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## Biosynthesis of titanium nanoparticles using locally *Lactobacillus* spp .isolated from different sources

Kawther HkeemIbrahem<sup>1\*</sup>, Jehan Abdul Sattar Salman<sup>1</sup>, Fattma Abodi Ali<sup>2</sup>

1.Department of Biology/College of Science/Al-Mustansiriya University/ Baghdad- Iraq

2 Department of microbiology/college of medicine/Hawler Medical university/Erbil-Iraq

### ABSTRACT

Microorganisms play a very important role in the eco-friendly and green synthesis of metal nanoparticles. The inherent, clean, nontoxic and environment friendly. The present study focuses on the rapid synthesis of titanium nanoparticles by Iraqi *Lactobacillus* isolates. Seventy five isolates from (120) samples included 35 vaginal swabs from healthy women, 45 infant stools and 40 yoghurt were isolated and identified to species, then tested for TINPs production. Results showed that only six (8%) isolates produced titanium nanoparticles, titanium nanoparticles were characterized with Atomic Force Microscopy (AFM) and found the average size ranges from 70.98 to 105.37 nm. The best of our study, this is the first report on synthesis of titanium nanoparticles by Iraqi *Lactobacillus* isolates from different sources.

**Keywords:** *Lactobacillus*, Atomic Force Microscopy

\*Corresponding Author Email: [kewther2001@gmail.com](mailto:kewther2001@gmail.com)

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## INTRODUCTION

Lactobacilli are part of the normal oral, intestinal and vaginal microflora. Lactobacilli have long been subject of interest of the food, especially of the dairy industry. Consumption of lactobacilli has been connected to the positive effects of human health for a long time. In general, lactobacilli have not been associated with disease and have been regarded as nonpathogenic members of the intestinal and urogenital flora<sup>1</sup>. Moreover, many of lactobacilli have been recognized as probiotics. Probiotics have been defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”<sup>2</sup>. The role of probiotics in conferring benefit to the host is being increasingly realized. However, most reports are on probiotics in dairy products which are of industrial importance and employed in fermented food item<sup>3</sup>. Development of technologies for producing nanoparticles, because of their key role in the future world could be one of the most valuable findings of human being. Nanoparticles have a wide range of use in different fields of industry, medicine and basic science. Microbes produce inorganic materials either intra- or extracellularly often in nanoscale dimensions.<sup>4</sup>

## MATERIALS AND METHODS

### **Isolation of Lactobacilli from vagina**

Thirty five sterile swabs were rolled over high vaginal swabs were taken from healthy women using serial swab stick wall by physician and placed in sterile screw cap tubes containing selective media MRS (deMan, Rogosa and Sharpe) broth incubated at 37C<sup>0</sup> anaerobically for (24-48)hours. A representative single colony was selected from each isolate directly inoculated on to MRS agar and Gram stained.<sup>5</sup>

### **Isolation of Lactobacilli from infant feces**

*Lactobacillus* isolated from healthy infants as previously reported by .<sup>6</sup> Fresh feces (1mg ) suspended and sequentially diluted in 9 ml sterile water. Aliquots of 100 µl of the diluted samples were spread onto MRS agar plates and incubated at 37C<sup>0</sup> for 24 to 48 hours. The colonies that displayed sufficient growth were cultivated in MRS medium at 37C<sup>0</sup> for 24 to 48 h.

### **Isolation of Lactobacilli from dairy products**

Forty conventional yogurt samples were procured from the local market in sterile plastic bags and homogenized by dissolving in 100 ml of sterilized phosphate buffer saline. Samples from both sources were diluted serially in phosphate buffer saline and then inoculated on MRS agar plates by pour plate method<sup>7</sup>. MRS agar plates were incubated at 37C<sup>0</sup> for 48 hours anaerobically. Morphologically distinct and well isolated colonies were picked and transferred to

new MRS agar plates by streaking. Finally, pure colonies were obtained.

