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Research Article

Characterization of Titanium dioxide (TiO_2) Nanoparticles Biosynthesized using *Leuconostoc* spp. Isolated from Cow's Raw Milk

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Abstract: Nanotechnology is a continually expanding field for its uses and applications in multiple areas i.e. medicine, science, and engineering. Biosynthesis is straightforward, less-toxicity, and cost-effective technology. TiO_2 NPs biosynthesis has attained consideration in recent decades. In this study, probiotic bacteria were isolated from cow's raw milk samples, and then were identified by using the Vitek2 system; as *Leuconostoc* spp. included *Leuconostoc mesenteroides* subsp. *mesenteroides* (Leu.1), *Leuconostoc mesenteroides* subsp. *cremoris* (Leu.4), and *Leuconostoc pseudomesenteroides* (Leu.14). All *Leuconostoc* spp. isolates showed an ability for TiO_2 NPs bio-production, after being incubated at anaerobic conditions (30 °C/ 24 h) in DeMan Regosa and Sharpe (MRS) broth medium. The biosynthesized TiO_2 nanoparticles were characterized using the following apparatuses: UV-Vis spectroscopy, X-ray diffraction (XRD) apparatus, Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FE-SEM) in addition to Energy Dispersive X-ray analysis (EDX) spectra. The characterized biosynthesized TiO_2 NPs were spherical-shaped, nanostructure anatase crystals with an average size range of 53.35-59.41 nm. The UV absorption spectrum was observed at the wavelength 344-248 nm; the topography AFM 2D and 3D images result showed the height and roughness of biosynthesized TiO_2 NPs that were in the range of 1.137-18.88 nm. Absorption peaks in the FTIR spectra were located in a region typical of TiO_2 NPs, and biosynthesized TiO_2 nanoparticles' main IR topographies (408.21- 445.80) cm^{-1} belonged to anatase Titania (Ti-O-Ti) bridge.

Keywords: Biosynthesis, *Leuconostoc* spp., TiO_2 Nanoparticles

1. INTRODUCTION

Leuconostoc species are Gram-positive (G +ve), mesophilic, non-motile, non-spore forming and facultative anaerobic/ aero tolerant bacteria [1]. They are negative (-ve) for oxidase and catalase tests and they appear under the microscope as pairs and chains [2, 3]. *Leuconostoc* can be isolated from the raw milk of goats, camel, plant matter and vegetables like carrots, sauerkraut, and banana [4, 5]. They are involved in food production and industrial processes such as the production of extracellular homopolysaccharides like α glucans (dextran and alternan), and β fructans (levan and inulin) from a substrate that contains sucrose [5, 6]. There are three kinds of approaches for nanoparticle production, physical; chemical and biological methods [7]. When compared to traditional chemical and physical procedures, the biological method offers an eco-friendlier option for producing nanoparticles. In addition, biological procedures have been widely used recently, allowing for NPs to synthesize in varying forms, sizes, and contents [8]. Eco-friendly, rapid, easy, safe, energy-efficient, affordable, and less toxic, green synthesis of biogenic nanoparticles from plants or microbes or their products has become promising. Remarkably, NPs have a crucial role in tumor diagnosis in its early stages by enabling cellular visualization [9, 10].

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Extensive research has shown that probiotic bacteria can yield a wide variety of metals and oxides nanoparticles, including TiO_2, Gd_2O_3, Se, Ag, Au, CdS, ZnO, Sb_2O_3, besides many others [11,12] developed a reliable, eco-friendly, and rapid process for extracellular titanium dioxide nanoparticles based on *Lactobacillus crispatus* isolated from healthy women vagina.

In addition to their applications in photocatalytic activity and solar energy, TiO_2 NPs are attractive antimicrobial compounds for their non-toxicity, chemical stability and cost-effectiveness [13, 14]. The strong oxidizing power by free radical generation greatly improved TiO_2 NPs potential as antimicrobial candidates [14]. The current study aimed to use *Leuconostoc* spp. isolated from cow's raw milk for TiO_2 NPs biosynthesis and then confirm this result by characterization. It's an important alternate, safe, inexpensive method besides being green as eco-friendly for nanoparticle microbial (non-pathogenic bacteria) biosynthesis [13].

2. MATERIALS AND METHODS

2.1 Isolates of Bacteria

Leuconostoc spp. were isolated from cow's raw milk, and then the identification was throughout the cultural [MRS agar media was used for incubation, at anaerobic conditions (30 °C/24 h)], microscopically and biochemical test [15]; besides using Vitek2 system.

