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CARACTERIZAÇÃO ANTIBACTERIANA DE NANOPARTÍCULAS DE TITÂNIO NANOSSINTETIZADAS POR *STREPTOCOCCUS THERMOPHILUS*

ANTIBACTERIAL CHARACTERIZATION OF TITANIUM NANOPARTICLES NANOSYNTHESIZED BY *STREPTOCOCCUS THERMOPHILUS*

التوصيف الضد البكتيري لجزيئات التيتانيوم النانوية الحيوية المصنعة من بكتيريا *STREPTOCOCCUS THERMOPHILUS*

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Received 22 December 2019; received in revised form 30 January 2020; accepted 11 February 2020

RESUMO

Os metais que contêm nanomateriais têm potencial para serem empregados no controle de diferentes tipos de infecções; no entanto, informações limitadas são conhecidas sobre suas propriedades antibacterianas. Este estudo foi realizado para investigar a nanossíntese de nanopartículas de titânio (NPTi) (utilizando *Streptococcus thermophilus* e analisando suas ações biológicas como antibacterianas). As colonias bacterianas isolados foram identificadas usando os primers universais 16S rRNA; posteriormente as sequências nucleotídicas do gene 16S rRNA foram alinhadas com as seqüências nucleotídicas das cepas obtidas no GeneBank através do software CLUSTAL X (versão 1.82). Nanopartículas de titânio foram nanossintetizadas por adição de dióxido de titânio 0,025M (TiO_2) ao sobrenadante livre de células de *Streptococcus thermophilus*. O TiO_2 foi utilizado como precursor para a nanobiossíntese de NPTi. A formação de NPTi foi indicada pela alteração da cor da solução do marrom claro para marrom escuro, indicando a produção de NPTi. A caracterização da nanobiossíntese foi realizada com UV-Visível (absorbância em 377 nm), Microscópia Eletrônica de Varredura, Difração de raios-X, Microscópia de Força Atômica, Espectroscopia de raios-X dispersiva de energia para distinguir a dimensão, forma (esférica) por MEV, análise de dispersão (homogênea) e elementar de nanopartículas. As NPTi biogênicas apresentaram atividade antibacteriana e antibiofilme contra a pneumonia por *Klebsiella* resistente a múltiplas drogas e ao *Staphylococcus aureus*. Como atividade antibacteriana, as NPTi inibiram significativamente *K.pneumoniae* (20 mm) na concentração de 500 µg/ml e *S. aureus* (16 mm) na mesma concentração e aumentando a concentração de NPTi, a zona de inibição aumentou. Enquanto a atividade antibiofilme das NPTi utilizando o método de tubos, nos tubos contendo suspensões bacterianas de *K.pneumoniae* e *S.aureus* com NPTi, os resultados demostraram que a formação de biofilme foi impedida e removida pelo efeito das NPTi.

Palavras-chave: *TiNPs biogênicos, nanopartículas, Streptococcus thermophilus, Atividade antibacteriana, Antibiofilme*.

ABSTRACT

Metals that contain nanomaterials have the potential to be employed in controlling different kinds of infection, however, very limited information is known about their antibacterial properties. This study has been done to investigate the nanosynthesis titanium nanoparticles (TiNPs) using *Streptococcus thermophilus* and analyzing their biological actions as antibacterial. The bacterial isolates identified using universal primers 16S rRNA; then the 16S rRNA gene nucleotide sequences were aligned with the nucleotide sequences of strains obtained from the GeneBank through the software CLUSTAL X (version 1.82). Titanium nanoparticles were nanosynthesized by adding 0.025M titanium dioxide (TiO_2) into cell-free supernatant for *Streptococcus thermophilus*. TiO_2 was used as a precursor for nanobiosynthesis TiNPs. The formation of TiNPs was indicated by the color alteration of the solution from the light brown into dark brown indicates for the production of TiNPs. The Characterization of nanobiosynthesis was accomplished with UV-Visible (absorbance at 377nm), Scanning Electron Microscope, X-ray diffraction, Atomic Force Microscope, Energy-dispersive X-ray spectroscopy was used to distinguish the dimension, form (spherical) by SEM, dispersal (homogenous) and elemental analysis of

