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Cytoplasmic Extract of the Bacteria *Lactobacillus Fermentum* for the Biosynthesis of Iron Oxide Nanoparticles

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ABSTRACT

The manufacturing of nanoparticles has occupied much of the scientific and technological community's attention during the last decade. Nanoparticle synthesis may be accomplished in a number of ways, however these methods are inefficient and expensive. Consequently, biologic methods for producing nanoparticles are required. Nanoparticles are manufactured using a variety of biological structures, including plants, algae, and microbes including bacteria, string molds, and yeasts. The aim of this research is to employ green chemistry as a basis for the manufacture of iron oxide nanoparticles using cytoplasmic extracts of the probiotic microbe *Lactobacillus Fermentum*. We incubated the bacterial cytoplasmic extract of *Lactobacillus Fermentum* for three weeks at 37 °C with 5% carbon dioxide after preparing it using the freeze-thaw technique. We then added an equal volume ratio of iron sulfate solution (III) at a concentration of 10⁻³ M. The use of X-ray diffraction (XRD) and transmission electron microscopy (TEM) allowed for the investigation of nanoparticle production. As soon as iron sulfate nanoparticles begin to form, the solution's color will change to black. The XRD examination demonstrated that the cytoplasmic extract of *Lactobacillus Fermentum* could generate nano crystals of iron oxide. According to the results of the transmission electron microscopy (TEM) analysis, the typical size of the nanoparticles was 10-15 nm, and they had a spherical form. When it comes to producing iron oxide nanoparticles, there is an efficient biological process that is both cost-effective and ecologically benign. This method involves using *Lactobacillus fermentum* cytoplasmic extract.

Keywords:

Iron oxide nanoparticles *Lactobacillus Fermentum* Biosynthesis Probiotic

Introduction

Various metal nanoparticles are the subject of much study in nanotechnology. Researchers are particularly interested in iron oxide nanoparticles with diameters less than 20 nm because of their unique combination of desirable magnetic characteristics and very low toxicity compared to other types of nanoparticles. There are several methods for producing nanoparticles, however they all have prohibitive costs, high energy requirements, and harmful environmental impacts. Thus, there is an ever-increasing need to manufacture nano-sized particles according to green chemistry principles. To do this, nanoparticles are produced using a wide range of biological structures, including plants, algae, bacteria, molds, yeasts, and filamentous molds. 1 Due to their diverse metabolic profiles, high substrate specificity, rapid growth rate, and ability to withstand extreme environments, microorganisms are ideal candidates for use as biocatalysts in two-phase systems containing organic solvents and water, as well as the primary employees in environmentally friendly nanofactories that manufacture and assemble nano-sized Things that have metal in them are very important. 2-3 Small factories employing microorganisms in the microbial approach to nanomaterial production can synthesize metal nanoparticles (ranging in size from 1 to 100 nm) at room temperature or temperatures higher than thermophiles, using their inexpensive and renewable reducing agents, such as Laktatyaastat. Microbes may reduce metals in two ways: within cells and outside of them. Ions' reduction positions and

Therefore, the kind of microbes and enzymes used in the reduction process, whether they are located within or outside of the cell, dictate nanoparticle creation. 4 Consistent with green synthesis goals, this study synthesized iron oxide nanoparticles using a cytoplasmic extract of *Lactobacillus fermentum*.

1. Results and Discussion

After three weeks, the incubated solution's hue changed, which was the first indicator of iron oxide nanoparticle development (Fe₃O₄). The iron sulfate solution went from being colorless to black after the cytoplasmic extract of *Lactobacillus fermentum* reduced the iron ions. XRD results of Fe₃O₄ nanoparticles are shown in Figure 1. According to the Debye-Scherrer technique and the XRD graph, the particles had a size of 15 nanometers. In agreement with the sizes determined using the XRD diagram, TEM images of the sample [Fe₃O₄] (Fig. 2) showed that the nanoparticles had a spherical shape and averaged 10-15 nm in diameter. In biological production, we see the reduction of enzymes, extracellular polysaccharides, and reducing materials, all thanks to various microorganisms that may reduce metal ions. To lessen the harmful effects of metal ions in the bacteria' growing environment, germs produce metal nanoparticles. To do this, they use an enzyme called NADH reductase or nitrate reductase to convert harmful metal ions into less harmful metallic

components. Not to mention that certain polysaccharides are present and

microorganisms inside the cells create organic materials, and culture media lead to the synthesis of metal nanoparticles.

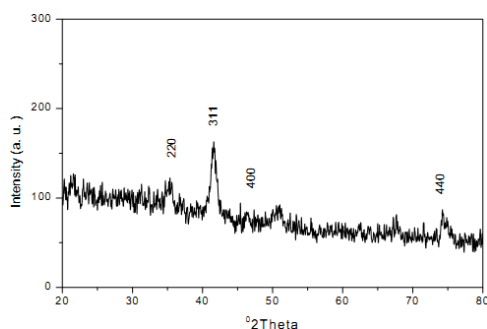


Figure 1. XRD spectrum of iron oxide nanoparticles [Fe_3O_4]: using Debye- Scherrer method, particles sizes were calculated 15-20 nm.