2.2 Biosynthesis of TiO_2 Nanoparticles

DeMan Regosa and Sharpe (MRS) liquid medium (40 mL) provided with 2 % (9×10^8 CFU/ mL) of *Leuconostoc* spp. The pure culture was added to TiO_2 (20 mL, 0.025 M). Additional two formulations were prepared as mentioned above with either bacterial growth or TiO_2 eliminated to serve as the control. After stirring for 60 min, the preparations were incubated anaerobically (30 °C/ 24 h). Compared with the control, the preparation containing bacterial growth along with TiO_2 changed to dark brown sediment, indicating an initial production of TiO_2 NPs [16]. The product was purified by repeated centrifugation (5000 rpm/5 min.) and washing with deionized water. The pellet was dried at 50 °C for 1 h [17].

2.3 Biosynthesized TiO_2 NPs Characterization

Biosynthesized TiO_2 NPS were characterized via the following apparatus: UV-Vis at Ibn Sina Research Center (Ministry of Industry and Minerals/ Iraq); while XRD apparatus, AFM, FE-SEM besides EDX spectra were done by sending all samples as a powder to Research Center in (Tehran/ Iran). In addition, FTIR was measured at Mustansiriyah University/ College of Science/ Physics Department.

3. RESULTS AND DISCUSSION

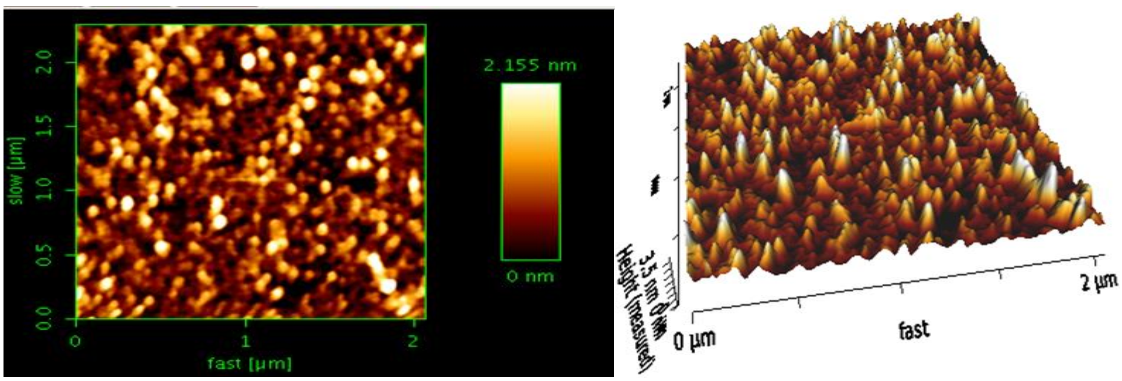
3.1 Identification of Probiotics Bacteria

Thirteen isolates, *Leuconostoc mesenteroides* subsp. *cremoris* (8)*, Leuconostoc mesenteroides* subsp. *mesenteroides* (3)*,* and *Leuconostoc pseudomesenteroides* (2) were identified using the Vitek2 system (its probability identification ratio ranged from 98-95 %, 97-90 % and 88, 86 % for all 13 *Leuconostoc* spp. respectively).

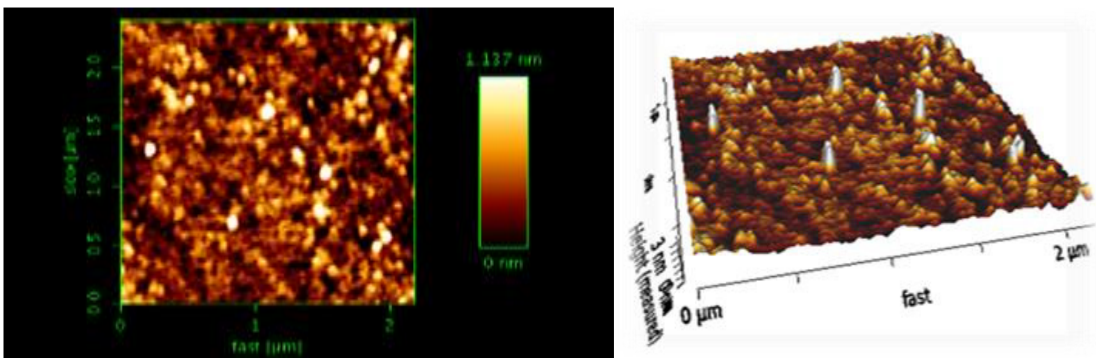
3.2 Characterization of TiO_2 NPs

The results showed that *Leuconostoc* spp. have been capable of biosynthesizing TiO_2 NPs intracellularly. UV absorption spectrum observed at the wavelength 344-348 nm for all 3 was the same as those obtained with previous TiO_2 nanoparticles synthesized by probiotics isolates, demonstrating anatase TiO_2 NPs formation [18]. The UV spectra reported by Landage *et al*. (2020) indicated a distinctive absorption peak approving the anatase phase of TiO_2 nanoparticles that were biosynthesized by *Staphylococcus aureus* and it was detected at 324 nm [19].