nanoparticles. Biogenic TiNPs have displayed antibacterial and antibiofilm activity against both multidrug-resistant *Klebsiella pneumonia* and *Staphylococcus aureus*. As an antibacterial activity, the TiNPs inhibited significantly *K.pneumoniae* (20 mm) with concentration (500 µg/ml), and *S. aureus* (16 mm) with the same concentration and increasing the concentration of TiNPs the inhibition zone increased. While as antibiofilm activity of TiNPs using the tube method, the tubes containing bacterial suspension *K.pneumoniae* and *S.aureus* with TiNPs, the results demonstrated that the biofilm formation was prevented and removed by the effect of TiNPs.

Keywords: *Biogenic TiNPs, nanoparticles, Streptococcus thermophilus, Antibacterial Activity, Antibiofilm.*

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الملخص

المعادن التي تحتوي على مواد متناهية الصغر لديها القدرة على أن تستخدم في السيطرة على أنواع مختلفة من العدوى ، ومع ذلك ، هناك معلومات محدودة للغاية معروفة عن خصائصها المضادة للبكتيريا. تم إجراء هذه الدراسة لاستقصاء جسيمات التيتانيوم النانوية (TiNPs) بإستخدام بكتيريا *Streptococcus thermophilus* (العقدية المحبة للحرارة) وتحديد فعالياتها البيولوجية كمضادات للبكتيريا. تم تحديد العزلات البكتيرية باستخدام باديء عام 16S rRNA؛ ثم تمت محاذاة متواليات النيوكليوتيدات الجينية 16S rRNA مع متواليات النيوكليوتيدات الخاصة بالسلالات التي تم الحصول عليها من بنك الجينات من خلال برنامج CLUSTAL X (الإصدار 1.82). تم تصنيع جزيئات التيتانيوم النانوية عن طريق إضافة M0.025 ثاني أكسيد التيتانيوم إلى راشح حاوي فقط على خلايا بكتيريا *Streptococcus thermophilus*. تم استخدام ثاني أكسيد التيتانيوم كباديء لجزيئات التيتانيوم النانوية الحيوية. ان الدليل على تكوين جزيئات التيتانيوم النانوية تم بواسطة تغيير لون المحلول من البني الفاتح إلى البني الداكن الذي يشير إلى إنتاج جزيئات التيتانيوم النانوية. تم تشخيص التركيب النانوي الحيوي بإستخدام الأشعة فوق البنفسجية المرئية (الامتصاص عند 377 نانومتر) ، المجهر الإلكتروني الماسح، حيود الأشعة السينية ، مجهر القوة الذرية ، ومطياف الاشعة السينية المشتتة للطاقة لتمييز البعد والشكل (كروي) بواسطة المجهر الالكتروني الماسح ، التشتت (متجانسة) والتحليل الاولي للجسيمات النانوية. لقد أظهرت جزيئات التيتانيوم الحيوية المنشأ فعالية ضد بكتيرية وضد الغشاء الحيوي لكل من بكتيريا *K.pneumoniae* و *S.aureus*المتعددة المقاومة للأدوية. كفعالية ضد بكتيرية؛ لوحظ أن جزيئات التيانيوم النانوية تثبط K.pneumoniae معنوياً بقطر (20 ملم) عند تركيز (500 µg/ml) وبكتيريا *S. aureus* بقطر (16 ملم) عند نفس التركيزومنطقة التثبيط تزداد بأزدياد تركيز جزيئات التيتانيوم النانوية. بينما فعالية ضد الغشاء الحيوي لجزيئات التيتانيوم النانوية بإستخدام طريقة الأنبوب، أظهرت النتائج أن الأنابيب التي تحتوي على معلق بكتيري *K.pneumoniae* و *S.aureus* مع جزيئات التيتانيوم النانوية، بأنه تم مُنع تكوين الأغشية الحيوية وإزالتها بواسطة تأثير جزيئات التيتانيوم النانوية.

الكلمات المفتاحية: جزيئات التيتانيوم النانوية الحيوية ، جزيئات نانوية، *Streptococcus thermophilus* ، فعالية ضد بكتيرية، ضد غشاء حيوي.

