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Biosynthesis and Characterization of TiO_2 Nanoparticles by *Lactococcus lactis* ssp. *lactis*

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ABSTRACT

Lactococcus lactis ssp. *lactis* isolated from raw milk was used for titanium dioxide (TiO_2) nanoparticles biosynthesis. Biosynthesized TiO_2 nanoparticles were characterized using UV.Vis spectroscopy, Atomic Force Microscopy (AFM) (1.97 nm), X-ray diffraction (XRD) apparatus, Field Emission Scanning Electron Microscopy (FE-SEM), Energy dispersive X-ray analysis (EDX) spectra and Fourier Transform Infrared Spectroscopy (FTIR). Result was 408.21 cm^{-1} that belong to anatase Titania. *L. lactis* ssp. *lactis* isolates had the ability to synthesize TiO_2 nanoparticles, the characterization results presented that the biosynthesized nanoparticles were at wavelength (344-347) nm; approving the formation of anatase phase of TiO_2 NPs; spherical crystals, with particles average diameter of 47.22 nm.

KEYWORDS: Probiotics bacteria, *Lactococcus* spp., Biosynthesis, TiO_2 nanoparticles.

الخلاصة

اختبرت قابلية بكتيريا المعززات الحيوية العائدة للجنس *Lactococcus lactis* ssp. *lactis* المعزولة من الحليب الخام لإنتاج الجسيمات النانوية لثنائي اوكسيد التيتانيوم (TiO_2). وصفت هذه الجسيمات النانوية المصنعة باستخدام جهاز مطياف الاشعة المرئية وفوق البنفسجية (UV.Vis)، وجهاز حيود الأشعة السينية (XRD)، مجهر القوة الذرية (AFM) (1.97) نانوميتر، جهاز التحليل الطيفي بالأشعة تحت الحمراء (FTIR) حيث كانت احد القمم الناتجة 408.21 سم$^{-1}$ والتي تعود لطور الاناتيز لجسيمات التيتانيوم النانوية، المجهر الإلكتروني الماسح بالانبعاثات الالكترونية (FE-SEM) ومطيافية الأشعة السينية المشتتة للطاقة EDX). *L. lactis* ssp. *lactis*) لها ألقدرة لتصنيع التيتانيوم النانوي, حيث أكدت نتائج توصيف جسيمات ثنائي اوكسيد التيتانيوم النانوية ان الجسيمات المصنعة كانت عند الطول الموجي (344-347) نانوميتر, كروية الشكل ذات توزيع موحد للبلورات النانوية نوع anatase وبمعدل حجم (47.22) نانوميتر.

INTRODUCTION

Probiotics are defined as living microorganisms that might be found in cultured milk and fermented food; they can be used in infant's food preparations [1]. Control on and reduce the serum cholesterol are some of the benefits of probiotic bacteria besides enhancing food products nutritional values, suppressing antibiotic-associated diarrhea and preventing gut infections [2, 3].

Lactococcus lactis subsp. *lactis* was isolated from milk or milk fermented products and plant material too. It was used for the fermentation of milk and buttermilk as well as wide types of cheeses [4]. *L. lactis* enhanced the microbial balance of the gastrointestinal tract by suppressing the growth of hydrogen sulfide (H_2S) producing microorganisms in the intestine of mice [1]. *L. lactis* was used in vaccines as a live vector. It is considered as a promising candidate for heterologous expression of protein and biotechnological application [5].

Green synthesis of biogenic nanoparticles (NPs) from plant or microbial sources became an emergent field due to being eco-friendly, fast, simple, safer, energy-efficient, cheap, and less toxic [6]. Bacterial applications for biomanufacturing of NPs have several advantages such as the short incubation time and easy control, for example, bacteria are considered as an excellent tool for extracellular synthesis for silver and gold nanoparticles [7, 8].