### **Identification of *Lactobacillus* isolates**

Macroscopic appearance of all the colonies was examined for cultural and morphological characteristics on MRS agar. The bacterial isolates were assayed for characteristics, such as Gram stain and biochemical examinations included catalase, oxidase, gelatinase, growth at 15c° and 45c° and carbohydrate fermentation tests<sup>8,9</sup>.

### **Detection for TINPs production**

Three tubes will used for each isolates and each tube were filled with 40 ml of MRS broth solution . Then 20 ml of TiO<sub>2</sub>(0.025m) were added to the first and second tube respectively and both were shacked for half hour by magnetite stirrer while the third tube contain MRS Broth only. Final concentration ultimately would be equivalent. Each isolates will cultured in first and third tube into CO<sub>2</sub>-incubator at 37C° for 24,48,72 hours. Second tube was used as blank for first one , the change in color from light brown to dark brown observed and production of sediment will observed as primary detection of produced TINPs<sup>10</sup>.

### **Characterization of titanium nanoparticles by Atomic Force Microscopy:**

Atomic Force Microscopy image was taken using Park system AFM XE 100. The aqueous titanium nanoparticles were deposited onto a freshly cleaved mica substrate. The sample aliquot was left for 1 min and then washed with deionized water and left to dry for 15 min. The images were obtained by scanning the mica in air inn on – contact mode<sup>11</sup>. The size, shape, and dispersity mode of TINPs will characterized by Atomic Force Microscopy .

## **RESULTS AND DISCUSSION**

Nanotechnology is the prompt emerging discipline in the field of life science. Biologists are highly interested in synthesizing bio-nanoparticles using many of the precious metals. A comprehensive study of rapid extracellular biosynthesis of TI-NPs from probiotic isolates were carried out in this research work.

A total of 120 samples were collected from different sources (40 yoghurt ,35 vaginal swabs,45 infant stools) (Table-1-). Morphological examination of all *Lactobacillus* isolates grown on MRS agar medium showed white, large, smooth, round colonies with entire margin. In microscopic examination, the cells of *Lactobacillus* reacted positively with Gram stain, rods with rounded ends that occurred singly, in pairs or in short chains and non-spore forming. All bacterial isolates were catalase, oxidase and gelatinase negative. The results showed that 75 isolates of *Lactobacillus* were obtained from 120 samples, all isolates were then identified to the

species (Table-2-). The isolates of *Lactobacillus* from human and yoghurt are distributed as ; (14) *L. delbrueckii* subsp. *bulgaricus*, (9) *L. paracasei*, (3) *L. rhamnosus*, (13) *L. fermentum*, (23) *L. gasseri*, (4) *L. acidophilus* and (9) *L. crispatus*. (Table-3-).

**Table -1- : Incidence of *Lactobacillus* isolates from different samples.**

Source of isolation	No. of samples	No. of lactobacillus isolates	Percentages%
Yoghurt	40	25	62.5
Vaginal swabs	35	25	71.43
Infant stools	45	25	55.55
Total	120	75	62.5

**Table-2- : Biochemical tests of the *Lactobacillus* isolates**

Test	<i>L.gasseri</i>	<i>L.crispatus</i>	<i>L.paracasei</i>	<i>L.rhamnosus</i>	<i>L.fermentum</i>	<i>L.delbrueckii</i> sub <i>sp.bulgaricus</i>	<i>L.acidophilus</i>
Catalase	-	-	-	-	-	-	-
oxidase	-	-	-	-	-	-	-
gelatinase	-	-	-	-	-	-	-
Growth at 45°C	+	+	-	+	+	+	+
Growth at 15°C	-	-	+	+	-	-	-
Glucose	+	+	+	+	+	+	+
Mannitol	-	-	+	+	-	-	+
Maltose	v	+	-	-	+	-	+
Mannose	+	+	+	+	+	-	+
Sucrose	+	+	+	+	+	-	+
Salicin	v	+	+	+	-	-	+
Lactose	+	+	-	-	+	+	+
Raffinose	v	-	-	-	+	-	+
Trehalose	v	-	+	+	+	-	+
Xylose	-	-	-	-	-	-	-
fructose	+	+	+	+	+	+	+
Arabinose	-	-	-	+	+	-	-
cellobiose	+	+	+	+	-	-	+