1. INTRODUCTION

Due to the high photocatalytic activity of titanium dioxide TiO_2, it has been used in most daily life applications (Allen, 2008). It is well-known that titanium nanoparticles have incredible gas-sensitive properties (Chen and Mao, 2007). TiO2 shows perfect stability and non-toxicity behavior (Sugimoto, 2003). Titanium dioxide is suitable to split H_2O due to its optical properties (Rao *et al*., 1980). It exhibits broadband UV absorption and sunscreen applications (Sung *et al*., 2002). The cure for infectious diseases is still concerned. Consequently, resistance has expanded becauseof the insensible take of antimicrobial agents (Jain *et al*., 2009).

Antimicrobial Resistance (AMR) is triggered by antimicrobial-resistant microorganisms. Many antimicrobials that are treated numerous microbial infections are quickly mislaying their efficiency, as bacterial species and further microbes improve resistance (O'Neill, 2016; Ali, 2018).

More than 700,000 persons in the world die from AMR. Death could increase to 10,000,000 persons by 2050. Essentially, people should decrease the danger of bacteria emerging resistance by reducing the overuse and misuse of antibiotics and avoiding the infection (O'Neill, 2016).

The antimicrobial characteristics of metals such as zinc, silver, titanium, and copper have been known for the last decades, which facilitate the way to be exploited in many modern medical applications in order to control some microbial infection diseases (Weber & Rutala 2001). Several chemical forms have arisen from these metal-containing nanomaterials, including either solid nanoparticles (NPs) of metal or metal oxides such as Ag NPs and TiO_2 NPs (Han *et al*. 2010).

To- date, the specific mechanism of bacterial toxicity of nanometals is still not fully understood, but there is a theory saying that the free metal ion toxicity arising from the surface of the nanoparticles may play a vital role in infection control (Kim *et al*. 2007). In the case of using the titanium nanoparticles, by photocatalysis, the TiO_2 surface reacts with water to enable the release of the hydroxyl radical, which leads

subsequently to form the superoxide (Linsebigler *et al*. 1995). This superoxide could then be a potential element that able to attack the polyunsaturated phospholipids in bacteria (Wong *et al*. 2006) and lead to site-specific DNA damage (Hirakawa *et al*. 2004).

It has been proved that TiNPs have inhibition actions and avoidance the biofilm development, in addition to distinctive physicochemical and biological chattels (Wijnhoven *et al*., 2009). Development of nanobiosynthesis that can be potential in many biomedical applications and development novel materials that inhibit the Multidrug resistance (MDR) microbe (Chaudhari *et al*., 2012 and Franci *et al*., 2015). That why use bacterial species in nanobiosynthesis (Gade *et al*., 2008).

S. thermophilus have antibacterial and antioxidative possessions, regulating the balance of intestinal flora (Songisepp *et al*., 2004).

The primary goal of this study was to nanobiosynthesis of TiNPs with *S. thermophiles*, and these TiNPs were chosen for their variety physico-chemical properties and, therefore, likely modes of action, TiO_2 NPs is a stable metal oxide. A secondary goal of this study was to come up with the ongoing test methods by evaluating both the antibacterial and antibiofilm activity.

2. MATERIALS AND METHODS

The Materials and Methods for this study as shown below in paragraphs:

2.1. 16S rRNA gene sequencing of Bacterial Isolates

The identification of species sequence was revealed via PCR with universal primers. The 16S rRNA was imperiled to DNA sequencing. Favour Prep total DNA Kit (Presto™ Mini gDNA Bacteria Kit, Geneaid ,Taiwan) was used to extract DNA following the company's instructions. PCR reaction mixture with final volume 20 µl consisted of 2µl for each 27F and 1492R primers (10 picomole), 9µl De-ionized water, and 7µl the DNA of the isolate were added into the AccuPower® *Taq* PCR PreMix tubes (Bioneer, South Korea); that contain (*Taq* DNA polymerase, dNTPs, KCl, $MgCl_2$, and buffer). PCR was done under the following conditions: 96°C, 6 min with 38 cycles: 35 sec at 96°C, 65 sec at 57°C, and 125 sec at 72°C, 5min 72°C. The products were separated at 1.5 % agarose gel, then 16S rRNA was sent for sequencing (Sambrook and Rusell, 2001).

