Biosynthesis, Characterization and Antibacterial Effect of Zno Nanoparticles Synthesized by Lactobacillus Spp

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Abstract

Biosynthesis of Zinc oxide (ZnO) nanoparticles using a probiotics bacteria Lactobacillus spp. which isolated from different sources was studied. The formation of ZnO nanoparticles was checked using X-ray diffraction (XRD) technique, Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopic (SEM) and Energy-dispersive X-ray analysis (EDX) spectra. The characterization results showed that the biosynthesized nanoparticles are hexagonal ZnO crystal structure, spherically shaped with a diameter of about (30.3 - 38) nm. The antibacterial effect of the synthesized ZnO nanoparticles was determined through the minimum inhibitory concentration (MIC) against pathogenic bacteria isolated from skin infections included Pseudomonas aeruginosa, Acinetobacter baumanii, Klebsiella pneumonia, and Staphylococcus aureus. The synthesized ZnO nanoparticles were effective against all the tested pathogenic bacteria at different degrees. The best effect has been shown against A. baumanii with MIC recorded at 12.5mg/ml, but the lowest effect has been recorded on P. aeruginosa(50mg/ml).

Keywords: Biosynthesis, ZnO nanoparticles, Lactobacillus spp., Antibacterial activity, Skin infections.

Introduction

Lactobacillus is a major and the largest of the genera included in the group of lactic acid bacteria, characterized as Gram-positive, non-motile, non-spore forming, favoring anaerobic conditions, fastidious, acid-tolerant, and obligate fermentative [1].

Lactobacilli are the important group of probiotic bacteria that inhibit undesirable microflora in the human gut. Probiotics are defined as living microorganisms that extend beneficial effects on health of human [2, 3]. They are the normal flora, like lactobacilli and Bifid bacterium [4].

Nanomaterials defined as a manufactured or natural material containing particles, in an unbound or as an agglomerate or aggregate state; showing of 50% or more of particles with one or more external dimensions with size (1–100) nm [5].

Biosynthesizing nanoparticles acting as biological factories approach a nontoxic, clean and eco-friendly method with a enormous range of shapes, sizes and compositions [6]. Lactobacillus finding its ability to synthesize nanoparticles of ZnO [7], Sb2O3 [8], BaTiO3 [9], CdS [9] Ag [10], TiO2 [11] and Fe3O4 [12].

Zinc oxide nanoparticles are non-toxic to cells of human, as antibacterial agents, and good biocompatibility to human cells [13]. Different physical, chemical and biological methods used to produce Zinc oxide nanoparticles.

The biological methods included using living microorganisms’ cells or plant extracts, are being characterized as low-cost and environmentally friendly [14].

In biological process, different forms of zinc have roles in a wide metabolic process like nucleic acid, lipid, and carbohydrate and protein synthesis [15].

The current study describes an eco-friendly and simple method for the biosynthesis of
zinc oxide nanoparticles by Iraqi Lactobacillus spp. isolates and evaluation of its antibacterial effect against pathogenic bacteria isolated from skin infections.

Materials and Methods

Microorganism

Lactobacillus SPP

Fifteen isolates of Lactobacillus spp. included four isolates belonged to Lb. Plantarum, four isolates to Lb. gasseri, three isolates to Lb. acidophilus, two isolates to Lb. fermentum and two isolates belonged to Lb. salivarius were isolated from different sources, then identified throughout cultural, microscopically, biochemical test according to [16] and Vitek2 system.

Pathogenic Bacteria

Eight isolates of pathogenic bacteria isolated from skin infections included two isolates for each of Pseudomonas aeruginosa, Acinetobacter baumanii, Klebsiella pneumonia and Staphylococcus aureus.

These isolates were identified throughout cultural test, microscopically test, biochemical test according to Forbes [17] and Vitek2 system.

Biosynthesis of ZnO Nanoparticles

A pure culture of Lactobacillus spp. (9×10^6 CFU/ml) was inoculated into the flask containing (MRS) De Man Rogosa and Sharpe broth and incubated for 24h at 37 °C. At the end of incubation time pH of the culture media broth was adjusted to 6 using 1 M Noah to delay the transformation process.

Zinc Chloride (ZnCl₂) with a solution of 0.25(M) was added to the flask containing culture solution and heated up to 80°C for 5-10 min on a water bath [8].

