *Bacillus coagulans* are used as probiotics to treat various diseases and we also know that probiotic therapy showed us a big ray of hope to build up our body's immunity. Thus, this article deals with an approach towards the safety of the human body's immunity.

ACKNOWLEDGEMENT:

This study was supported by Department of Science and Technology, Govt. of India (SR/NM/NS-08/2014), Depart of Biotechnology, Govt. of India (BT/PR26647/NNT/28/1365/2017), Indian Space Research Organization (ISRO/RES/3/827/19-20) and Indian Statistical Institute (ISI) (ISI/TAC/PROJECT 1/ 2020-21). We are also grateful to Mrs. Sutanuka Mitra (Ph.D. scholar ISI, Kolkata) for her assistance in sample delivery for TEM image and laboratory supporters Mr. Ashim Dhar and Mrs. Piyali Maitra for their assistance in field work.

REFERENCES:

Arruda SCC, Silva ALD, Galazzi RM, Azevedo RA, Arruda MAZ (2015) Nanoparticles applied to plant science: a review. Talanta 131:693–705. https

://doi.org/10.1016/j.talanta.2014.08.050

Atif M, Farooq WA, Fatehmulla A, Aslam M, Ali SM (2015) Photovoltaic and impedance spectroscopy study of screen-printed TiO2 based CdS quantum dot sensitized solar cells. Materials 8(1):355–367. https ://doi.org/10.3390/ma801 0355

Brandão F, Fernández-Bertólez N, Rosário F, et al. Genotoxicity of TiO_2 Nanoparticles in Four Different Human Cell Lines (A549, HEPG2, A172 and SH-SY5Y). *Nanomaterials (Basel).* 2020;10(3):412. Published 2020 Feb 27. doi:10.3390/nano10030412

D.A. La Van, D.M. Lynn, R. Langer, Moving smaller in drug discovery and delivery, Nat. Rev., Drug Discov. 1 (2002) 77–84.

Dobson, A. and Carper, R., 1993. Biodiversity. *Lancet,* 342: 1096- 1099.

Hartmann M, Berditsch M, Hawecker J, Ardakani MF, Gerthsen D, Ulrich AS (2010) Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S and PGLa as revealed by transmission and scanning electron microscopy. Antimicrob Agents Chemother 54(8):3132–3142. https ://doi.org/10.1128/ AAC.00124 -10

Helgeland L, Brandtzaeg P (2000) Development and function of intestinal B and T cells. Microbecol health dis 12(2):110–127. https :// doi.org/10.3402/mehd.v12i2 .8071

Hong F, Zhou J, Liu C, Yang F, Wu C, Zheng L, Yang P (2005) Effect of nano-TiO2 on photochemical reaction of chloroplasts of spinach. Biol Trace Elem Res 105(1–3):269–279. https ://doi.org/10.1385/BTER:105:1-3:269

Jeelani, P.G., Mulay, P., Venkat, R. *et al.* Multifaceted Application of Silica Nanoparticles. A Review. *Silicon* **12,** 1337–1354 (2020). https://doi.org/10.1007/s12633-019-00229-y

Jiang W, Mashayekhi H, Xing B (2009) Bacterial toxicity comparison between nano-and micro-scaled oxide particles. Environ Pollut 157(5):1619–1625. https ://doi.org/10.1016/j.envpol.2008.12.025

L.R. Hirsch, R.J. Stafford, R.A. Bankson, S.A. Sershen, B. Rivera, R.E. Price, J.D. Hazle, N.J. Halas, J.L. West, Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13549– 13554.

Mousavi SR, Rezaei M (2011) Nanotechnology in agriculture and food production. J Appl Environ Biol Sci 1(10):414–419

Mukherjee, N., Mitra, S., Adak, S. *et al.* Evaluation of the growth response of spore forming lactic acid *Bacillus–Bacillus coagulans* in presence of oxide nanoparticles. *ApplNanosci* **10,** 4075–4086 (2020). https://doi.org/10.1007/s13204-020-01512-w

Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B (2016) Probiotic approach to prevent antibiotic resistance. Ann Med 48(4):246–255. https ://doi.org/10.3109/07853 890.2016.11612 32

RahmaniKukia N, Rasmi Y, Abbasi A, et al. Bio-Effects of TiO2 Nanoparticles on Human Colorectal Cancer and Umbilical Vein Endothelial Cell Lines. *Asian Pac J Cancer Prev.* 2018;19(10):2821-2829. Published 2018 Oct 26. doi:10.22034/APJCP.2018.19.10.2821

Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea- Torresdey JL (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem 59(8):3485–3498. https ://doi.org/10.1021/jf104 517j