The 16S rRNA gene nucleotide sequences were aligned with the nucleotide sequences of reference strains obtained from the GeneBank database through the software CLUSTAL X (version 1.82) (Phalakornkule and Tanasupawat, 2006). The SnapGene tool was used for overall applications of editing and analysis. The similarity between the 16S rRNA gene nucleotide sequence and the most related species was exposed from the NCBI public database. BLASTn tool was used for accomplishing nucleotide sequences similarity against nucleotide sequence references that available in the Genbank database (Hall, 1999; Altschul *et al.,* 1997).

2.2. Preparation of cell-free supernatant of *Streptococcus thermophilus*

S. thermophilus was inoculated in prepared and autoclaved broth. Then, the culture was incubated in aerobic conditions at 37°C for 18 hrs**.** After the incubation period, centrifugation at 6000 rpm, 10 min. The cells were precipitated at the bottom of the tube and were discarded later, calmed was filtrated to TiNPs nanobiosynthesis (Chaudhari *et al.,* 2012).

2.3. Nanobiosynthesis of TiNPs with supernatant

Titanium dioxide (TiO_2) was used as originator to TiNPs nanobiosynthesis by *S. thermophilus*. 5 ml of titanium dioxide (0.025M) solution were added to 5 ml of cell-free supernatant of *S. thermophilus* that distributed in sterilized test tubes and mixed well. The resultant solutions were incubated at 37 °C for 18 h in a shaking incubator at 160 rpm. After incubation, the color has changed, then the reaction mixture was centrifuged at 10000 rpm for 20 minutes, the cell-free filtrate was rejected and substituted with water and re-centrifuged for several times, the precipitated pellets which represent the assemblage of TiNPs and then dryness, 40°C for 18-24 hours. The residues were assemblage plus kept aimed at use (Prasad *et al.,* 2007).

2.4. Nanocharacterization of TiNPs

The color of the reaction mix was observed by determining the absorbance of the reaction mix. TiNPs were examined with UV-Vis Spectra. (Shimadzu,1600). X-ray diffraction (XRD) was used to analyze the purity of the TiNPs (Huang *et al.,* 2018). Atomic Force Microscope (AFM) was accomplished for the examination of the TiNPs. Scanning Electron Microscope (SEM) was employed to determine

the morphology properties of TiNPs (Caroling *et al*., 2013).

2.5. Antibacterial activity of TiNPs

Nanobiosynthesized TiNPs were tested to evaluate their antimicrobial activity against Multidrug resistance (MDR) *K. pneumoniae* and *S. aureus* using agar well diffusion method. Four different concentrations of TiNPs (100, 300, 400, and 500 µg/ml) were used, and the experiment has been done using a well plate of 6 mm. 100µl of each concentration was added into each well. One Petri-dish subcultured for each pathogenic bacteria and used as a control (without testing for antibacterial activity), and incubation conditions were at 37 °C for 24 h. (Rajeshkumar and Malarkodi, 2014).

2.6. Antibiofilm activity of TiNPs

Tube method (TM) was achieved to evaluate the biofilm development and antibiofilm action by TiNPs as follows: BHI was prepared and sterilized by autoclave, then inoculated each pathogenic bacteria and keep warm for 24 hrs at 37°C. The next day, BHI was prepared again, 2% of sucrose was added to BHI after sterilization by filtration, 60 µl from BHI was distributed in sterilized tubes, then 30 µl of each overnight bacterial suspension and 30 µl of TiNPs with concentration of 500 µg/ml were added separately to each tube, the control tube normally contained only the bacterial suspension without TiNPs and incubated under the same incubation conditions (Kumar *et al.,* 2012). After incubation, discharge the bacterial suspension and phosphate buffer saline (PBS) pH 7.4 from the tube and dryness at room temperature. The tubes were dealt with 1% crystal violet for 10 min and incubated at room temperature, and the excess stain was removed and washed with distilled water, the tubes were placed upside down to drain. Biofilm formation was observed as a positive when a visible film lined the wall and bottom of the tubes. The effect of TiNPs on biofilm formation of clinical bacteria was observed through inhibition of the formation of biofilm (Mathur *et al.,* 2006).