A white precipitate indicates the process of transformation, and then the heating was stopped by removing the flask from the water bath and incubated for 12 h at 37 °C [18].

The net product was filtered, deionized water was added to nanoparticles and centrifugation was done at 3000 rpm for 10min, after every centrifugation, the pellet was washed with deionized water followed by drying at 40 °C for 8h till it was totally dry. The powder of ZnO nanoparticle was obtained.

Characterization

The formation of ZnO nanoparticles was checked by Atomic Force Microscopy (AFM), X-ray diffraction (XRD) technique, Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopic (SEM) and Energy-dispersive X-ray analysis (EDX) spectra.

Antibacterial Activity of Synthesized ZnO Nanoparticles

The antibacterial effect of the synthesized ZnO nanoparticles was determined through the MIC using micro dilution method.

Further dilutions were prepared to concentrations ranging from (100-0.04) mg/ml. 125 µl of Muller Hinton broth was added to the first column of the 96-well microplate and other 125 µl of the same media in the remaining wells.

Then, 125 µl of ZnO nanoparticles solution in PBS (100 mg/ ml) was added to the first column and mixed with the medium; serially, 125 µl were transferred to the following wells, 125 µl of the mixture in the last column was discarded, the final volume for each well was 125 µl. All wells were inoculated with 2.5 µl of pathogenic bacteria culture (1.5× 10^8 CFU/ml) [19] with modification. Covered microplates were incubated for 24 h at 37 °C. The MIC was detected at a concentration which no visible growth could be observed after sub culturing on Nutrient agar.

Results and Discussion

Biosynthesis and Characterization of ZnO nanoparticles

In the present study the white precipitate of all Lactobacillus spp. isolates indicate all ZnO nanoparticles are deposited.

Then all samples of nanoparticles analyzed by Atomic Force Microscope (AFM), different average size diameter of synthesized ZnO nanoparticles recorded of Lactobacillus spp. isolates with smallest size 68.41nm for Lb. salivarius isolated from the fish intestine and 69.03nm for Lb. gasseri isolated from the vagina (Table 1).

Atomic Force Microscopy (AFM) was used to record the ZnO nanoparticles topography. The micrograph shows uniform single-crystalline nature of the ZnO nanoparticles.
with many voids. The particle size and roughness of ZnO are found to be increased with the density of the ZnO nanoparticles. Figure (1) shows 2D and 3D AFM images, height histogram and section analysis (5 μm × 5 μm²) of ZnO nanoparticles powder synthesized by Lb. gasseri and Lb. salivarius.

### Table 1: Average size diameter of zinc oxide nanoparticle synthesized by *Lactobacillus* spp. analyzed by Atomic Force Microscope (AFM)

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Isolation source</th>
<th>Average size diameter (nm)</th>
<th>Bacterial isolates</th>
<th>Isolation source</th>
<th>Average size diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lb. plantarum</em></td>
<td>Raw milk</td>
<td>113.28</td>
<td><em>Lb. gasseri</em></td>
<td>Vagina</td>
<td>100.08</td>
</tr>
<tr>
<td><em>Lb. plantarum</em></td>
<td>Raw milk</td>
<td>84.90</td>
<td><em>Lb. acidophilus</em></td>
<td>Vagina</td>
<td>123.41</td>
</tr>
<tr>
<td><em>Lb. plantarum</em></td>
<td>Fish intestine</td>
<td>113.55</td>
<td><em>Lb. acidophilus</em></td>
<td>Vagina</td>
<td>89.19</td>
</tr>
<tr>
<td><em>Lb. fermentum</em></td>
<td>Fish intestine</td>
<td>68.41</td>
<td><em>Lb. gasseri</em></td>
<td>Vagina</td>
<td>108.26</td>
</tr>
<tr>
<td><em>Lb. salivarius</em></td>
<td>Fish intestine</td>
<td>86.08</td>
<td><em>Lb. gasseri</em></td>
<td>Vagina</td>
<td>69.03</td>
</tr>
<tr>
<td><em>Lb. plantarum</em></td>
<td>Infant stool</td>
<td>102.86</td>
<td><em>Lb. fermentum</em></td>
<td>Infant stool</td>
<td>89.09</td>
</tr>
<tr>
<td><em>Lb. gasseri</em></td>
<td>Infant stool</td>
<td>101.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1:** AFM results of ZnO nanoparticles synthesized by *Lactobacillus* spp (A) *Lb. gasseri* (B) *Lb. salivarius*

**X-ray Diffraction**

Zinc oxide nanostructure films synthesized by *Lb. gasseri* and *Lb. salivarius* were analyzed by X-ray diffraction.