Many studies indicated that probiotic bacteria produce a lot of metal nanoparticles/oxide nanoparticles involving Gd_2O_3, Sb_2O_3, Ag, CdS, Se, Au, ZnO, and TiO_2 [9]. Ibrahem *et al.* (2014)

used a biological method for the synthesis of TiO_2 NPs besides studying the effect of different culture media on TiO_2 nanoparticles that were biosynthesized by *Lactobacillus crispatus* [10].
Nanoparticles reveal distinctive, catalytic, optical, magnetic, and electronic properties that are not the same as bulk metals [11]. The importance of TiO_2 NPs was related to their chemical stability; photocatalytic activity; low-priced; non-toxicity besides being Generally Recognized as Safe (GRAS) [12].
Studies on metal oxide NPs proved that they act as potential antimicrobial agents [13]. Depending on the foodstuff, the type of packaging matrix is of great importance; the addition of metal nanoparticles as (Ag, Cu, Hg, and TiO_2) to polymer matrices, mechanical properties, thermal resistance, gas barrier properties, and antimicrobial properties is considered to be improved in comparison with the usual polymers [14]. This study aimed to use green synthesis of TiO_2 NPs by probiotic's bacteria *Lactococcus lactis* ssp. *lactis.*

MATERIALS AND METHODOLOGY

Lactococcus lactis ssp. *lactis* isolates

Buffalo and cows' raw milk was used as a source for *Lactococcus lactis* ssp. *lactis* isolation, their identification was dependent on the cultural, microscopical, and biochemical tests [15]; in addition to the Vitek 2 system.

Biosynthesis of TiO_2 Nanoparticles

Pure culture of *L, lactis* ssp. *lactis* **(**9×10^8 CFU/ml), inoculated at 2% in De Man Rogosa and Sharpe (MRS) broth flasks; then were incubated at 30 ^oC, for 24 hrs. under anaerobic conditions. TiO_2 NPs detected by color-changing (light to dark brown) and sediment production [16]. The product was transferred later to centrifuge tubes; the centrifugation process was done at 5000 rpm for 5 min. After that, the pellet was washed with deionized water; this step was repeated several times until pure solution was acquired. After the final centrifugation, the pellet was dried for 1 hr. at 50 °C until TiO_2 nanoparticles were converted to powder formula [17].

Characterization of Biosynthesized TiO_2 nanoparticles

The characterization of biosynthesized TiO_2 nanoparticles was done by using: UV.Vis spectroscopy [18]; Atomic Force Microscopy (AFM) [19]; X-ray diffraction (XRD) [20]; Fourier Transform Infrared Spectroscopy (FTIR) [21]; Field Emission Scanning Electron Microscopy (FE-SEM) and Energy dispersive X-ray analysis (EDX) [21].

RESULTS AND DISCUSSION

Six isolates of *L. lactis* ssp. *lactis* were identified from raw milk samples. *L. lactis* ssp. *lactis* isolates had the ability for synthesizing TiO_2 NPs and this result was observed by the sediment formation and color-changing from light brown to dark. The detected absorption spectrum corresponds with those gained with TiO_2 nanoparticles synthesized by *Lactococcus* isolates, with an absorption band in the UV range and its cut-off wavelength was (344-347) nm (Figure 1); approving the formation of anatase phase of TiO_2 NPs [16]. The absorption band was at 406 nm for TiO_2 nanoparticles biosynthesized extracellularly by *Lactobacillus johnsonii* in the anatase phase [22]. The UV spectra indicated a distinct absorption peak revealing anatase phase similar to the peak detected of TiO_2 NPs synthesized via *Staphylococcus aureus* at 324 nm wavelength [19].

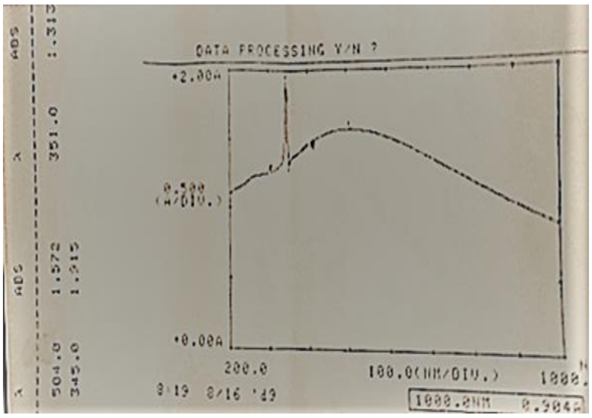

Figure 1. The UV.Vis spectroscopy for *Lactococcus lactis* ssp. *lactis*.

The topographical 2D and 3D images with AFM microscope for TiO_2 nanoparticles biosynthesized by *L. lactis* ssp. *lactis* result was (1.97 nm) as in Figure 2. Atomic Force Microscopy depicted anatase formation formulas of TiO_2 nanoparticles as well surface morphology as a result of incidence of some aggregates and single particles. Roughness with no linear trend; which verified that the highest TiO_2 concentrations would lead to smoother layers of materialization [19].