3. RESULTS AND DISCUSSION:

3.1 Results

The results of this study as shown below in paragraphs:

3.1.1. 16S rRNA gene sequencing of Bacterial Isolates

The 16S rRNA sequence was considered as a more discerning. Thus, 1260 bp of 16S rRNA was amplified with PCR. From alignment with the database in GenBank by the BLAST program, bacteria were recognized with 99 % certainty to be *S. thermophiles* (Chagnaud *et al.,* 2001).

3.1.2. Nanobiosynthesis of TiNPs

The supernatant of *S. thermophilus* demonstrated ability in synthesis for TiNPs using Titanium dioxide (5mM) as an initiator for nanosynthesis TiNPs, and after shaking incubation for 18 hrs, 150 rpm 38°C, *S. thermophilus* changed the color from light brown into dark brown as an indicator for nanosynthesis of the TiNPs.

3.1.3. UV-visible spectrophotometer analysis

UV-Visible spectrophotometric is a proven technique that is usually used for the analysis of the nanoparticles. After 24 hours of incubation of the reaction mixture, color-changing was observed, which indicated the formation of the nanoparticles in the reaction mixture. The absorption spectrum has a peak at 377nm for TiNPs. This is an indication of the TiNPs formation.

3.1.4. SEM exploration of TiNPs

SEM enables the high-resolution imaging of single nanoparticles (NPs) with sizes well below 10 nm, In the current study, SEM analysis exhibited well-spread of TiNPs, regular with 18-30nm in diameter to each nanoparticle, with inconstant shape mostly spherical form (Figure 1).

3.1.5. EDS analysis of TiNPs

Titanium was quantified by Energy-dispersive X-ray spectroscopy (EDS) analysis through observing the optical absorption peaks of titanium elements. The presence of elemental titanium indicated the reduction of titanium ions in the reaction mixture. The EDS appeared solid indications from Ti atoms. The weight proportion of TiNPs was 70.50 % Titanium and 29.50% for O2 (Figure 2). The peak appeared at 4.5keV for TiNPs.

3.1.6. XRD analysis of TiNPs

The average size of TiNPs was detected by X-ray crystallography diffraction (XRD) analysis, and the *S. thermophilus* produce TiNPs with average size was 21 nm (Figure 3).

3.1.7. AFM analysis of TiNPs

The average diameter and the three-dimensional structure of TiNPs were detected by the Atomic Force Microscope (AFM) analysis, and the *S. thermophilus* produce TiNPs with an average diameter of 54.32 nm (Figure 4).

Depending on description and nano-characterization of nanoparticles by the color-changing, XRD and AFM. The morphology, size, distribution, and presence of metals nanoparticles were nanocharacterized and therefore, TiNPs were used for further study.

3.1.8. Antibacterial activity of TiNPs

Antibacterial activity of nano-biosynthesized TiNPs was used to evaluate their ability for inhibition growth of clinical bacteria. Agar well diffusion method was used for detecting the antibacterial activity of different concentrations of TiNPs. The study presented that TiNPs inhibited bacterial growth (Table 1; Figure 5). TiNPs inhibited significantly *K.pneumoniae* (20mm) with concentration (500µg/ml), and *S. aureus* (16mm) with the same concentration. It was observed when increased the concentration of TiNPs the inhibition zone increased, (500 µg/ml) showed a large inhibition zone than 100 µg/ml , 300 µg/ml and 400 µg/ml respectively.

3.1.9. Antibiofilm activity of TiNPs

The tube method (TM) was used for impost of biofilm creation by MDR *K.pneumoniae* and *S.aureus* and antibiofilm by TiNPs.

Results revealed that biofilm formation was observed in control tubes containing only bacterial suspension (without nanoparticles), which was considered positive by showing a visible film lined the wall and bottom of the tube in tested bacteria, as showed in Table 2.

While the tubes containing bacterial suspension *K.pneumoniae* and *S. aureus* with TiNPs, the results showed biofilm formation was prevented and removed by the effect of TiNPs, that have the ability on formation biofilm in moderate and weak degree respectively.