(-) : negative result, (+) : positive result, variable(-.+)

**Table- 3- :*Lactobacillus* isolates from different sources .**

Isolate No	<i>Lactobacillus</i> species	Isolate No.	<i>Lactobacillus</i> species	Isolate No.	<i>Lactobacillus</i> species
LV <sub>1</sub>	<i>L.rhamnosus</i>	LS <sub>1</sub>	<i>L.fermentum</i>	LY <sub>1</sub>	<i>L.delbrueckii</i> subsp <i>bulgaricus</i>
LV <sub>2</sub>	<i>L.gasseri</i>	LS <sub>2</sub>	<i>L.fermentum</i>	LY <sub>2</sub>	<i>L.delbrueckii</i> subsp <i>bulgaricus</i>
LV <sub>3</sub>	<i>L.gasseri</i>	LS <sub>3</sub>	<i>L.gasseri</i>	LY <sub>3</sub>	<i>L.delbrueckii</i> subsp <i>bulgaricus</i>
LV <sub>4</sub>	<i>L.gasseri</i>	LS <sub>4</sub>	<i>L.gasseri</i>	LY <sub>4</sub>	<i>L.delbrueckii</i> subsp <i>bulgaricus</i>
LV <sub>5</sub>	<i>L.fermentum</i>	LS <sub>5</sub>	<i>L.gasseri</i>	LY <sub>5</sub>	<i>L.delbrueckii</i> subsp <i>bulgaricus</i>
LV <sub>6</sub>	<i>L.gasseri</i>	LS <sub>6</sub>	<i>L.fermentum</i>	LY <sub>6</sub>	<i>L.rhamnosus</i>
LV <sub>7</sub>	<i>L.gasseri</i>	LS <sub>7</sub>	<i>L.gasseri</i>	LY <sub>7</sub>	<i>L.parscasei</i>

LV <sub>8</sub>	<i>L.gasseri</i>	LS <sub>8</sub>	<i>L.gasseri</i>	LY <sub>8</sub>	<i>L.paracasei</i>
LV <sub>9</sub>	<i>L.gasseri</i>	LS <sub>9</sub>	<i>L.gasseri</i>	LY <sub>9</sub>	<i>L.paracasei</i>
LV <sub>10</sub>	<i>L.fermentum</i>	LS <sub>10</sub>	<i>L.crispatus</i>	LY <sub>10</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>11</sub>	<i>L.gasseri</i>	LS <sub>11</sub>	<i>L.crispatus</i>	LY <sub>11</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>12</sub>	<i>L.gasseri</i>	LS <sub>12</sub>	<i>L.crispatus</i>	LY <sub>12</sub>	<i>L.paracasei</i>
LV <sub>13</sub>	<i>L.acidophilus</i>	LS <sub>13</sub>	<i>L.gasseri</i>	LY <sub>13</sub>	<i>L.paracasei</i>
LV <sub>14</sub>	<i>L.acidophilus</i>	LS <sub>14</sub>	<i>L.fermentum</i>	LY <sub>14</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>15</sub>	<i>L.acidophilus</i>	LS <sub>15</sub>	<i>L.gasseri</i>	LY <sub>15</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>16</sub>	<i>L.acidophilus</i>	LS <sub>16</sub>	<i>L.fermentum</i>	LY <sub>16</sub>	<i>L.rhamnosus</i>
LV <sub>17</sub>	<i>L.crispatus</i>	LS <sub>17</sub>	<i>L.fermentum</i>	LY <sub>17</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>18</sub>	<i>L.crispatus</i>	LS <sub>18</sub>	<i>L.fermentum</i>	LY <sub>18</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>19</sub>	<i>L.crispatus</i>	LS <sub>19</sub>	<i>L.fermentum</i>	LY <sub>19</sub>	<i>L.delbruekiispbulgaricus</i>
20 LV	<i>L.crispatus</i>	LS <sub>20</sub>	<i>L.fermentum</i>	LY <sub>20</sub>	<i>L.delbruekiispbulgaricus</i>
LV <sub>21</sub>	<i>L.crispatus</i>	LS <sub>21</sub>	<i>L.fermentum</i>	LY <sub>21</sub>	<i>L.paracasei</i>
LV <sub>22</sub>	<i>L.crispatus</i>	LS <sub>22</sub>	<i>L.gasseri</i>	LY <sub>22</sub>	<i>L.paracasei</i>
LV <sub>23</sub>	<i>L.gasseri</i>	LS <sub>23</sub>	<i>L.gasseri</i>	LY <sub>23</sub>	<i>L.paracasei</i>
LV <sub>24</sub>	<i>L.gasseri</i>	LS <sub>24</sub>	<i>L.fermentum</i>	LY <sub>24</sub>	<i>L.paracasei</i>
LV <sub>25</sub>	<i>L.gasseri</i>	LS <sub>25</sub>	<i>L.gasseri</i>	LY <sub>25</sub>	<i>L.delbruekiispbulgaricus</i>