This technique gives an indication of formation material type, the grain size and strain and dislocation density of the prepared thin film at optimum conditions (900°C and 4h). Generally, of ZnO thin film, the peak of the XRD was observed between (30°-40°). The presence of diffraction peaks indicates that the film is single crystalline with a hexagonal crystal structure without amorphous phase.

The sprayed film had peak corresponding to (101) directions of the hexagonal ZnO crystal structure that corresponding to the position (2θ =36.261°), with the full width at half maximum (FWHM) equal to (0.1224) as a preferred growth orientation which agree with the card of Joint Committee of Powder Diffraction Standard (JCPDS card. No 36-1451) as shown in Figure (2).
Figure 2: XRD pattern of ZnO nanoparticles synthesized by Lactobacillus spp. (A) Lb. gasseri (B) Lb. salivarius

Scanning Electron Microscope (SEM)

The surface micrograph of zinc oxide nanoparticles powder synthesized by Lb. gasseri and Lb. salivarius had spherical-shaped nanostructure grains with a diameter (30.3 and 38) nm respectively. The film had a good adhesiveness with the substrate at optimum conditions (900 °C and 4 h). The cross-section of pure ZnO nanoparticles and the thickness of pure ZnO thin film nanoparticle layer is measured (Figure 3).

Figure 3: SEM picture of ZnO nanoparticles synthesized by Lactobacillus spp. (a) Lb. gasseri (b) Lb. salivarius

Energy-dispersive X-ray analysis (EDX) spectra

The energy-dispersive X-ray analysis (EDX) spectra of the ZnO nanoparticle powder synthesized by Lb. gasseri and Lb. salivarius as shown in Figure (4), which confirm that all the films contain the elements (Zn, O).
Figure 4: EDX results of ZnO nanoparticles synthesized by Lactobacillus spp. (a) Lb. gasseri (b) Lb. Salivarius,

FTIR Spectra Measurement

Results of Fourier Transform Infrared spectroscopy (FTIR) indicate the phase composition and the process in which O₂ is bound to metal ions. FTIR spectra for ZnO nanoparticles synthesized by Lb. gasseri and Lb. salivarius shows that the peak of absorption is around (400–800) cm⁻¹ broken spectra that related to the all zinc oxide powder nanoparticles prepared. The main IR features of pure ZnO at (786.96 ) and (437.42 cm⁻¹ ) are assigned to Zn-O and stretching vibration bonds (609 cm⁻¹), it is showed that all the transmittance peaks are related to the ZnO respectively Figure(5-a). A change in an oxidation powder composition was recognized as shown in Figure (5-b). For the oxide formation, indicate kinetic energy of the Zn atoms that decreased through collision with oxygen, the formation of (O⁺2) ion. Chemical bonding (Zn–O) vibration stretching was formed.

Figure 5: FTIR results of ZnO nanoparticles synthesized by Lactobacillus spp. (a) Lb. gasseri (b) Lb. salivarius
The characterization of biosynthesized ZnO nanoparticles using Lactobacillus spp. showed agreement with the literature report, XRD analysis of ZnO nanoparticles synthesized using Lactobacillus plantarum reveals that the nanoparticles synthesized were pure and crystalline in nature with hexagonal phase were formed and The SEM images of the ZnO nanoparticles from L. plant arum have spherical nanoparticles clusters [18].

XRD analyses indicated that ZnO nanoparticles synthesized by Lactobacillus sporogens have a hexagonal unit cell and formation of crystalline ZnO nanoparticle [8, 20] about the results obtained in current study, Lactobacillus spp.

Had the ability to formation ZnO nanoparticles, this bacteria possession of negative electrokinetic potential which attracts the cations and triggers the synthesis of nanoparticles. The grow capacity of Lactobacilli in the oxygen presence makes it more capable of metabolically process [18].