3.2. Discussions

Nano-biosynthesis of titanium nanoparticles by *S. thermophilus* supernatant was indicated by changing the color of reaction mixture from light brown color into dark brown, the changing in color may be attributed to reduction of (TiO_2) into Titanium nanoparticles due to the action of extracellular proteins, and other biomolecules present in the culture of *S. thermophilus* mediated the hydrolysis of the anionic complexes and resulted in the nanobiosynthesis of titanium nanoparticles (Jha and Prasad, 2010; Ahmad *et al.,* 2013).

Not all the organisms are found to be competent for the nanobiosynthesis of nanoparticles. The exact process in nanobiosynthesis by all organisms is yet to be elucidated (Gurunathan *et al.,* 2009).

The nano-characterization of the biogenic nanoparticles was performed by UV-visible spectroscopy, SEM, and EDS analysis. The preliminary confirmation of the extracellular nanobiosynthesis of nanoparticles was obtained by the contrast color change due to the surface Plasmon resonance (SPR) phenomenon. This was observed with the UV spectroscopic study of the colloidal solution, the maximum absorption peak of the Titanium nanoparticles centered at 377nm (Vidyasagar *et al.,* 2018).

The plasmon resonance band showing the sharp absorbance and indicates little aggregation of the particles in solution. The absorption of brown color due to excitation of surface plasmon vibration in particles, surface plasmon absorption strongly depends on the particle size, shape dielectric medium, and chemical surrounding the UV-Vis absorption spectra of nanoparticles dispersed in water (Mariselvam *et al.,* 2013).

The current study displayed well-dispersed TiNPs with 50-150nm with spherical shape (Ibrahem and Salman,2014; Aldujaili *et al.,* 2015). The weight percentage of Ti was 70.50%. indicated to elemental titanium that directed the reduction to Ti metals in the mix (Bhakya *et al.,* 2016: Chaudhari *et al.,* 2012).

Due to the appearance and rise of AMR, nanomaterials are a substitute for antibiotics. Nano-particles (NPs) were reflected good-looking for the making of a novel Antibacterials (Rai *et al.,* 2012). The TiNPs seemed to have particular effects on *K. pneumoniae* (20 mm) with concentration (500 µg/ml), and *S. aureus* (16 mm) (Silhavy *et al.,* 2010; Taglietti *et al.,* 2012).

Results from the present study and other studies showed differences in inhibition zones of bacterial species to TiNPs, and this may be

returned to the variances liability of bacterial species depends on commotion of elements (Gad *et al.,* 2007; Blake and Neill, 2013; Hadi and Melconian, 2013), As well as when increase concentration of TiNPs showed an increase in the inhibition activity (Shrivastava *et al.,* 2007).

The steady release of ions from the degradation of nanoparticles is a critical function of nanoparticles. Ions bind to the protein and genomic negative charge, causing changes in the cell wall, membrane, DNA, and RNA of bacteria. Ion interacts with functional groups such as imidazoles and indoles. The TiNPs also injury membranes and encourage the discharge of ROS (Wu *et al.,* 2014). It has been proposed that NPs inhibit DNA replication, Ribosomes Denaturation, and modulation the signal transduction in bacteria (Shrivastava *et al.,* 2007; Jung *et al.,* 2008; Davod *et al.,* 2011).

Nanosized particles showed more inhibition activity than large particles. Bactericidal activity of AgNPs of Nanosized particles (<30 nm) was optimum alongside *S. aureus and K. pneumonia* (Wu *et al.,* 2014).

The morphology of nanomaterials has important characterization in the inhibition commotion of TiNPs. Hexagonal NPs showed a high effect in comparison with further shapes, and this was endorsed to the particular surface areas (Hong *et al.,* 2016).

Several different mechanisms that may work together to confer resistance which include prevention of the antimicrobial from reaching its target by reducing its ability to penetrate the cell or expulsion of antimicrobial from the cell via general or specific efflux pumps or by modification or degradation of antimicrobial (Alekshun and Levy, 2007).

Organisms generally possess mechanisms for detoxifying metals. this including exclusion from the cell, isolating the metal in the cytoplasm by concentrating it in granules, precipitating it in the cell wall, or transforming it (e.g., by oxidation or reduction) into a harmless form in the organism (Brown,1991).