Shin, M.D., Shukla, S., Chung, Y.H. *et al.* COVID-19 vaccine development and a potential nanomaterial path forward. *Nat. Nanotechnol.* **15,** 646–655 (2020). https://doi.org/10.1038/s41565-020-0737-y

Yang F, Liu C, Gao F, Su M, Wu X, Zheng L, Hong F, Yang P (2007) The improvement of spinach growth by nano-anatase TiO2 Treatment is related to nitrogen photoreduction. Biol Trace Elem Res 119(1):77–88. https ://doi.org/10.1007/s1201 1-007-0046-4

Zheng L, Hong F, Lu S, Liu C (2005) Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. Biol Trace Elem Res 104(1):83–91. https ://doi.org/10.1385/BTER:104:1:083

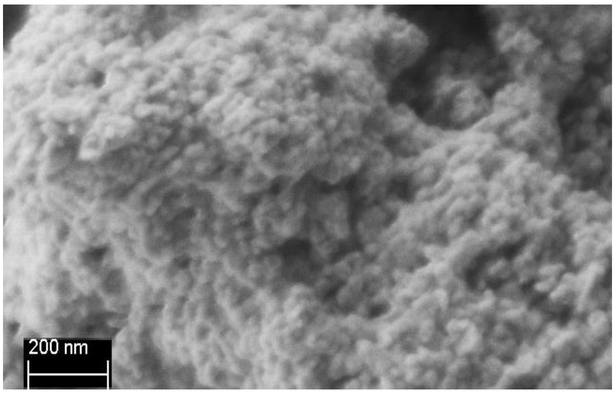

Fig 1 a. FESEM image of nano TiO2 anatase

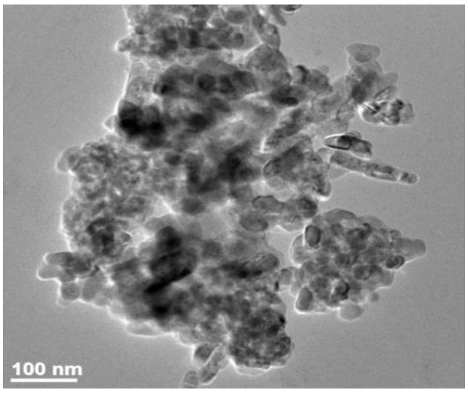

Fig 1 b. TEM image of nano TiO2 anatase

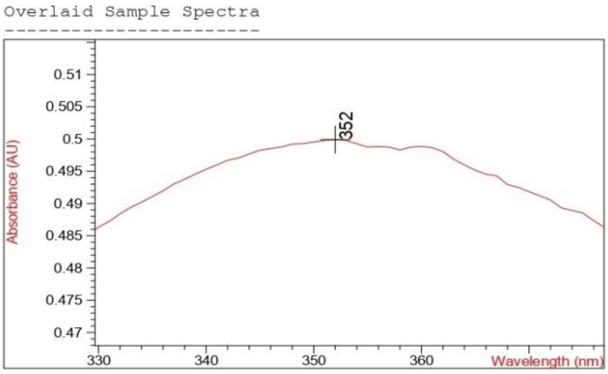

Fig 1 c. UV-VIS of nano TiO2 anatase

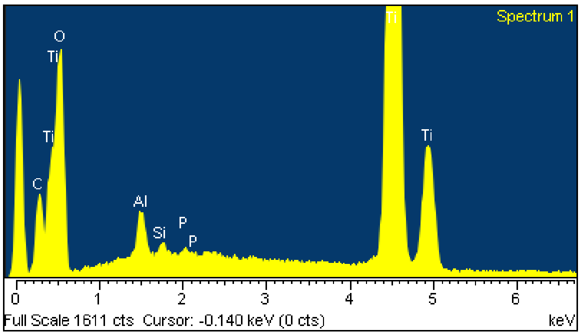

Fig 1 d. EDS spectrum of nano TiO2 anatase

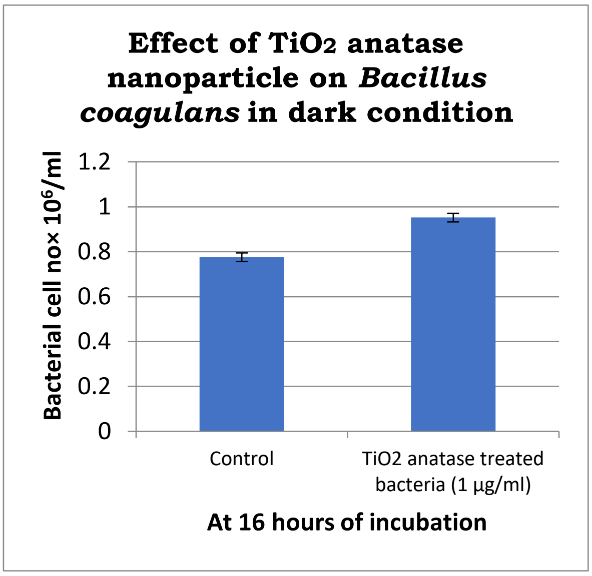

Fig 2 a. Effect of TiO2 Anatase nanoparticle on bacterial growth at 16 hours of incubation in dark condition.