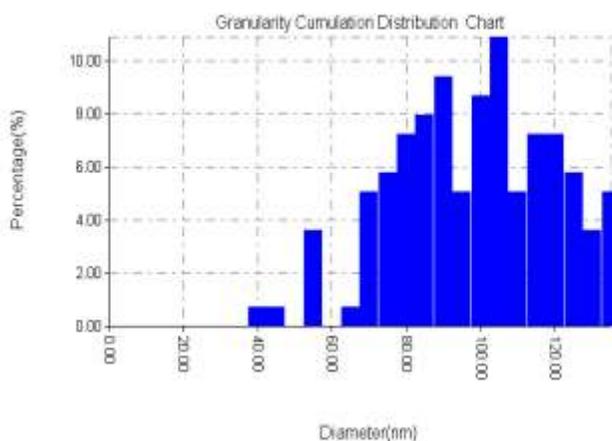
LS : isolated from stool, LV: isolated from vagina, LY: isolated from yoghurt

All 75 isolates were tested for TINPs production. Titanium dioxide exposing to the bacteria was reduced and nanoparticles of titanium formed. Solution color changed from light brown to dark brown in samples of six producer isolates included *L. gasseri* (L V4), *L. rhamnosus*(LV1), *L. acidophilus* (Lv14), *L. gasseri*(LV8), *L. crispatus*(Lv20) from vagina and *L.delbruekiisubsp. bulgaricus* (LY2) from yoghurt, where as the non producers demonstrated no color changing as well the solutions of second tube as controls as shown in figure (1).

