Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Biosynthesis and evaluation of TiO_2 and ZnO nanoparticles from *in vitro* stimulation of *Lactobacillus johnsonii*

Hind A. Al-Zahrani[1], Amr A. El-Waseif[2*], Dina E. El-Ghwas[1,3]

[1]*Biology Dept., Faculty of Science, University of Jeddah, Saudi Arabia.*
[2]*Botany and Microbiology Dept., Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt.*
[3]*Chemistry of Natural and Microbial products Dept., National Research Center, Dokki, Egypt.*

Key words: TiO_2 and ZnO Nanoparticles, UV, FTIR, TEM and Lactobacillus.

***Corresponding Author: Amr A. El-Waseif,** Botany and Microbiology Department, Faculty of Science (Boys); Al-Azhar University, Cairo, Egypt.

Abstract

The microbial synthesis of nanoparticles is advantageous more other chemical and physical methods. Biosynthesis of TiO_2 and ZnO Nanoparticles (NPs) were carried out using *Lactobacillus johnsonii* that isolated from human gut and identified biochemical in previous study. Results of molecular identification using 16S DNA of Lactobacillus strain revealed that similarity in phylogenic tree is 100% with *Lactobacillus johnsonii.* Synthesis in MRS broth and evaluation of TiO_2 and ZnO nanoparticles from *Lactobacillus johnsonii* carried out using UV, FTIR, and TEM. Charts results of UV experiment for both nanoparticles indicated that absorbance at 409 and 492 nm is typically for TiO_2 and ZnO Nanoparticles respectively. Also, FTIR peaks of TiO_2 chart has confirmed that the stronger ability of proteins to bind metal, and grow the possibility of coating the metal nanoparticles with proteins to prevent agglomeration of the particles. TEM images at different magnification explain the shape and size range of TiO_2 and ZnO Nanoparticles. TEM of TiO_2 NPs recorded irregular shapes but, ZnO NPs spherical, results shows also, average size between 4-9 nm in both cases.

Introduction

The synthesis of nanoparticles (NPs) with various methods; chemical physical and biological make a change in sizes, and shapes of nanoparticles. Biological methods have advantageous more than other chemical methods as they are low cost and do not use of temperatures, toxic chemicals, energy and high pressure [1, 2]. The synthesis of nanoparticles by biological method is eco-friendly, simple and can be used as catalysts composition, which the classical methods cannot be produced it, nanoparticles applications in medicine and sensors are envisaged. Also, the bacterial nanoparticles can be used to control the human pathogens [3].

The biosources used for Nanoparticles synthesis such as fungi, yeast, bacteria, plant extract; the green chemistry principles is compatible with using microorganisms: the microorganism is eco-friendly and reducing agent employed [4]. Wide classes of Gram positive and negative bacteria can used as to adsorb and capture up of heavy metal ions. Bacterial system advantages include easy handling and hereditarily manipulated easily [5]. The NPs synthesized biologically have various applications like biolabelling, in cancer management and coating of medical products [6]. The oxidized form of synthesized NPs was more useful, because these NPs have good magnetic, electrical and optical properties [7].

The essential trace element Zinc with ionic state is a necessary for animals, plants and microbes [8]. The great significance material Titanium dioxide (TiO_2) which, apply in many fields, such as, biomaterials, solar cell devices, photo catalysis, and gas sensors [9]. Titanium found as non- toxic and biocompatible Titanium applications in medical filed like bone tissue engineering and industry of pharmaceutical [10, 2]. Numerous contaminants like environmental toxin can be inhibit and degradation with TiO_2 catalysts that confirmed to be excellent and efficient in photocatalysts. Wide applications of TiO_2 include cleaning of surface, air and water [11]. Desalinization of plants by Titanium is recommended because Titanium strong resistance to corrosion from sea water. The titanium pins are effective in medical uses because Ti not react nature when contacting flesh and bone [12]. The TiO_2 nanoparticles can synthesis by microorganisms such as *Saccharomyces cerevisae* and *Lactobacillus* sp. [13]. Few reports only are available on the *Lactobacillus sp.* l production of TiO_2NPs.

