Original Article

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Biosynthesis of Iron Nano-Particles by Bacillus Megaterium and Its Anti-Bacterial Properties

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ABSTRACT

BACKGROUND AND OBJECTIVE: According to the environmental pollution caused by chemical and physical methods of synthesis of nanoparticles and their incompatibility in medical, the aim of this study is biosynthesis of iron nano particles and its antibacterial activity.

METHODS: In this experimental study, Bacillus megaterium PTCC1656 was cultured in nutrient broth medium, Then bacterial suspension was combined at a ratio of 1:1 with 0.1 M iron nitrate.Synthesis of iron nanoparticles confirmed with Uv-vis, XRD and SEM techniques. Antibacterial properties of nanoparticles were evaluated by disk diffusion method and dilution tube MIC (Minimum inhibitory concentration) on standard strains of Staphylococcus aureus PTCC 1431, Bacillus cereus 1015 PTCC and Escherichia coli PTCC 1399, pseudomonas aeruginosa PTCC 1571.

FINDINGS: The peak of absorbtion for iron nanoparticle was 220 nm. Iron nanoparticles was cuboid shape. The average size of the nanoparticles was 40-60 nm. MIC was measured for Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa 0.031, 0.031, 0.0078, 0.015 mg/ml respectively.

CONCLUSION: Results showed iron nanoparticle have antibacterial effect on pathogenic bacteria. The highest inhibition zone diameter by biological iron nitrate nanoparticles was observed for E. coli and the minimum diameter of inhibition was observed for Bacillus cereus.

KEY WORDS: *Bacillus Megaterium, Anti-Bacterial Activity, Iron Nanoparticles.*

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Introduction

Nanotechnology is one of the most important emerging sciences in the 21st century. Research and development in this field has grown rapidly around the world. One of the important products of this area is metal nanoparticles and is defined in size less than 100 nm. Metallic nanoparticles have been considered for their unique optical properties, catalytic properties, electrical and magnetic applications (1). Nanotechnology has influenced various sciences and has provided many products that have entered the field of biology and medicine, and have had many effects in this regard, including the reduction of the side effects of chemotherapy drugs (2).

Nanotechnology has also entered into action to address the underlying problem in microbiology, the counteracting of antibiotic resistant microorganisms. Today, the antimicrobial properties of silver nanoparticles have been proven and presented commercially (3-8). Among Different methods of biosynthesis of nanoparticles, the use of bacteria is of particular importance. Gold and silver are among the most important metals that most of articles have been published regarding the biosynthesis of these two ions (9). One of these is the biosynthesis of silver nanoparticles by the Yarrow's herbal medicine, a very nature-friendly method (10).

Another well-known research is the use of fusarium oxysporum in the synthesis of these nanoparticles (11). In the latest research on the synthesis of gold nanoparticles, the cultivation of bacteria Bacillus subtilis was used to revive trivalent gold ions from a chloride solution to nanoparticles of 5 to 25 nm in the form of quadrilateral pyramids, at ambient temperature and pressure (12). Nanotechnology services are not limited to the gold and silver nanoparticles. The entry of iron oxide nanoparticles into biotechnology and medical sciences has led researchers to make research on this issue, with the FDA only using nanoscale particles of iron oxide coated with dextran for medical and medical purposes (13). Also, iron oxide nanoparticles were more attractive to researchers (15, 14) due to their proper magnetic properties, low toxicity, high biocompatibility, and relative ease of synthesis compared to other nanoparticles. These compounds also have antimicrobial properties and there are numerous reports of the antimicrobial effects of these nanoparticles against gram-negative and gram-positive bacteria and fungi (16-18). A number of physical, chemical and biological methods have been used to develop metal nanoparticles (19). Chemical methods used to synthesize nanoparticles usually result in environmental contamination rather than the presence of a number of toxic reactants and the non-use of nanoparticles in biological applications, as well as chemical and physical methods are very expensive and hazardous and may there is an inappropriate effect on medical use. Thus, the synthesis of nanoparticles by using biological methods can help to solve this problem (20, 1). Therefore, the aim of this study is to biosynthesize iron nanoparticles with Bacillus megatrium and to study the antimicrobial activity of iron nanoparticles against gram-negative and gram-positive bacteria.

Methods

Materials and devices: In this study, all used microbial culture media were prepared by the Merck-Germany Company, and all used standard bacterial strains were prepared by the Center for the collection of fungi and industrial and infectious bacteria of Iran's scientific and research organization and synthesis confirmation tests of nanoparticles including SEM, Uv vis and XRD were performed at Imam Hossein University's research center.