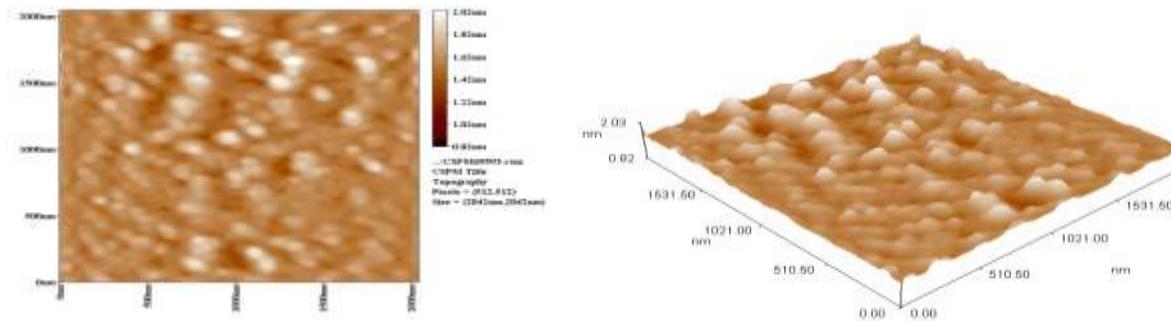


Figure(1):left tube Lactobacillus spp in MRS broth without TIO<sub>2</sub>taken as control , right tube with bacteria and TIO<sub>2</sub> (0.025 mM) while middle tube is blank TIO<sub>2</sub> and MRS broth only. Ability of eukaryotic and prokaryotic microorganisms, plants system to form the metal nanoparticles is particularly important in the development of nanobiotechnology. Biologically synthesized nonmaterial's has vital role in material science and nano-biotechnology. TI nanoparticles of various sizes were synthesized and characterized in terms of their morphologies, dimensions, and surface charges. This study covered particle size was analyzed by Atomic Force Microscopy. AFM was used to view the nanoparticles both in surface and three Dimensional

view ,and found the average size of particles(95.83,102.35 ,89.94, 71.78,105.37,70.98) nm. Synthesized by *Lactobacillus* isolates by *L. gasseri* (L V4),*L. rhamnosus*(LV1) ,*L. delbrueckii*subsp. *bulgaricus* (LY2), *L. acidophilus* (Lv14), *L. gasseri*( LV8),*L. crispatus*(Lv20) respectively Fig. (2,3,4,5,6,7).Microbial resistance to most toxic heavy metals is due to their chemical detoxification as well as due to energy-dependent ion efflux from the cell by membrane proteins that function either as ATPase or as chemiosmoticcation or proton anti-transporters .Azhar<sup>10</sup> found extracellular *Lactobacillus*-mediated Biosynthesis of Titanium Nanoparticles in MRS-broth Medium occurred anaerobically after 72h with nanoparticle size 150nm from dairy products in Iran,whileJha&Parsad<sup>12</sup> discovered that Silver and titanium dioxide nanoparticles are synthesized using *Lactobacillus* spp. procured from yoghurt and probiotic tablets. Titanium nanoparticles of spherical aggregates of 40–60 nm were produced extracellularly using the culture filtrate of *Lactobacillus* spp. at room temperature , in the other hand fungus (*Fusariumoxysporum*) mediated synthesis of titania nanoparticles has been reported by Bansal<sup>13</sup> TiO<sub>2</sub> is considered to be good candidate material for photoelectrochemical energy production. Jha and Prasad<sup>14</sup> found the baker's yeast (*Saccharomyces cerevisiae*) synthesized TiO<sub>2</sub> nanoparticles, the particles are found almost spherical in shape having a size of the order of 8–35 nm. The non-toxic and biocompatible properties of titanium finds its applications in biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries. These titanium nanoparticles were lighter in weight and high resistance to corrosion and have enormous applications in automobiles, missiles, airplanes, submarines, cathode ray tubes and in desalting plants and has promising future role in cancer chemotherapy and gene delivery. Antibacterial effects of TiO<sub>2</sub> on *Lactobacillus acidophilus* would also be used in orthodontic appliances, such as pit and fissure sealants, toothbrushes, dental implants, and screws.<sup>15</sup>