Objectives of this idea includ, we study the biological synthesis of TiO_2 and ZnO nanoparticles using *Lactobacillus johnsonii* and evaluate the properties of TiO_2 and ZnO NPs using UV, FTIR and TEM. Focus on the biological source for nanoparticles synthesis specially the co-friendly bacteria *Lactobacillus.*

Experimental

Materials and Methods

Bacterial culture and media

Bacterial strain was used in this study was isolated from human gut grown in MRS broth (10 g Peptone, 8 g Meat extract, 4 g yeast extract, 20 g D(+) Glucose, 2 g Dipotassium hydrogen phosphate, 5 g Sodium acetate trihydrate, 2 g Triammonium citrate, 0.2 g Magnesium sulfate heptahydrate, 0.05 g Magnesium sulfate tetrahydrate, 1L Dist. water, Final pH 6.2) at 30°C for 48 h [14].

Molecular identification of Lactobacillus strain

Selected strain was examined microscopically for cellular morphology and biochemical properties in previous study**.** PCR was used to amplify the 16S ribosomal DNA gene of strain. The 16S ribosomal DNA sequence was determined by direct sequencing. Total DNA was isolated by using Wizard genomic DNA purification kit (Promega, Madison, USA). Primers used for PCR and DNA sequencing. The PCR amplification was performed with the primer pair SPO/SP6 targeted against regions of 16S ribosomal DNA StrepF; 5-AAGAGTTTGATCCTGGCTCAG-3. and StrepR; 5-CTACGGCTACCTTGTTACGA-3**.** Amplification of DNA was performed in a Mastercycler personal thermal cycler (Eppendorf). PCR conditions included a hot start at 96°C (5 min.), 35 cycles consisting of hybridation at 50°C (1 min), polymerisation at 72°C (2 min.), denaturation at 96°C (1 min) and a final extension at 72°C (2 min.). PCR products were resolved by electrophoresis in 1% (w/v) agarose gel and visualized by ethidium bromide (1 µl/10 ml) staining. 16S ribosomal DNA PCR applicants were purified following the microcon YM-100 kit (Bedford, MA, USA) and sequenced using the Big Dye Terminator V3.0 kit as specified by the supplier with primers while automated sequencing of both strands of the PCR products gene sequencer (ABI, Forster, USA). The sequences obtained (500–750 bp) were then assembled in silico (Vector NTI) using overlapping zones between the various sequences to form the contiguous sequence. Phylogenetic analysis was realized by an alignment of sequence consensus of the 16S ribosomal DNA genes collected in an international database (Gene bank). The results were then expressed in percentage of homology between the submitted sequence and the sequences resulting from the database [15].

Synthesis of TiO_2 and ZnO nanoparticles using *Lactobacillus johnsonii* in MRS broth

Two flasks were used; each flask was filled with 40 ml of MRS (De-Man Rogosa Sharpe) broth. Then 20 ml of TiO_2 (0.025m) and 20 ml of ZnO 0.1 (g/mL) were added to the first and second flask respectively and both were stirred for half hour on a magnetic stirrer while the third flask contain MRS broth only. Final concentration ultimately would be equivalent. The fresh culture 24h of *Lactobacillus johnsonii* was inoculated the first and second flask. Then, transfer to incubator at 37Co for 24 hours. Then, they were centrifuged at 5000 rpm for 5 min and washed with distilled water several times then dried in an oven at 50°C for one hour and kept for further use [16].

Evaluation of TiO_2 and ZnO Nanoparticles

Ultraviolet (UV) spectrum

The UV spectrum analysis was carried out using: T80+UV/VIS Spectrometer, PG Instrument Ltd. Range: 190-1000 nm.

FTIR spectrum measurement

FTIR spectrum was obtained by mixing with potassium bromide at 1 : 100 ratio which was compressed to a 2 mm semi-transparent disk for 2 min. spectra over the wave length (4000-400 cm^{-1}) were recorded using Nexus 670 FTIR spectrophotometer (Iclet Co., USA).