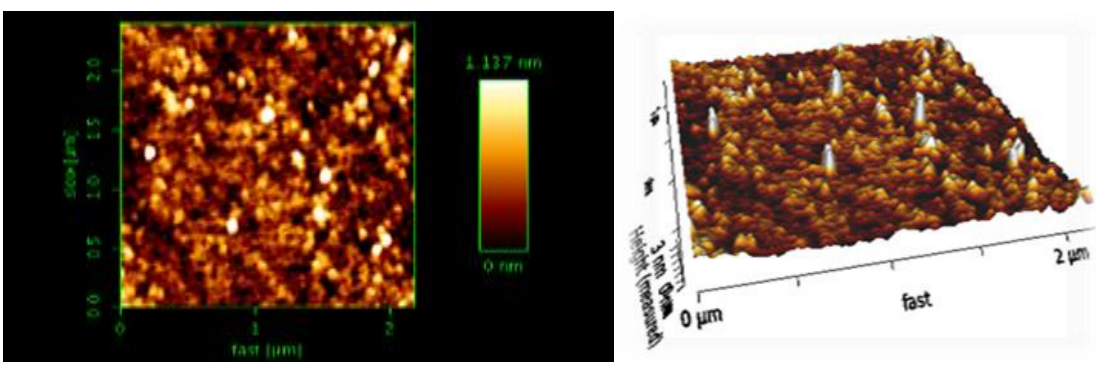
The topography AFM images with 2D and 3D images were demonstrated in Figure (1A, 1B, 1C) for biosynthesized TiO_2 nanoparticles from selected *Leuconostoc* spp. (*L. mesenteroides* subsp. *mesenteroides* (Leu.1)*, L. mesenteroides* subsp. *cremoris* (Leu.4)*, L. pseudomesenteroides* (Leu.14) as 2.155, 1.137, and 18.88 nm respectively. AFM notably depicted the formation of anatase formulas in TiO_2 NPs, besides particles surface morphology that was owing to the occurrence of some aggregates and individual particles. No linear trend was seen



A. *L. mesenteroides* subsp. *mesenteroides* (Leu.1), the height of the particle was 2.155 nm.



B. *L. mesenteroides* subsp. *cremoris* (Leu.4), the height of the particle was 1.137 nm.

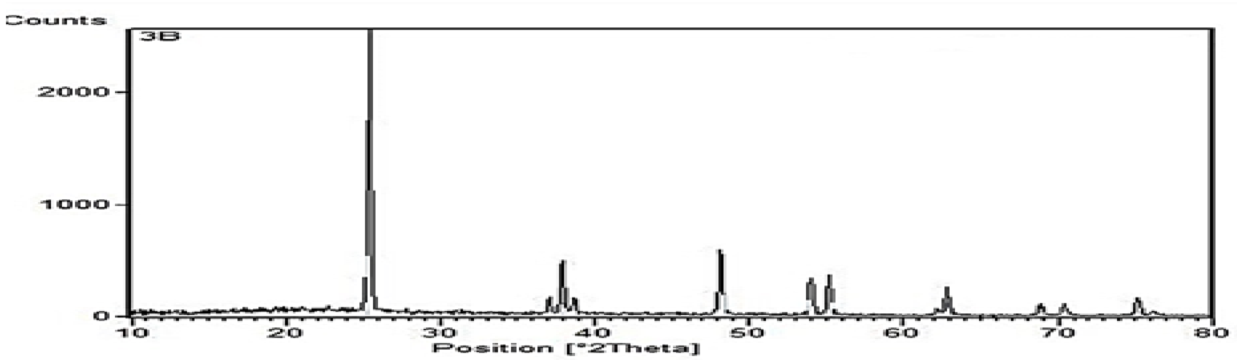


C. *L. pseudomesenteroides* (Leu.14), the height of the particle was 18.88 nm.

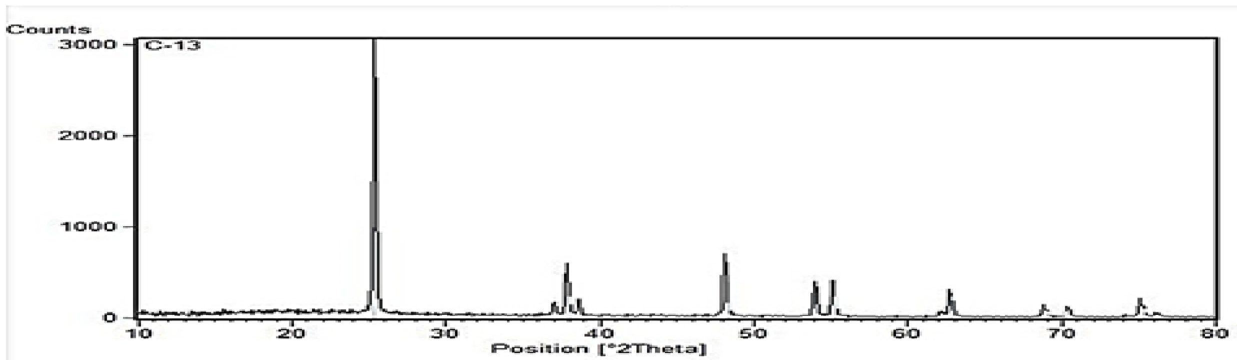
Fig. 1. AFM of TiO_2 nanoparticles biosynthesized by *Leuconostoc* spp. the highest of TiO_2 surface asperities were belong to (Leu.14), while the lowest was related to (Leu.4).

in roughness; however, that proved the highest of TiO_2 surface asperities lead to the materialization of smoother layers [19].