Synthesis of iron nanoparticles: In this experimental study, Bacillus megaterium (PTCC1656) was cultured in a fresh broth medium and incubated for 32 hours at 24 ° C. After assuring the growth of the bacteria, the bacterial suspensions were combined with 0.1 molar iron nitrate solution at a ratio of 1: 1, and the nanoparticle synthesize was done at room temperature over a period of 20 minutes. A suspension containing bacteria and iron nanoparticles was first sterilized from Whatman filter paper and then sterilized using a microbiological filter of 0.22 microns.

Characterization of iron nanoparticles

Uv-vis: Absorption spectra of synthesized nanoparticles was done by a Uv-vis spectrophotometer (SERIES8000CECIL). The absorption of iron nanoparticles in the wavelength range of 200-700 nm was investigated.

XRD: The solution containing the synthesized nanoparticles was centrifuged three times for 20 minutes with round (12000 rpm), then the supernatant solution was discarded and the obtained precipitate was stored in the oven for drying. Finally, dried powder of iron nanoparticles was investigated for X-ray powder diffraction analysis of crystalline nanoparticles.

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SEM: Using the SEM device, model of the HITACHI S-4500, the shape and size of the synthesized iron nanoparticles were investigated. So that 15 microliter of iron nanoparticles were sprayed onto the SEM electron microscope gradients and after drying, they were examined by an electron microscope.

Determination of the Antibacterial Properties

Disk diffusion method: in the investigation of anti-bacterial properties, standard strains of Staphylococcus aureus 1431 PTCC, bacillus cereus 1015 PTCC as gram-positive bacteria and Escherichia coli 1399 PTCC, Pseudomonas aeruginosa 1571PTCC as gram-negative bacteria were used. 100 µl of each bacterium with a concentration of 0.5 McFarland was cultured on the Muller Hinton Agar (Merck, Germany) medium. In this method, for each 4 bacteria, 4 plates containing 4 discs were considered as follows:

A 6 mm diameter sterile disc was dipped with 6 µL of 0.1 molar iron nitrate solution to evaluate the effect of iron, a sterile disk dipped with 6 µl of a suspension containing synthesized nanoparticles by Bacillus megatrium, a sterile disk dipped with 6 µl iron nanoparticle and a sterile disk dipped with 6 µL of Bacillus megatrium bacterial suspension as control were used. Then they were placed in optimum temperature for bacterial growth for 24 hours. After 24 hours, the diameter of the inhibition zone was measured and recorded as antimicrobial properties of the samples based on millimeters. To avoid the possibility of error in all stages of operation, these tests were repeated 3 times and the results were compared with each other.

MIC tube dilution method: In the tube dilution method, the minimum inhibitory concentration of bacterial growth (MIC) in a culture medium (Tryptic Soy Broth = TSB) was performed in four series of 10 sets of tubes containing 1 cc sterile TSB medium, 1 cc nanoparticle solution of iron nitrate was added to the first tube. After shaking, 1 ml of the first tube was added into the second tube, then 1 ml of the second tube was added to the third tube, and then up to the last tube, and finally 1 ml of the last tube was poured out. All tubes were added 0.1 ml of active bacterial suspension, which was standardized in accordance with the standardized semi-MacFarlend tube. Next to each serial, a tube containing bacteria and culture media was used for positive control and a tube containing culture medium and a solution of iron nitrate nanoparticles for control. All tubes were placed in a suitable temperature incubator for 24 hours. After 24 hours, due to the opacity in the tubes, the MIC was determined (21).

Results

Spectrophotometer (UV-Vis): In the analysis of iron nanoparticles with Uv-vis spectrophotometry, absorption peak, b was observed at 220 nm wavelength, indicating the presence of iron nanoparticles in the reaction solution (Fig 1). According to valid sources, the highest absorption peak of iron nanoparticles is at a wavelength of 200 to 300 nm (22).

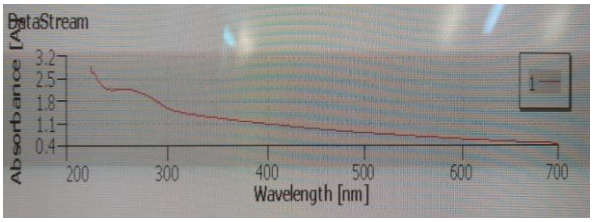


Figure 1. A: Uv-vis absorption spectrum of iron nanoparticles

X-ray diffraction (XRD): In the study, XRD analysis was performed to prove the formation of iron nanoparticles. Based on the obtained results of x-ray editing at the angle θ 2 and in degrees with a range of 10-60, 5 couriers were shown at degrees 4, 30, 48/35, 53/54, 59/54, 83/56 which were related to the levels of 200, 311, 400, 422, and 511, respectively, which were consistent with the X-ray diffraction pattern (Fe3O4) and no other phase was observed (Fig 2).