Transmission electron microscopy (TEM)

This study was undertaken to know the size and shape of Titanium dioxide and Zinc oxide Nanoparticles. The TEM image was carried out using: Electron probe micro-analyzer JEOL – JXA 840A, Model Japan. Thin films of the sample were prepared on a coated copper grid by just placing a very small amount of the sample on the grid. Then the film on the TEM grid was allowed to dry and the images of nanoparticles were taken.

Results and Discussion

Molecular identification of Lactobacillus strain

Lactobacillus strain that isolated from human gut and identified as Gram positive bacilli and biochemical in previous study. Results of molecular identification using 16S rDNA of the Lactobacillus strain revealed that similarity in phylogenic tree 100% with *Lactobacillus johnsonii*. The sequence was compared with available sequence in Gene Bank databases, (Figure 1).

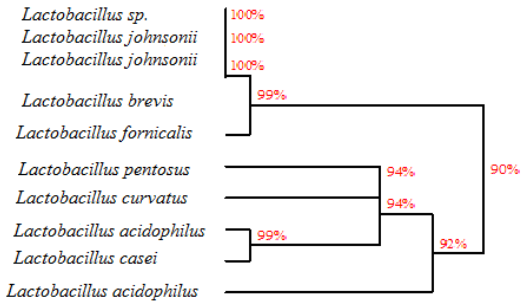


Figure 1. The phylogenetic tree of *Lactobacillus* isolate based on 16S rDNA sequences.

Evaluation of TiO_2 and ZnO Nanoparticles synthesis from *Lactobacillus johnsonii*

UV- spectrophotometer

The extracellular biosynthesis TiO_2 nanoparticles by the culture broth of *Lactobacillus johnsonii* was carried out in our paper. In Figure 2 chat of UV–spectrum the broad peak of the absorption band at 406 nm. The time factor play role in biosynthesis the maximum synthesis can be reported at 24 hours from the inoculation, But, after 48 hours of incubation the production are reduced. The similar result observed with biosynthesis of TiO_2 NPs [17].

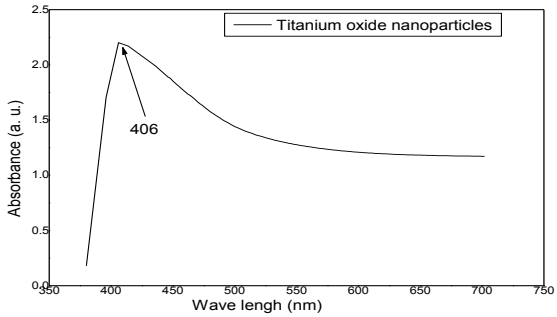


Figure 2. UV-spectrum of TiO_2 NPs synthesized using *Lactobacillus johnsonii.*

UV spectroscopy revealed that the optical properties of the ZnO NPs Figure 3. Chart results indicated that ZnO NPs absorption peak at 392 nm was presence. The same clear result revealed from *Lactobacillus plantarum* VITES07 [18]. Absorbance peaks at 409 and 492nm is typically for TiO_2 and ZnO Nanoparticles respectively.

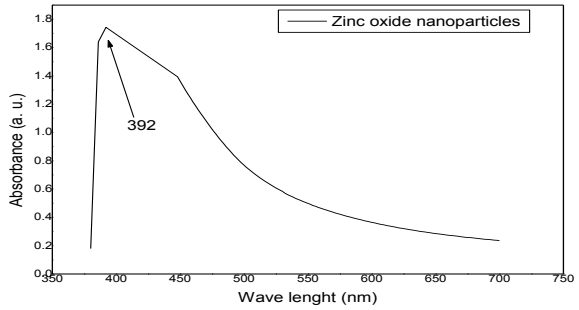
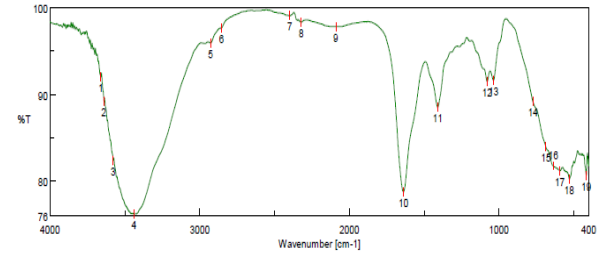