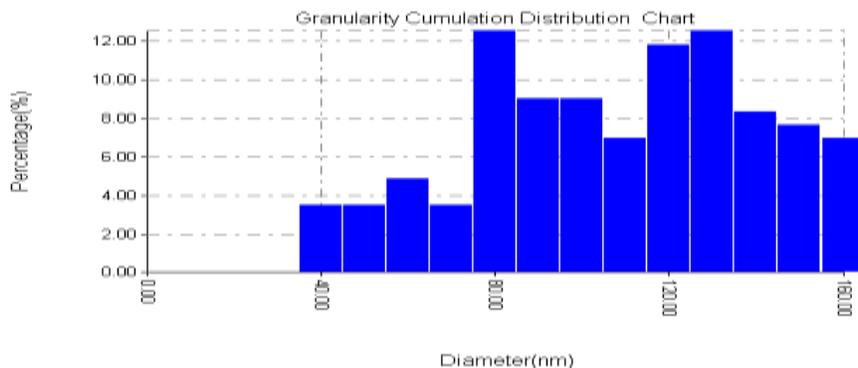


-A-

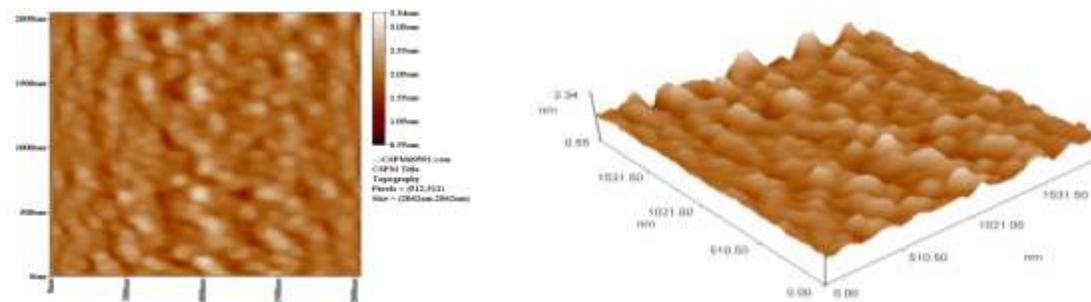


-B-

Figure: (2). Atomic Force Microscopy image of titanium nanoparticles synthesized by *Lactobacillus gasseri*(L V4) which isolated from vagina A-Diameter percentage ,B- Surface and three Dimensional view

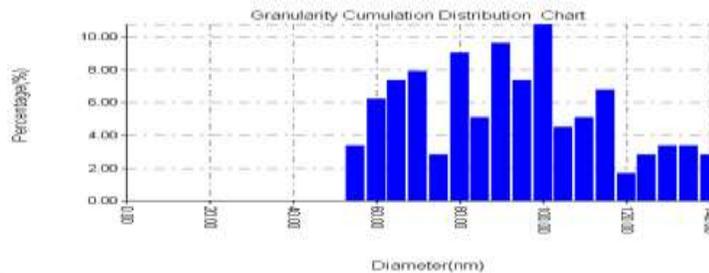


-A-

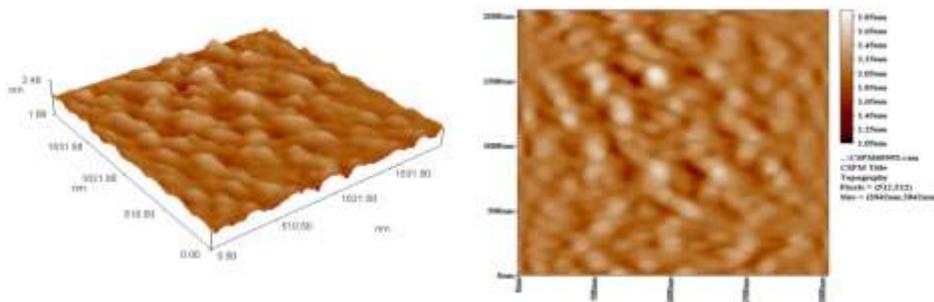


-B-

Figure: (3). Atomic Force Microscopy image of titanium nanoparticles synthesized by *Lactobacillus rhamnosus*(LV1) which isolated from vagina A-Diameter percentage ,B- Surface and three Dimensional view