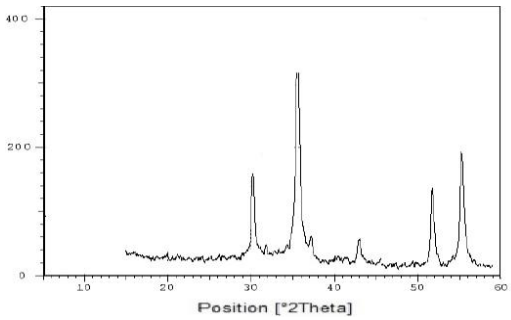


Figure 2. XRD pattern of Fe3O4 nanoparticles

Scanning electron microscope (SEM): Scanning electron microscope (SEM) images, synthesized iron nanoparticles are shown in Figure 3. This is used to confirm the morphology and size of the nanoparticles of iron. According to SEM nanoscale images, iron particles have a cubic shape and the size of nanoparticles is at a magnification of 200 nm between

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40 and 60 nanometers, which is within the size range of nanoparticles.

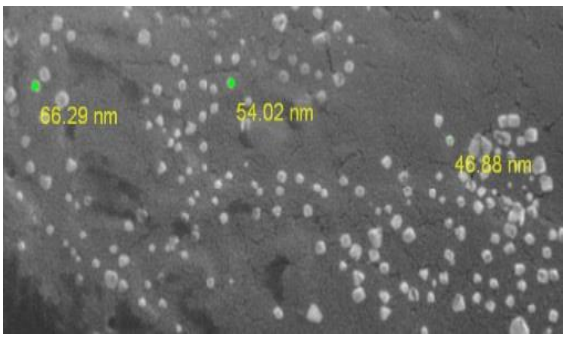


Figure 3. SEM images of cube nanoparticles of iron

Disc diffusion: After the synthesis of nanoparticles and their structural analysis, antimicrobial activity of iron nanoparticles was investigated.

The diameter of the inhibition zone was measured disc diffusion method for 4 strains of Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa. Based on these results, the most antimicrobial activity of the biological nanoparticle of iron was measured on E. coli with a diameter of 23 mm inhibition zone and the lowest antimicrobial property of the biological nanoparticle of iron was measured in Bacillus cereus with a diameter of 14 mm inhibition zone (table 1).

Also, in the case of Bacillus cereus, chemical nanoparticles exhibited more antibacterial properties than biological nanoparticles. But in the case of E. coli bacteria, the antibacterial properties of biological and chemical nanoparticles were equal and the diameter of the inhibition zone was 23 mm in both cases (Fig 4).

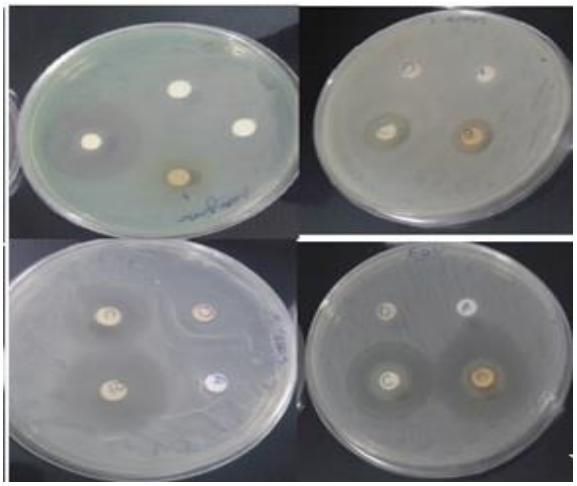


Figure 4. Inhibition zone of bacteria against nanoparticles of iron

Table 1. Antibacterial test results (mean diameter of the bacteria growth inhibition zone)

Bacterium	Bacillus megaterium (mm)	Chemical nanoparticle (mm)	Iron nanoparticle (mm)	Iron nitrate (mm)
B.cereus	0	26	14	0
p.aeruginosa	0	0	20	0
S. aureus	0	13	15	0
E.coli	0	23	23	0

(MIC=Minimum inhibitory concentration): Minimum inhibitory concentration: The minimum MIC value was obtained by biological nanoparticles of iron for E. coli, equal to chemical nanoparticle with amount of 0.0078 mg / ml, and the highest MIC value was obtained by biological nanoparticles of iron in the case of Staphylococcus aureus and Bacillus cereus with amount of 0.31 mg / ml. In addition, the amount of MIC obtained from the iron nanoparticles on pseudomonas aeruginosa is equal to zero, which corresponds exactly to the results of the diffusion test (table 2).