Figure 3. UV-spectrum of ZnO NPs synthesized using *Lactobacillus johnsonii.*

FTIR spectrum measurement

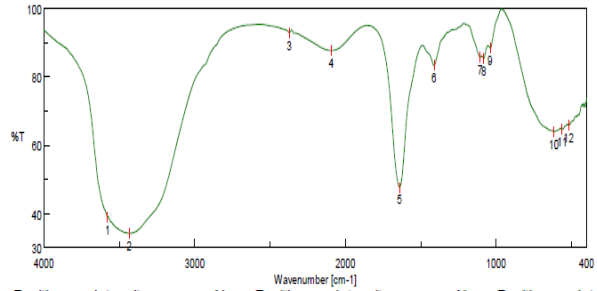
The FTIR spectrum data shows in Figure 4, revealed that peak at 529cm^{-1} indicates the Ti-O stretching vibrations , 1638 cm^{-1}, 1409 cm^{-1},and 1078 cm^{-1} had the slight peak level, 686 cm-^{1} O-H stretching vibrations and bands revealed N-H stretching vibrations of primary and secondary amines respectively. 3438 cm^{-1} are identified as the phenol groups arise due to the O-H stretching vibrations. - C=C- stretching vibrations can be assigned at 1638 cm^{-1} and 1409 cm^{-1} indicated C-H in plane bending vibrations of alkenes respectively. The aliphatic amines C-N stretching vibrations band at 1078 cm^{-1} was indicated. The amines linkages of proteins have the stronger ability to bind metal chart has confirmed that, so that the proteins possibly coating the metal nanoparticles to prevent agglomeration of the NPs and stabilizing in the medium. According to [17] that synthesized TiO_2 nanoparticles by using *Bacillus subtilis* either through the amines residues in the proteins and lipids can obtain similar results.



No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3661.19	92.0326	2	3639.98	89.2211	3	3578.27	82.435
4	3438.46	76.2253	5	2926.45	95.9257	6	2853.17	97.6772
7	2398.05	99.0722	8	2321.87	98.3181	9	2090.46	97.7847
10	1638.23	78.7343	11	1409.71	88.5129	12	1078.98	91.5027
13	1037.52	91.6423	14	773.315	89.1596	15	686.534	83.9333
16	637.358	81.7988	17	593.004	81.2248	18	529.364	80.2578
19	416.549	80.6752						

Figure 4. FTIR-spectrum of TiO_2 NPs synthesized using *Lactobacillus johnsonii.*

FTIR spectra is used to access the details of functional groups involved in the biosynthesis of ZnO NPs FTIR spectra exhibited prominent peaks at 3434, 1640, 1412, 1039, and 519 cm^{-1}. The symmetric stretching mode of water molecules is attributed as broad vibrational band observed at 3434 cm^{-1}. The band observed at 1640, 1412 and 1039 cm^{-1} is assigned to the bending vibrational mode of water molecules. Figure 5 reveals the peak observed at 519 cm^{-1} corresponds to the stretching vibrations of ZnO NPs. The same result shows from *Lactobacillus plantarum* VITES07 [18]. Biological methods have advantageous more than other chemical methods as they are low cost and do not use of temperatures, toxic chemicals, energy and high pressure [1, 2].



No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3578.27	39.0515	2	3434.6	34.1429	3	2372.01	92.9238
4	2092.39	87.6448	5	1640.16	47.6999	6	1412.6	83.2405
7	1105.98	85.7476	8	1082.83	85.3803	9	1039.44	88.414
10	617.109	63.8713	11	562.148	64.5583	12	519.722	65.7921

Figure 5. FTIR-spectrum of ZnO NPs synthesized using *Lactobacillus johnsonii.*

Transmission electron microscopy

The Figure 6 shows Transmission Electron microscopy images of TiO_2 nanoparticles. The TiO_2 nanoparticles were viewed at different magnification and NPs showed approximately in the range of 4 to 9 nm (Scale bar 50 nm). The TEM image clearly indicates the particles were formed irregular Shape and they agglomerated. Few particles were spherical in shape. Other results reported similar shape of the TiO_2 nanoparticles by using *Lactobacillus sp.* but, differentiated in size [17].