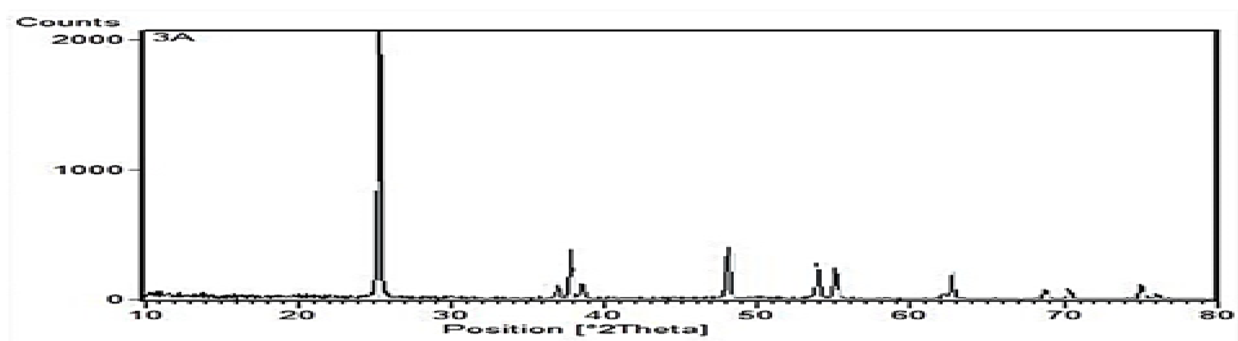
Identification of minerals based on their diffraction pattern is one of the main applications of XRD research. In addition to phase determination, XRD provides information on how interior stresses and defects cause the real structure to diverge from the ideal one [20]. Results of biosynthesized TiO_2 nanoparticles were analyzed by XRD; TiO_2 peaks were detected at (25.2^o), corresponding to 101, characteristic line broadening of diffraction peak was because of the nano size nature of anatase crystals (Figure 2). 2^{θ}=25.2, matches (101) the anatase crystallographic plane of TiO_2 NPs, demonstrating that the structure of nanoparticles agrees with anatase crystalline which was regarded as an indicator of TiO_2 NPs synthesized biologically [20]. Between TiO_2 crystalline phases, the anatase phase was defined as the greatest active photocatalytic one [21]. Additionally, TiO_2 layers



A. *L. mesenteroides* subsp. *mesenteroides* (Leu.1).



B. *L. mesenteroides* subsp. *cremoris* (Leu.4).



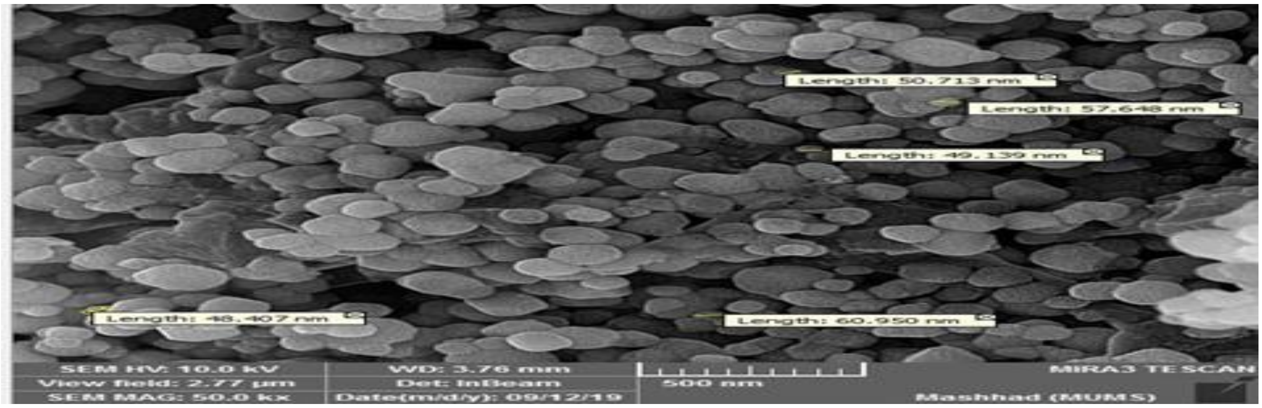
C. *L. pseudomesenteroides* (Leu.14).