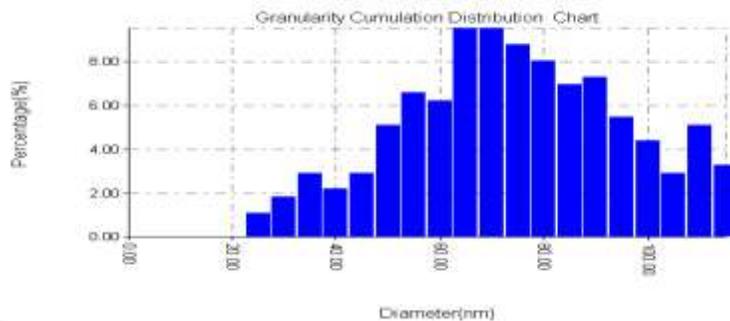


-A-

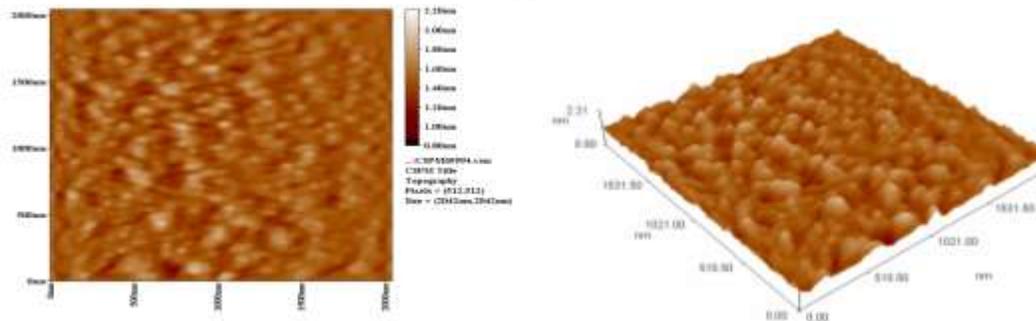


-B-

Figure: (4). Atomic Force Microscopy image of titanium nanoparticles synthesized by *Lactobacillus delbrueckii subsp. bulgaricus* (LY2) which isolated from yoghurt A-Diameter percentage ,B- Surface and three Dimensional view