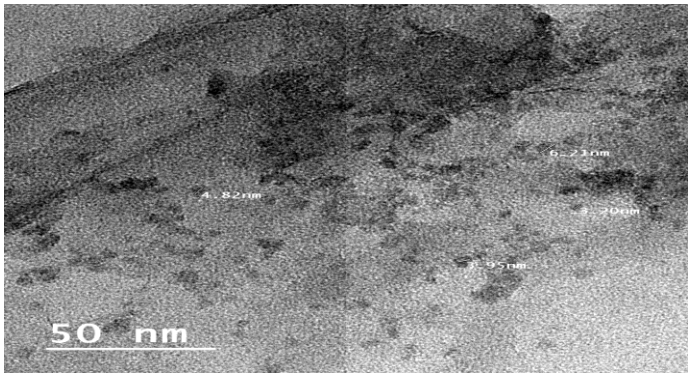


Figure 6. TEM images of TiO_2 NPs synthesized using *Lactobacillus johnsonii.*

The TEM images of the prepared ZnO NPs at 50 and 200 nm scales are shown in the Figure 7. We can observed that ZnO NPs are roughly spherical in shape and polydispersed with maximum particles in size range within 5–9 nm. Particle size distribution determined from TEM is shown in Figure 7. The result revealed from *Lactobacillus plantarum* VITES07 recorded larger particles but, nearly shapes [18].

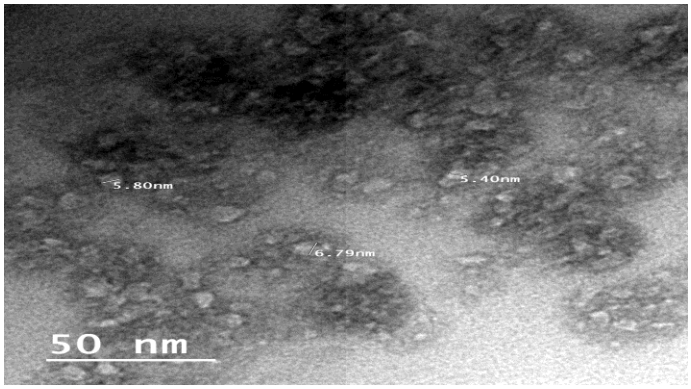


Figure 7. TEM images of ZnO NPs synthesized using *Lactobacillus johnsonii.*

The synthesis of nanoparticles by biological method is eco-friendly, simple and can be used as catalysts composition, which the classical methods cannot be produced it, nanoparticles applications in medicine and sensors are envisaged. Also, the bacterial nanoparticles can be used to control the human pathogens [3].

Conclusion

Symbiotic microorganisms can use as safe source of nanoparticles. The microbial synthesis of nanoparticles is advantageous more other chemical and physical methods. Nanoparticles applications in medicine and sensors are envisaged. Also, the bacterial nanoparticles can be used to control the human pathogens. Biosynthesis of TiO_2 and ZnO Nanoparticles (NPs) using *Lactobacillus johnsonii* that identified using 16S DNA. Evaluation of TiO_2 and ZnO nanoparticles carried out using UV, FTIR, and TEM indicated that absorbance at 409 and 492 nm is typically for TiO_2 and ZnO Nanoparticles respectively. Also, FTIR peaks of TiO_2 chart has confirmed that the stronger ability of proteins to bind metal, and grow the possibility of coating the metal nanoparticles with proteins to prevent agglomeration of the particles. TEM images of TiO_2 NPs recorded irregular shapes but, ZnO NPs spherical, results shows also, average size between 4-9 nm in both cases.