Fig. 2. XRD pattern of TiO_2 nanoparticles biosynthesized by *Leuconostoc* spp.

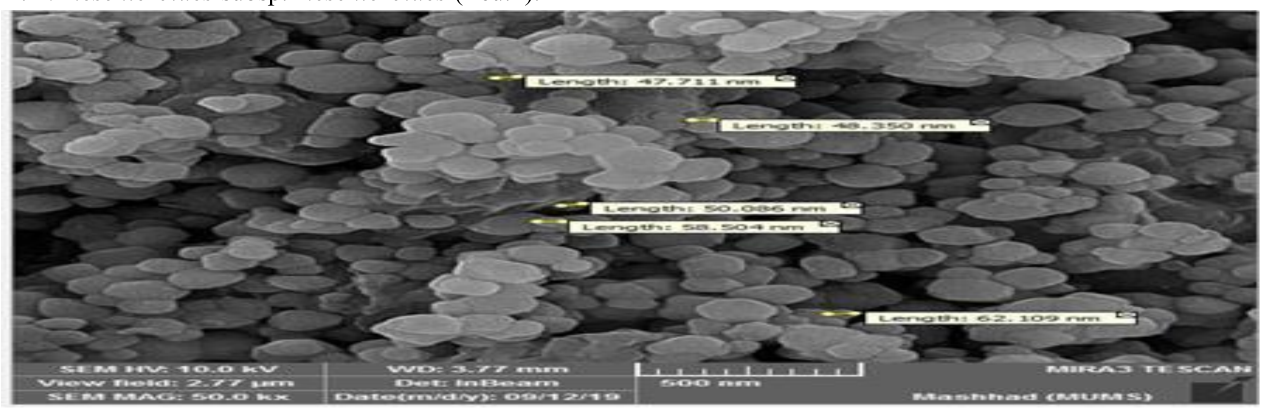
in the anatase phase typically work as a super hydrophilic surface [22]. High crystalline nature of TiO_2 NPs, which was indicated by the sharp peaks, favored photocatalytic activity [23]. Due to anatase's highest photocatalytic activity, has the most uses in industry and is the most widely utilized commercially [24].

The surface micrograph of titanium dioxide nanoparticles synthesized by *L. mesenteroides* subsp. *mesenteroides* (Leu.1), *L. mesenteroides subsp. cremoris* (Leu.4), *L. pseudomesenteroides* (Leu.14), consisted of a uniform distribution of spherical-shaped nanostructure crystals with an average diameter of each isolate (53.37, 53.35, 59.41) nm respectively (Figure 3).

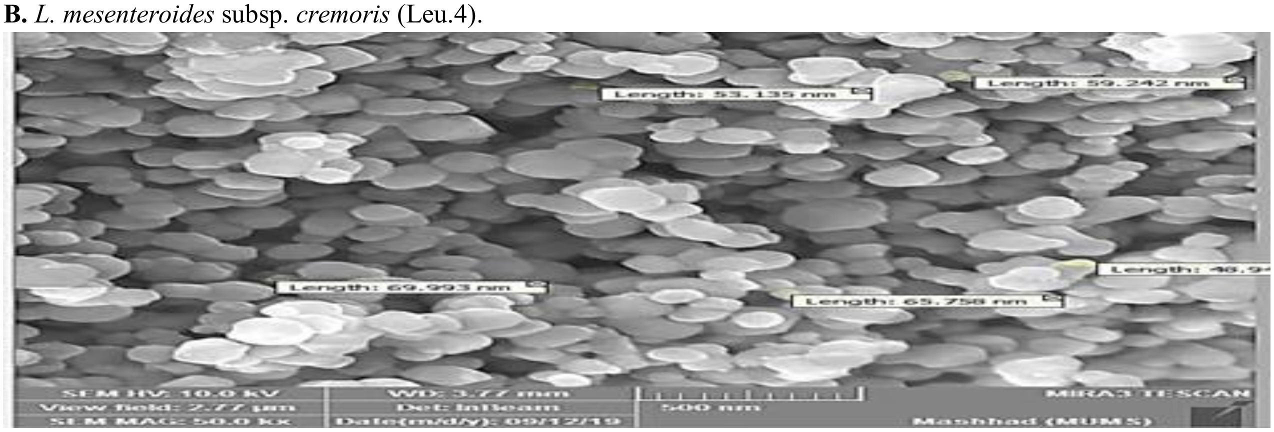
Field Emission Scanning Electron Microscopy identified the physical morphology and size of biosynthesized TiO_2 NPs. The particle size and shape, besides surface morphology that was studied by the SEM, were different. This might be related to different bacteria used in the synthesis process or because the TiO_2 nanoparticles were