Metal ions are reduced into metal nanoparticles by biological materials using functional groups including cysteine, histidine, aldehydes, and ketones. 7 This terrain has been the subject of several research. Omide and colleagues (2014) performed a research whereby *Lactobacillus Fermantom* supernatant was used to generate silver nanoparticles. These nanoparticles were found to be very tiny, spherical, highly impressionable, and, on average, 15 nanometers in size. 6 Zinc oxide nanoparticles were synthesized by Prasad and Jaha in 2009 using *Lactobacillus sprozhenz*. The produced nanoparticles were 5–15 nm in size and had a hexagonal shape, according to the results. 8 The current investigation shown that the cytoplasmic extract of *Lactobacillus Fermantom* may be effectively used to produce iron oxide nanoparticles. The construction and stabilization of nanoparticles by chemical processes results in hazardous byproducts that are harmful to the environment. Another common side effect is the absorption of harmful compounds from nanoparticle surfaces, which may compromise the efficacy of nanoparticle medications. In contrast, green synthesis is less harmful in addition to being easy, cheap, and cost-effective. 9 Thus, a green, efficient, and successful method for making metal nanoparticles is to synthesize iron oxide nanoparticles using cytoplasmic extracts of *lactobacillus fermentum*. The cytoplasmic extract of *Lactobacillus fermentm* seems to include reduction-oxidation enzymes and other reducing substances, which lead to the reduction of metal ions and the creation of iron oxide nanoparticles.

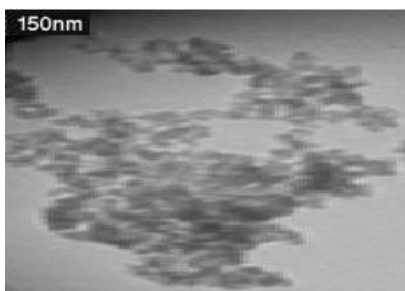


Fig 2. TEM image showed that the nanoparticles had average diameters of 10-15 nm.

The results also indicated that extracellular production of iron oxide nanoparticles is possible using *Lactobacillus fermentum*. Since in the extracellular production, metal ions on the surface of cells are trapped in which there is no need to extract the nanoparticles from inside the cells contrary to inside production. And this has led to the present method; even as compared to the intracellular production of nanoparticles which are also more affordable.¹⁰ In green synthesis, more time is needed to produce iron oxide nanoparticles. It may be due to slow reactions done by enzymes placed in the biological compounds.

2. Conclusion

Green synthesis is recommended as the best replacement for chemical synthesis because of its immense benefits such as environmental compatibility, low cost and toxicity. On the other hand, *Lactobacillus* are from the large family of probiotics, which are safe with no pathogenic effects that can be used for medical goals.

3. Experimental

3.1. Materials and methods

3.1.1. Purchase and cultivation of bacteria *Lactobacillus fermentum*

Lactobacillus fermentum strain with ID (PTCC 1638) was bought from microbial bank of Scientific and Industrial Research Organization of Iran. After moving in a MRS Broth medium was incubated for 24 hours at 37 °C.

3.1.2. Preparing *Lactobacillus fermentum* cytoplasmic extract through freeze thaw method

After 24 incubation, the MRS Broth mediums containing the bacteria were centrifuged at 3000 g for 15 minutes. Then supernatant was discarded and the resulted sediment was added phosphate buffered saline and centrifuged at 3000 g for 10 min [washing step was repeated three times]. Afterwards, for 15 minutes was placed inside the nitrogen tank [liquid nitrogen -196 °C] and then in steam bath [Ben Murray] for 15 minutes [37 °C]. At the end after centrifuging at 12,000 g for 30 minutes, the supernatant was collected as the cytoplasmic extract.⁵

3.1.3. Nanoparticles synthesis by cytoplasmic extract of *Lactobacillus fermentum*

The cytoplasmic extract of *Lactobacillus fermentum* was mixed with aqueous iron sulfate solution at a volume ratio of 10 parts iron sulfate by volume to produce iron oxide nanoparticles. After the pH was adjusted to 6.5, the solution was left to incubate for three weeks at 37 °C with 5% CO_2 . 6 Following this time, a shift in the ambient hue was seen along with the extracellular buildup of metal particles. The creation of iron oxide nanoparticles was indicated by a change in hue from colorless to black. Following this, sterile Falcon tubes were used to transfer the solution, and it was centrifuged at 2500 g for 10 minutes. Following the removal of the supernatant, the sediment was separated by washing it three

times with sterile deionized water and once with acetone. The sediment that was produced was then dried in an oven set at 40 °C for a day before being ground into a powder using a porcelain mortar. A magnet was used to study the magnetic property of iron oxide nanoparticles that were biosynthesized.

References

1. Reference: 1. "Appl. Microbiol. Biotechnol. 2006, 69, 485" by D. Mandal, M.E. Bolander, D. Mukhopadhyay, G. Sarkar, and P. Mukherjee.
Nanomaterials. 2011, 1, X. Li, H. Xu, Z. Chen, G.J. Chen. 3. Annu Rev Microbiol. 1994, 48, 773, S. Hagedorn, B. Kaphammer.
4. [Nanomedicine] by A. Nanda and M. Saravanan (2009), 5, 452.
Journal of Chemical Engineering, 2010, 17, 1836, by Z. Wang, L. Zhang, and T. Tan.
In BEPLS. 2014, 3, 186, B. Omid, S.J. Hashemi, M. Bayat, and K. Larijani wrote the article.
7. Invest. 2010, 19, 18 (K. Mollazadeh-Moghadda, J. Young, eds.).
129 (K. Prasad and A.K. Jha, 2009, in Natural Science).
H.L. Kashani, "Journal of Applied Biochemistry" (2012), 3, 7.
10. Article published in Bioimpacts in 2011 by M. Mohseniazar, M. Barin, H. Zarredar, S. Alizadeh, and D. Shanchbandi, page 149.