References

1. Tripathy, A., A., Raichur, M., Chandrasekaran, N., Prathna, T.C. and Mukherjee, A: Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of Azadirachtaindica (Neem) leaves. Journal of Nanoparticle Research 2010; 12 (1): 237-246.
2. Malarkodi,C., Chitra,K. Rajeshkumar, K., Gnanajobitha, K., Paulkumar, M., Vanaja, G. and Annadurai,G: Novel eco-friendly synthesis of titanium oxide nanoparticles by using Planomicrobium sp. and its antimicrobial evaluation. Der Pharmacia Sinica 2013; 4(3):59-66.
3. Popecu,M., Velea, A. and Lorinczi, A: Biogenic production of nanoparticles. Digest Journal of Nanomaterials and Biostructures 2010; 5(4): 1035 – 1040.
4. Parashar U.K., Saxena P.S., and Srivastava A.: Bioinspired Synthesis of Silver Nanoparticles. Digest J. Nanomater. Biostruct 2009; 4(1): 159-166.
5. Goldie O., Sunil P., Ashmi M. and Madhuri S.: Extracellular Biosynthesis of Gold Nanoparticles Using Salmonella typhyi. Der Chemica Sinica 2012; 3(5):1041-1046.
6. Prakash, A., Sharma, S., Ahmad , N., Ghosh, A. and Sinha, P.: Bacteria mediated extracellular synthesis of metallic nanoparticles. International Research Journal of Biotechnology 2010; 1(5): 071-079.
7. Sagadevan,S.: Synthesis and electrical properties of TiO2 nanoparticles using a wet chemical technique. American Journal of Nanoscience and Nanotechnology 2013; 1(1): 27-30.
8. Park, S., Lee, J.H., Kim, H.S., Park, H.J., Lee, J. C.: Effects of ZnO nanopowder dispersion on photocatalytic reactions for the removal of Ag ions from aqueous solution. J. Electroceram 2009; 22(1): 105–109.
9. Gong X.Q., and Selloni A.: Reactivity of Anatase TiO2 Nanoparticles. The Role of the Minority (001) Surface Surface. J. Phys. Chem. 2005; 109(42): 19560-19562.
10. Jha A.K., Prasad K., and Kulkarni A.R.: Synthesis of TiO2 Nanoparticles Using Microorganisms. Colloids and Surfaces B: Biointerfaces 2009; 71(2): 226–229.
11. Pan X., Ramirez I.M., Mernaugh R., Mernaughc R. and Liu J.: Nanocharacterization and bactericidal performance of silver modified titania photocatalyst. Colloids and Surfaces B: Biointerfaces 2010; 77(1):82–89.
12. Prasad K., Jha A.K., and Kulkarni A.R.: Lactobacillus assisted synthesis of Tritium Nanoparticles. Nanoscale Res Lett. 2007; 2(1): 248–250.
13. Jha, A.K, Prasad , K. and Kulkarni, A.R: Synthesis of TiO2 nanoparticles using microorganisms. Colloids Surf B Biointerfaces 2009; 71 (2):226–229.
14. De Man, J.C., M. Rogosa and E. Sharpe: A medium for the cultivation of Lactobacilli. J. Appl. Bacteriol. 1960; 23(1):130-135.
15. Ventura, M., M. Elli, R. Reniero and R. Zink: Molecular microbial analysis of bifidobacterium isolates from different environments by the species-specific amplified ribosomal DNA restriction analysis (ARDRA). FEMS Microbiol. Ecol. 2001; 36(1): 113–121.

16. Azhar,A.M., Behtas Ladan, S.A., Ajabadi Ebrahimi, M.T. and Heydari,M.: Lactobacillus-mediated biosynthesis of titanium nanoparticles in MRS broth medium . Brno, Czech Republic, EU. 2011; 9(1): 21– 23.
17. Malarkodi C., Chitra K., Rajeshkumar S., Gnanajobitha G., Paulkumar K., Vanaja M. and Annadurai G.: Novel eco-friendly synthesis of titanium oxide nanoparticles by using Planomicrobium sp. and its antimicrobial evaluation. Pelagia Research Library 2013; 4(3):59-66.
18. Selvarajan E., and Mohanasrinivasan V.: Biosynthesis and characterization of ZnO nanoparticles using Lactobacillus plantarum VITES07. Materials Letters 2013; 112(1): 180-182.