A. *L. mesenteroides* subsp. *mesenteroides* (Leu.1).



B. *L. mesenteroides* subsp. *cremoris* (Leu.4).



C. *L. pseudomesenteroides* (Leu.14)

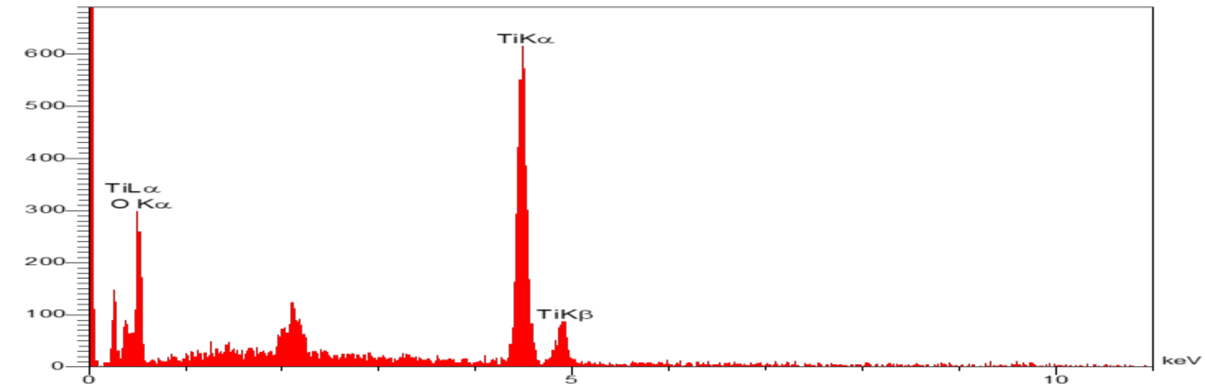
Fig. 3. FES-EM of TiO_2 nanoparticles biosynthesized by *Leuconostoc* spp.

being formed at different times [25]. According to the FE-SEM image, sphere-like particles have different diameters of 70–90 nm were observed for the biosynthesized TiO_2 nanoparticles, which might be related to particle aggregation phenomena [26]. Generally, the decrease in particle size is inversely proportional to the surface volume of the material. Consequently, the smaller the particle size, the quicker enters the toxic components along with the bacterial surface which drove decomposition [27].

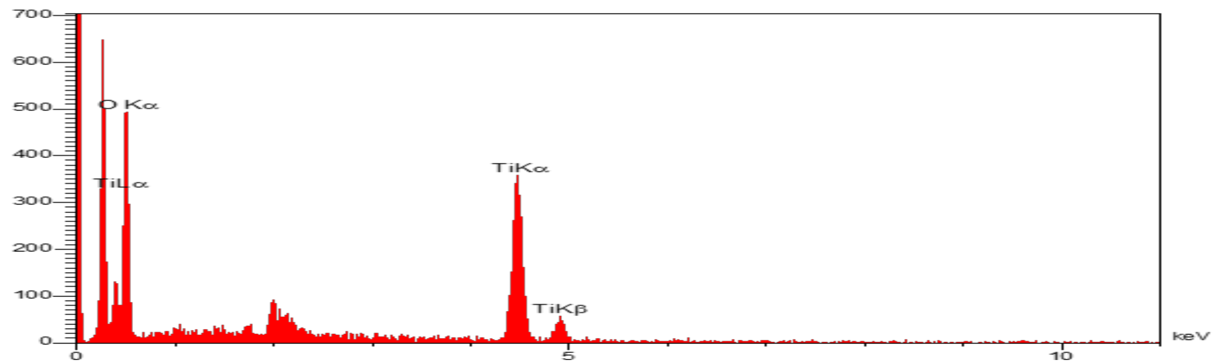
Energy dispersive X-ray analysis scales exhibited that, TiO_2 NPs powders prepared from *L. mesenteroides* subsp. *mesenteroides* (Leu.1), *L. mesenteroides* subsp. *cremoris* (Leu.4), *L. pseudomesenteroides* (Leu.14) contained (Ti and O), confirming the purity of biosynthesized TiO_2 nanoparticles (Figure 4).

The EDX result of TiO_2 demonstrated that particles were in crystalline nature plus indeed in metallic TiO_2 nanoparticles [28].

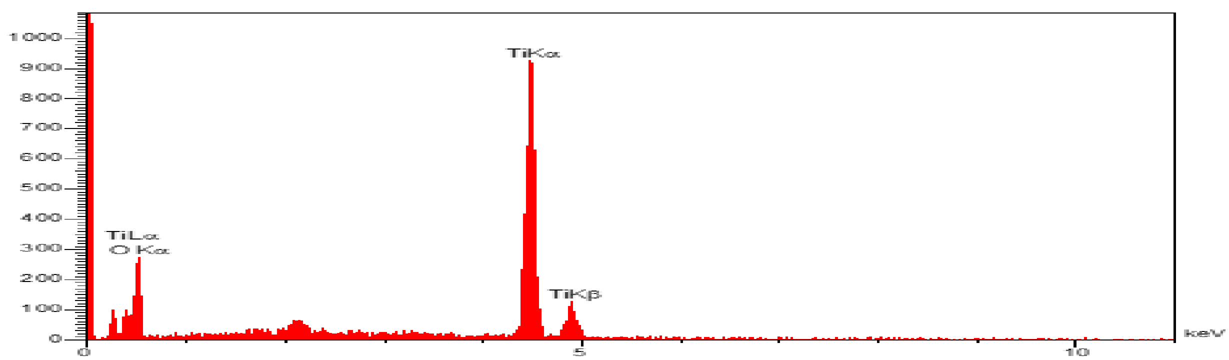
Fourier Transform Infrared Spectroscopy was