The media composition plays a main role in the metallic and/or oxide nanoparticles biosynthesis.

The factors affect on the biosynthesis of ZnO nanoparticles in the presence of Lactobacillus strain may be included the rH2 control by energy yielding material suitable carbohydrate , oxidation-reduction potential (Eh) of the culture medium and the ionic state of the culture medium pH[8].

**Antibacterial Activity of Synthesized ZnO Nanoparticles**

Antibacterial effect of ZnO nanoparticles was determined on the basis of the MIC for each pathogenic bacteria isolates.

The MIC of ZnO nanoparticles synthesized by Lb.gasseri was found to be 12.5mg/ml against A. baumannii and S. aureus isolates, the MIC against K. pneumoniae isolates was between (12.5–25) mg/ml while in P.aeruginosa was 50mg/ml (Table2). The best effect of ZnO nanoparticles synthesized by L b. salivarius has been shown against A. baumannii with MIC recorded at 12.5mg/ml, but the lowest effect has been recorded on P. aeruginosa (50mg/ml) (Table 3).This ZnO nanoparticle synthesized by Lactobacillus isolates had activity against all the tested pathogenic bacteria at various degrees, and A. baumannii was more sensitive compared with other bacterial under study. Zinc oxide nanoparticles synthesized by Lb.sporogenes inhibited the growth of Staphylococcus aureus [19].

Another study showed the antibacterial properties of different nanoparticles biosynthesized using Lactobacillus spp., Salman [10] observed a good antibacterial effect of Ag nanoparticles against MRSA S. aureus. Ibrahim et al.[21] showed that TiO2 nanoparticles synthesized by Lb. crispatus had an inhibitory effect against recurrent UTI causative bacteria. Antibacterial effect of nanoparticles is due to their surface area [22]. The positive charge of metal oxides and the negative charge of microorganism causing electromagnetic attraction between the metal oxides and microorganism surface, leads to oxidization and death [23].

ZnO nanoparticles inhibit a wide range of pathogenic bacteria growth [24], cell membrane destruction; the ZnO nanoparticles probably remain adsorbed on the dead bacterial surface. ZnO nanoparticles continue to release peroxides into the surrounding medium, after that all surface of a dead bacteria covered by ZnO nanoparticle, and showing bactericidal activity [25-26].

### Table 2: MIC (mg/ml) of zinc oxide nanoparticle (ZnO NPs) synthesized by Lactobacillus gasseri

<table>
<thead>
<tr>
<th>Pathogenic bacterial isolates</th>
<th>MIC(mg/ml)</th>
<th>Pathogenic bacterial isolates</th>
<th>MIC(mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae (K)</td>
<td>12.5</td>
<td>Acinetobacter baumannii (A)</td>
<td>12.5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (K)</td>
<td>25</td>
<td>Acinetobacter baumannii (A)</td>
<td>12.5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (P3)</td>
<td>50</td>
<td>Staphylococcus aureus (S)</td>
<td>12.5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (P11)</td>
<td>50</td>
<td>Staphylococcus aureus (S)</td>
<td>12.5</td>
</tr>
</tbody>
</table>

### Table 3: MIC (mg/ml) of zinc oxide nanoparticle (ZnO NPs) synthesized by Lactobacillus salivarius

<table>
<thead>
<tr>
<th>Pathogenic bacterial isolates</th>
<th>MIC(mg/ml)</th>
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<th>MIC(mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae (K)</td>
<td>25</td>
<td>Acinetobacter baumannii (A)</td>
<td>12.5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (K)</td>
<td>25</td>
<td>Acinetobacter baumannii (A)</td>
<td>12.5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (P3)</td>
<td>50</td>
<td>Staphylococcus aureus (S)</td>
<td>25</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (P11)</td>
<td>50</td>
<td>Staphylococcus aureus (S)</td>
<td>25</td>
</tr>
</tbody>
</table>
Conclusion

The present study focuses on the rapid and eco-friendly method of ZnO nanoparticles synthesis by Iraqi Lactobacillus isolates. Synthesized and characterized pure ZnO nanoparticles in the range (30.3-38) nm had the antibacterial activity against pathogenic bacteria isolated from the skin infections.

Acknowledgement

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References


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