Other studies have demonstrated that TiNPs failed to exhibit antibacterial activity, but upon combination with antibiotics, they were able to inhibit the growth of microorganisms (Roy *et al.,* 2010), contrary with some studies that revealed TiNPs have the ability to inhibit the microbial growth without any kind of combination (Vincent *et al.,* 2014).

Microbial biofilms are communities that have resistance to antibiotics and immunity. Nanoparticles may be used TiNPs to inhibition activity (Vincent *et al.,* 2014).

Clinical bacteria appeared their capability to create biofilm without treated by the nanoparticles, with TiNPs biofilm was disallowed in *K.pneumoniae* and *S. aureus*, TiNPs maybe alter genes regulation of biofilm, the effect on initial formation and maturation, leading to inhibition of biofilm-related infections (Martinez-Gutierreza *et al.,* 2013: Fayaz *et al.,* 2010: Sadekuzzaman *et al.,* 2015). More or less strains of species may be sensitive or resistant to nanoparticles (Vanaja andAnnadurai, 2013: Aldujaili, 2017).

4. CONCLUSIONS:

16S rRNA is a sensitive and reliable method for the identification of *Streptococcus thermophilus*. The Bacteria *S. thermophilus* competent for Nanobiosynthesis of nanoparticles because Not all the organisms are found to be competent for the nanobiosynthesis of nanoparticles. The TiNPs have particular effects on Multidrug-resistant *Klebsiella pneumonia* and *Staphylococcus aureus*, thus are a substitute for antibiotics.

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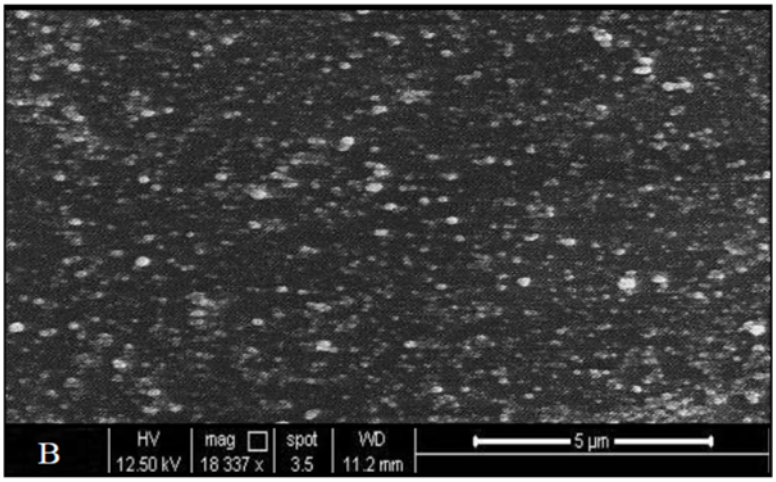