A. *L. mesenteroides* subsp. *mesenteroides* (Leu.1).



B. *L. mesenteroides* subsp. *cremoris* (Leu.4)

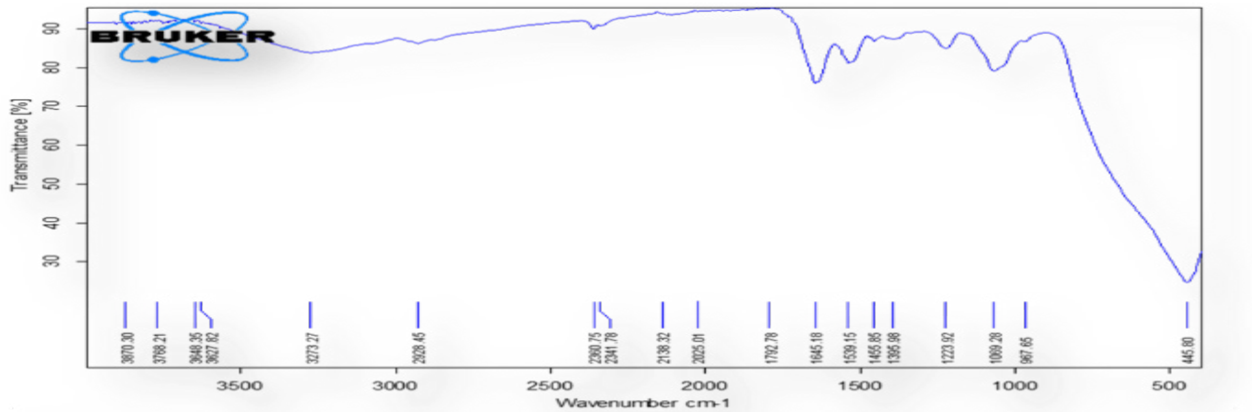


C. *L. pseudomesenteroides* (Leu.14)

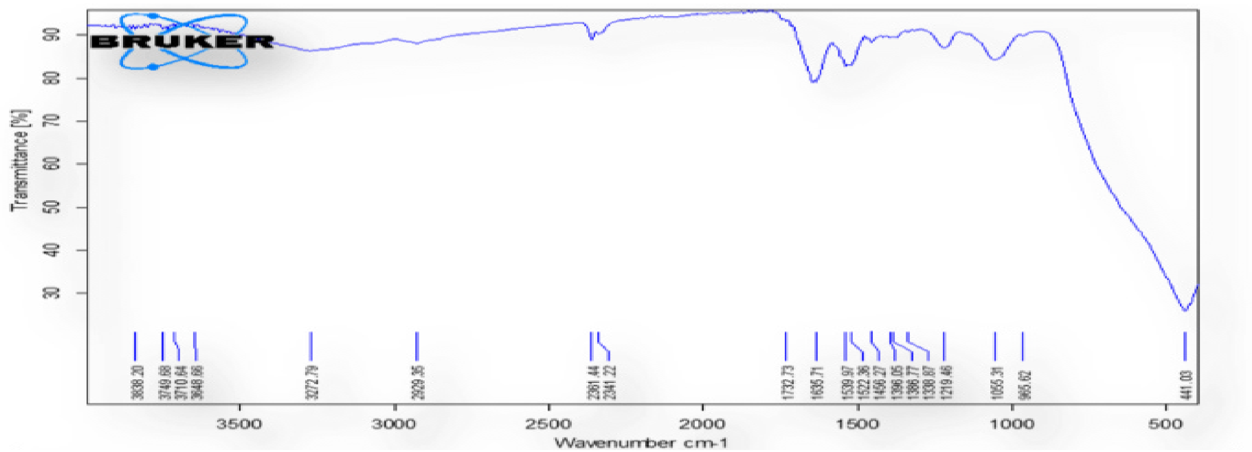
Fig. 4. EDX result of TiO_2 nanoparticles biosynthesized by *Leuconostoc* spp.