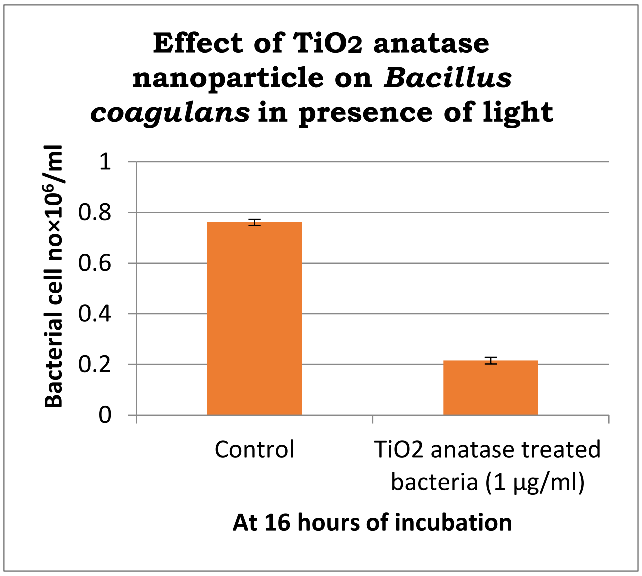

Fig 2 b. Effect of TiO2 Anatase nanoparticle on bacterial growth at 16 hours of incubation in presence of light.

e-ISSN 2347 – 517X
Original Article

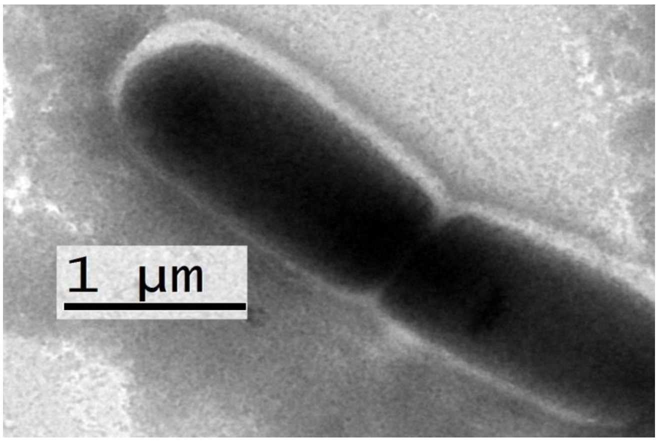

Fig 3 a. TEM images of bacteria without treatment (control)

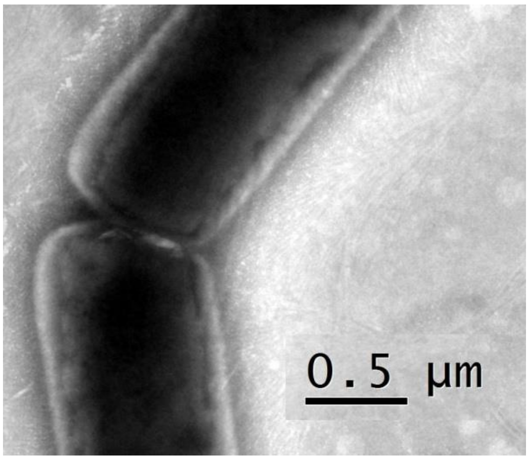

Fig 3 b. TEM images of bacteria treated with TiO2 Anatase nanoparticle (1 µg/ml) at 16 hours of incubation in dark condition.

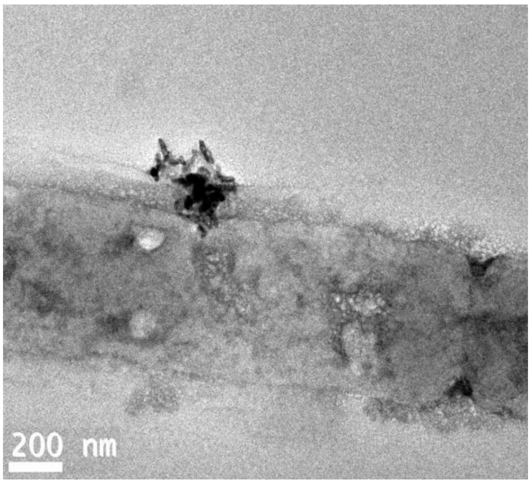

Fig 3 c. TEM images of bacteria treated with TiO2 Anatase nanoparticle (1 µg/ml) at 16 hours of incubation in presence of light.