Figure 1. SEM micrograph of nanobiosynthesized TiNPs from *S. thermophilus*

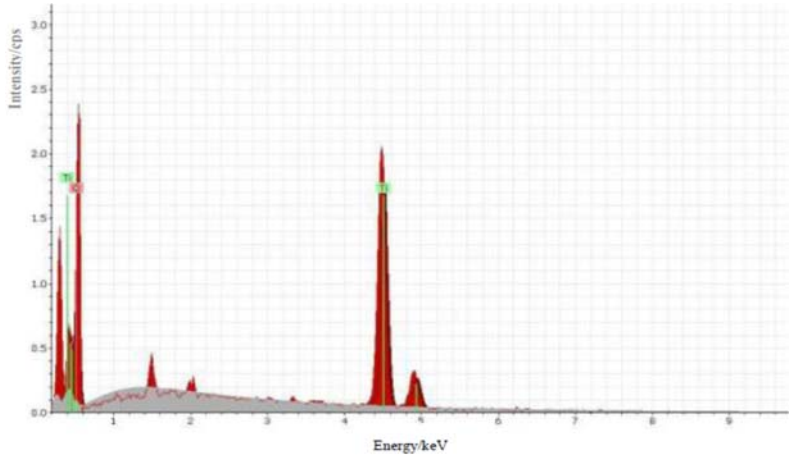

Figure 2. EDS analysis of nanosynthesized TiNPs using *S. thermophilus*

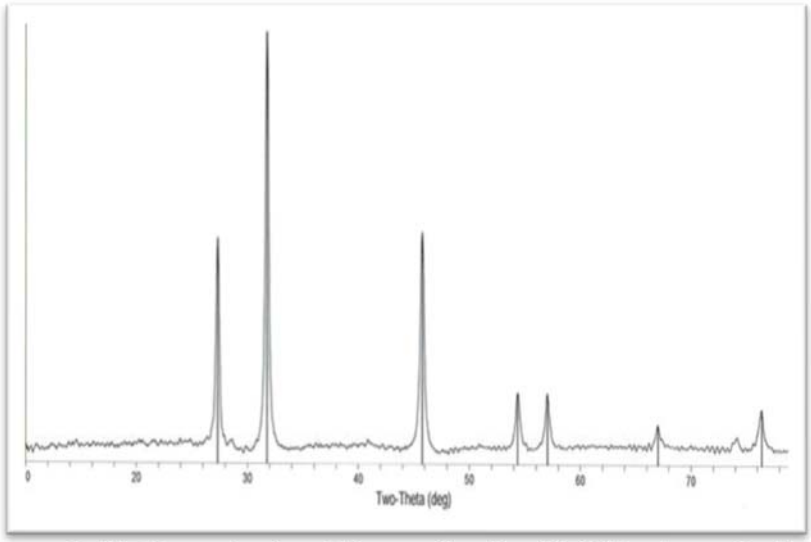

Figure 3. XRD analysis of biosynthesized TiNPs from *S. thermophilus*

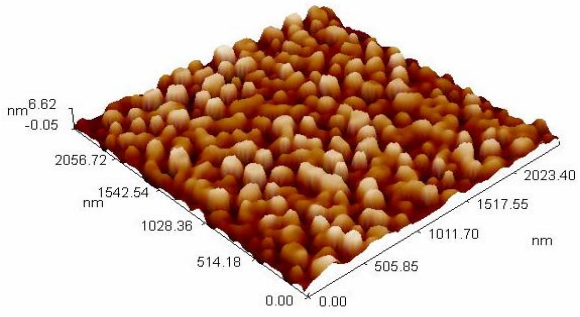

Figure 4. AFM analysis of nanobiosynthesized TiNPs from *S. thermophilus*

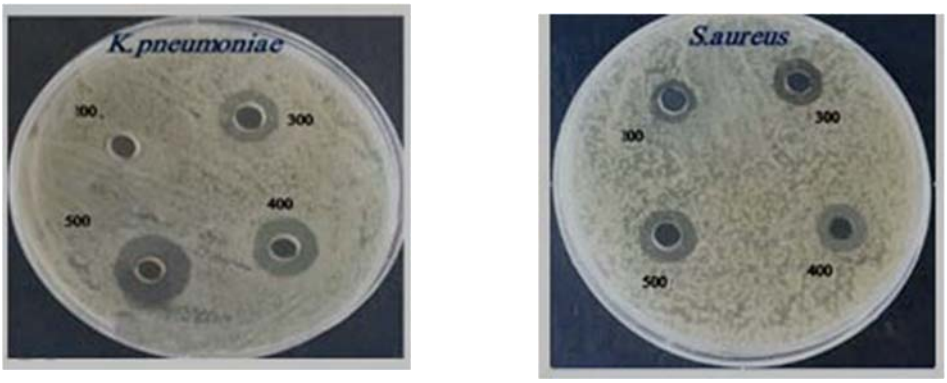

Figure 5. Inhibition zones of different concentrations of TiNPs against MDR *K.pneumoniae* and *S.aureus.*

Table 1. Inhibition zones of different concentrations of TiNPs against MDR *K.pneumoniae* and *S.aureus*

TiNPs con.(µg/ml)	100	300	400	500
K.pneumoniae	12	15	16	20
S.aureus	12	13	14	16

Table 2: Antibiofilm activity of TiNPs

Pathogenic bacteria	Qualitative of biofilm formation	Antibiofilm by TiNPs
K.pneumoniae	2	0
S.aureus	1	0