used for vibrational information of biosynthesized TiO_2 nanoparticles. It is responsible for information on the fingerprint regions of the chemical bonds within molecules. Figure (5A, 5B, 5C), demonstrated that an absorption peak was within the range spectrum that was related to TiO_2 NPs, and main IR topographies of TiO_2 nanoparticles biosynthesized by probiotics bacteria *L. mesenteroides* subsp. *mesenteroides* (Leu.1), *L. mesenteroides* subsp. *cremoris* (Leu.4), *L. pseudomesenteroides* (Leu.14) respectively, were: first peak at (445.80, 441.03, 408. 21) cm^{-1} respectively for *Leuconostoc* isolates mentioned above; these peaks were belonging to anatase Titania (Ti-O-Ti) bridge or called (Ti-O) stretching bond, as reported in the study of Praveen *et al*. (2014), Dodoo-Arhin et al. (2018), they mentioned the range of anatase titanium wavenumber as (400- 1000) cm^{-1} [29, 30].

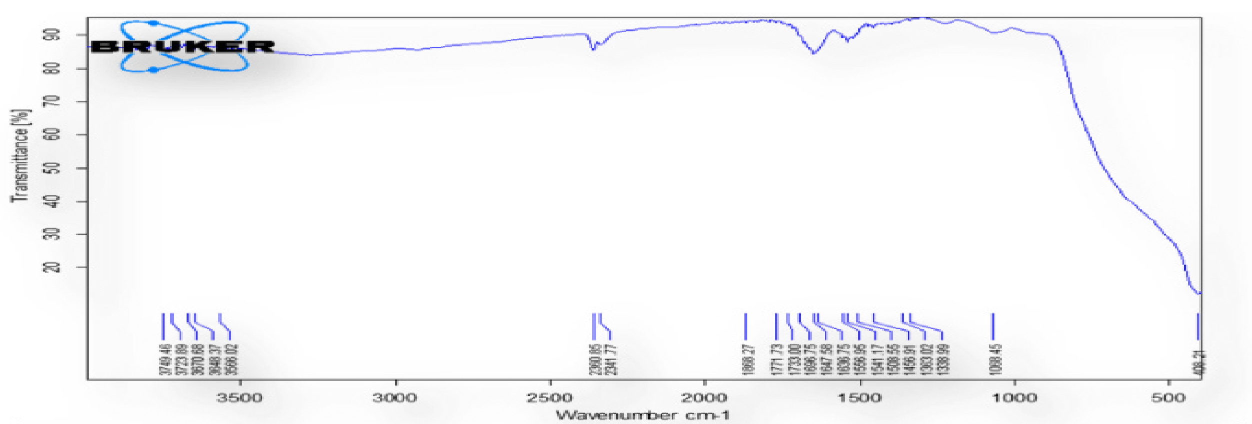
Peaks at (3273.27, 3272.79, 3273) in the 3600-3050 cm^{-1} region might relate to the O-H stretching mode of surface and adsorbed water molecules (Figure 5). The peaks at (2360.45, 2361.44, 2360.85) cm^{-1} for isolates in the same order. These peaks are assigned to the symmetric stretch (C–H) of CH_2 and CH_3 groups of aliphatic chains [31]; or



A. *L. mesenteroides* subsp. *mesenteroides* (Leu.1).



B. *L. mesenteroides* subsp. *cremoris* (Leu.4).



C. *Leuconostoc pseudomesenteroides* (Leu.14).

Fig. 5. FTIR result of TiO_2 nanoparticles biosynthesized by *Leuconostoc* spp.

might be related to hydrogens vibration from the hydroxyl layer [32].

The OH stretching band contributed either by molecularly adsorbed water or by surface OH. In addition to reducing particle aggregation, and retaining their photoactivity in high concentrations. High surface OH groups of TiO_2 nanoparticles can also obstruct electron-hole recombination. The increased bridging hydroxyls on the surface decrease the positive charge of TiO_2 and led to particle aggregation, negatively affecting catalytic activity [33].

Multiple studies have shown that increasing the number of surface hydroxyl groups on the TiO_2 surface enhances the material's adsorption capacity, mesoporous structure formation, and catalytic efficiency [34, 35].

4. CONCLUSION

The current study was focused on a quick and environmentally friendly process of creating TiO_2 NPs using *Leuconostoc* spp. isolated from cow's raw milk, the biosynthesized and characterized pure anatase TiO_2 nanoparticles within average size ranged from (53.35- 59.41) nm.

5. ACKNOWLEDGMENTS

Thanks to Mustansiriyah University-Iraq (www.uomustansiriyah.edu.iq) for supporting the current study.

6. CONFLICT OF INTEREST

The authors declared no conflict of interest

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8. DECLARATION

The authors declared that: (i) the results are original; (ii) the same material is neither published nor under consideration elsewhere; (iii) approval from all authors have been obtained; and (iv) in case the article is accepted for publication, its copyright will be assigned to the Pakistan Academy of Sciences. Authors give permission to reproduce, where needed, copyrighted material from other sources and that no copyrights are infringed upon.