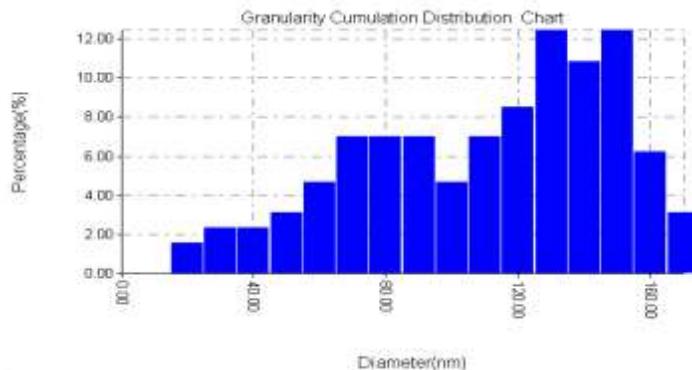


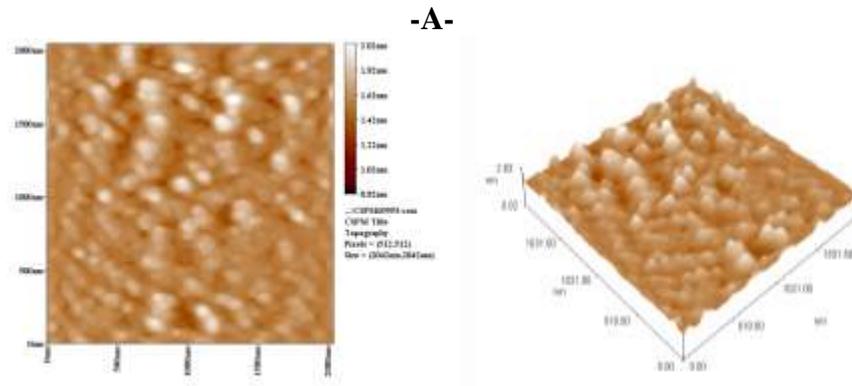
-A-



-B-

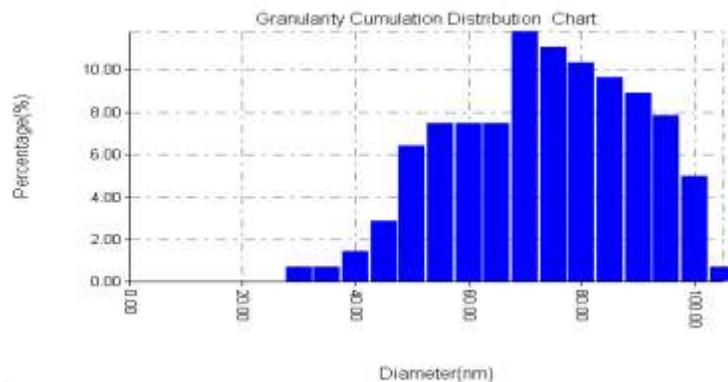
Figure: (5). Atomic Force Microscopy image of titanium nanoparticles synthesized *Lactobacillus acidophilus* (LV14) which isolated from vagina A-Diameter percentage ,B- Surface and three Dimensional view



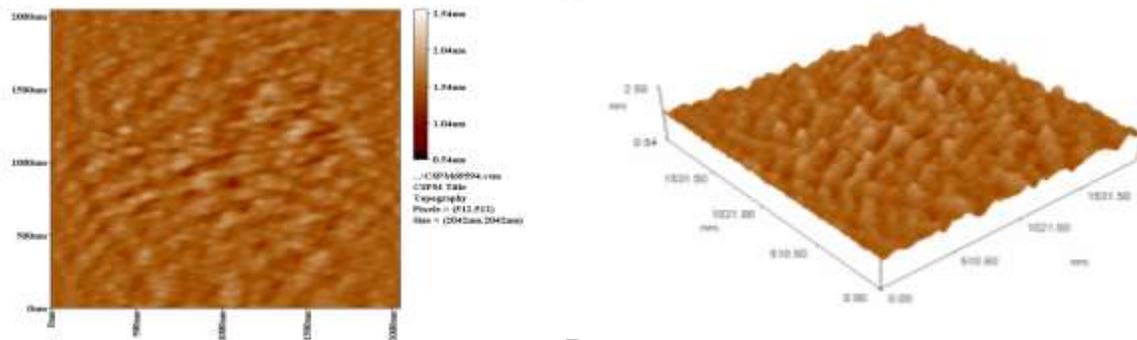


-B-

**Figure(6):Atomic Force Microscopy image of titanium nanoparticles synthesized by *Lactobacillus gasseri*(LV8) which isolated from vagina A-Diameter percentage ,B- Surface and three Dimensional view**



-A-



-B-

**Figure(7):Atomic Force Microscopy image of titanium nanoparticles synthesized by *Lactobacillus crispatus*(LV20)which isolated from vagina A-Diameter percentage , B- Surface and three Dimensional view**

## CONCLUSIONS

Nanotechnology offers important new tools expected to accelerate scientific as well as economic activities in medical research and development. Nanotechnology has the potential to make significant contributions to disease detection, diagnosis, therapy, and prevention. In the present

study we have reported a simple biological extracellular, easy, low cost, non toxic economical and ecofriendly approach for synthesizing titanium nanoparticles by using *Lactobacillus spp.*, which provides extraordinary opportunities to improve materials and medical devices. The titanium nanoparticles formed were characterized by AFM. The smallest size of the particles (70.98nm) was obtained from vaginal isolate *L.crispatus* (LV20)

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