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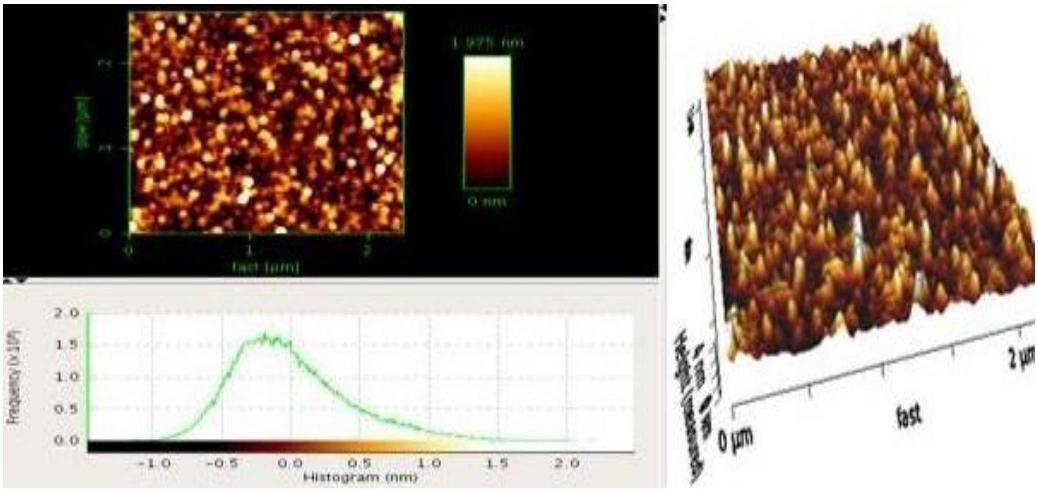

Figure 2. AFM of TiO_2 nanoparticles biosynthesized by *Lactococcus lactis* ssp. *lactis.*

The anatase phase is known as the most active photocatalytic one [23]. Furthermore, layers of anatase phase typically work as a super hydrophilic surface [24]. Attributable to the greater photocatalytic activity, anatase has the highest number of industrial applications as the most commercially used form [25].

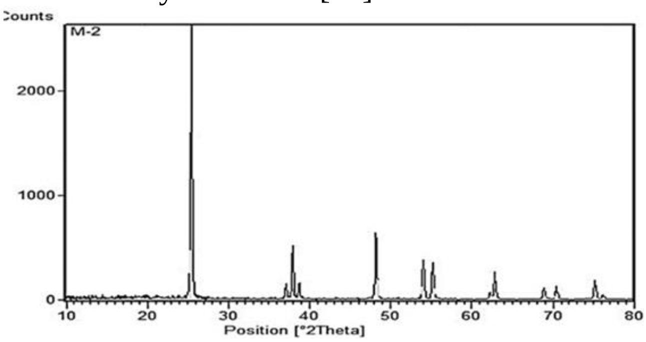

Figure 3. XRD pattern of TiO_2 nanoparticles biosynthesized by *Lactococcus lactis* ssp. *lactis*.

Titanium dioxide nanoparticles surface micrograph biosynthesized by *L. lactis* ssp. *lactis was* made up of a uniform distribution of spherical crystals in nanostructure; the size was 47.22 nm. The FE-SEM result was shown in (Figure 4) for biosynthesized TiO_2 NPs; this image evidently gives the size and physical morphology of particles. The surface morphology (shape and size) of NPs was analyzed by SEM varies in size of particles and might be correlated to the formation of TiO_2 nanoparticles at different times [21]. SEM result, spherical shapes with diverse particles diameter of 70–90 nm for the synthesized TiO_2 NPs, these differences may be associated with the aggregation phenomena of particles [26]. Decreasing particles size is inversely proportional to surface volume; therefore, the smaller particle size quickly penetrates the bacterial surface, which leads to the process of decomposition [27].