Table 2. The results of MIC for 4 strains of pathogenic bacteria

Concentration Bacterium	Biological iron nanoparticle mg/ml	Chemical iron nanoparticle mg/ml
B.cereus	0.031	0.0039
P. aeruginosa	0.015	0
S.aureus	0.031	0.031
E.coli	0.0078	0.0078

Discussion

The study found that Bacillus megatrium has the ability to synthesize iron nanoparticles, and these bioactive nanoparticles have antibacterial activity. Silver nanoparticles are also synthesized through microorganisms such as fungi, bacteria or microorganisms such as plants, algae, etc. In 2010, the synthesis of nanoparticles of silver was performed using cyanobacter, spirulina platensis and actinobacter Streptomyces spp. 211A and results showed the synthesis of silver nanoparticles in the range of 7-16 nm (23).

However, in the present study, the size of bio-synthesized nanoparticles were in the range of 40-60 nm that seems the type of microorganisms as well as the type of primary material in this field are effective. In a study by Natarajan and colleagues, the microbial synthesis of silver nanoparticles was carried out using

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Escherichia coli. The synthesis of silver nanoparticles was confirmed with a uv test at 400 nm (24). In addition, in a study by Rahimi et al., the synthesis of silver nanoparticles using three species of Persian Gulf algae was investigated. The results showed that all three species of algae were capable of synthesizing silver nanoparticles, (25) which confirmed the ability of bacteria and algae to synthesize metal nanoparticles. The results of the study by Pantidos et al. demonstrated that green methods of nanoparticles synthesis require less energy and lower costs, as well as are environmentally friendly green processes (26). In studies conducted by Pattanayak et al., and Eftekhari et al., the absorption peak of the synthesized iron nanoparticles was 220 nm (28, 27) which was consistent with the current research.

In another study by Awwad et al., the synthesis of iron nanoparticles was done using a coconut leaf extract, the absorption peak of the nanoparticles was 233 nm and a particle size of 8-5 nm was reported (29), which in terms of absorption peak is consistent with our study, but the size of the nanoparticles is different, which is certainly related to the construction technique using the plant extract. In study of Behera et al., iron oxide nanoparticles were made by chemical methods and their antimicrobial properties were measured, so that nanoparticles with an irregular size of 10-20 nm were made and the most antimicrobial property of these nanoparticles was on Bacillus ligniform formis and did not show any antimicrobial activity on two bacteria Pseudomonas aeruginosa and Shigella Flexneri, while the obtained nanoparticles in our study were more regular (40-60 nm) (30), and also in our study, the chemical nanoparticles had no antibacterial effect on Pseudomonas aeruginosa that was compatible with the mentioned investigation, but on the other hand, the nanoparticles synthesized by biological method had the antibacterial properties against Pseudomonas aeruginosa. Therefore, it can be concluded that iron nanoparticles have a significant antibacterial effect compared to iron chemical nanoparticles against Pseudomonas aeruginosa. In the present study, the antibacterial properties of the synthesized iron nanoparticles against E. coli bacteria were higher than other bacteria, but the results of a study by Mohamed et al. showed that iron nanoparticles on Bacillus subtilis had more antibacterial properties than E. Coli, S.aureus, and P. aeruginosa, while nanoparticles produced by Azzam et al. in 2015 were synthesized using Alternaria fungus and also the size of nanoparticles made in the range of 3 to 9 nanometers which is different from the present study (31).

In this study, the synthesis of iron nanoparticles was performed using Bacillus megatrium bacteria. Absorption peak of iron nanoparticles was observed at 220 nm wavelength, the synthesized iron nanoparticles had cube shape. The average size of iron nanoparticles was 60-40 nm. In this study, using XRD test, the presence and synthesis of iron nanocrystals by bacteria was ensured. All samples conformed to the X-ray diffraction pattern (Fe3O4) and no other phase was observed (33, 32).

In this study, Bacillus cereus chemical nanoparticles showed more antibacterial properties than biological iron nanoparticles, but in the case of E. coli, the antibacterial properties of biological and chemical nanoparticles were equal. In the case of Staphylococcus aureus and Pseudomonas aeruginosa, the antibacterial properties of biological nanoparticles were higher than chemical nanoparticles.

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