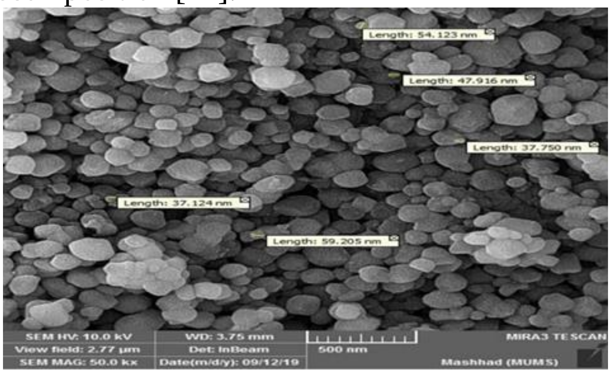

Figure 4. FESEM image of TiO_2 nanoparticles biosynthesized by *Lactococcus lactis* ssp. *lactis*.

L. lactis ssp. *lactis* EDX scales for biosynthesized TiO_2 NPs powders confirmed the existence of (Ti, O). No other peaks for other elements were detected in the spectrum, which confirmed the results of biosynthesized nanoparticles purity (Figure 5). This result verified that the particles were crystalline and metallic nanoparticles of TiO_2 [28].

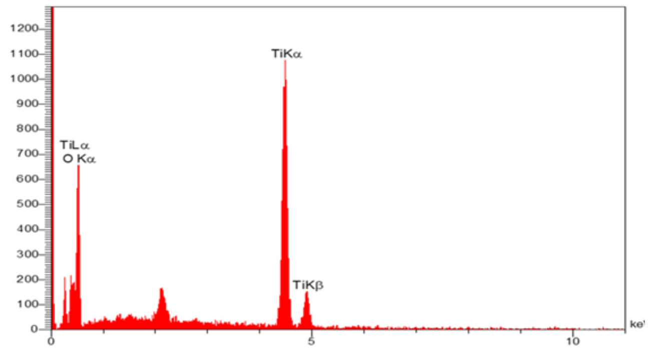

Figure 5. EDX of biosynthesized TiO_2 nanoparticles by *Lactococcus lactis* ssp. *lactis*.

The absorption peaks shown in the FTIR technique were within the spectrum associated to TiO_2 nanoparticles. The main IR topographies of TiO_2 NPs biosynthesized by *L. lactis* ssp. *lactis* showed at 408.21 cm^{-1} and belong to anatase Titania (Figure 6); the range of anatase Titania wavenumber (400- 1000) cm^{-1} as Ti-O-Ti bridge [29, 30] and 1060.88 cm^{-1} corresponds to the Ti-OH as mentioned in [31] the 1068 cm^{-1} band was attributed to Ti–OH bond. Another peak was measured at (1646.01, 3273.41) cm^{-1}, the first peak was attributed to the stretching of titanium carboxylate; while the second one was for O-H stretching mode of surface and the adsorbed water molecules; other vibrational bands were observed at 2929.47 cm^{-1} for *L. lactis* ssp. *lactis*, and assigned to C-H asymmetric stretching vibrations [30]. Xing *et al.*, 2021 noticed a similar band around 2920 cm^{-1} corresponding to the C–H stretching vibration of alkyl and aldehyde groups for TiO_2 nanoparticles [31].

Laisney *et al.*, 2021 mentioned that; TiO_2 NPs could be found at 1630 cm^{-1} corresponding to the Ti (TiO_2)-O (H_2O), vibrations between the titanium or oxygen from the TiO_2 surface and the oxygen from the hydroxyl layer [32]. The increased bridging hydroxyls on the surface reduce the positive charges of TiO_2 and cause particle aggregation, negatively affecting catalytic performance [33].

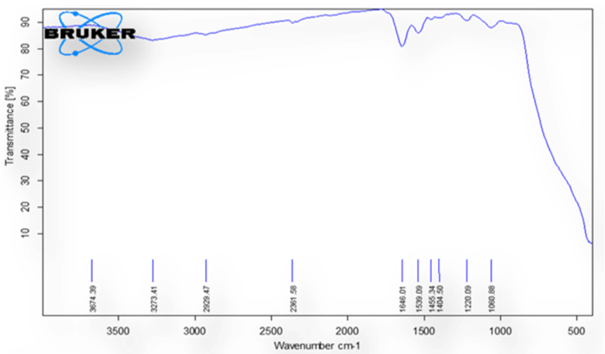

Figure 6. FTIR results of biosynthesized TiO_2 nanoparticles by *Lactococcus lactis* ssp. *Lactis*.

CONCLUSIONS

Iraqi probiotics isolates belong to *Lactococcus lactis* ssp. *Lactis* had the ability to synthesize pure anatase TiO_2 nanoparticles with particles diameter of 47.22 nm.

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