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GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES BY CYTOPLASMIC EXTRACT OF *LACTOBACILLUS ACIDOPHILUS* FOR BIOMEDICAL APPLICATIONS

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ABSTRACT
The past decade mainly focused on nanotechnology and the uses of nanoparticles to improve human life. Chemical and physical syntheses of nanoparticles involve use of hazardous chemicals or high temperatures, which make the process very costly. On the other hand, biological synthesis of nanoparticles is a cost-effective and environmentally friendly method. In the present work, iron oxide nanoparticles were synthesized using Lactobacillus acidophilus which is one of the most common types of probiotics. It can be found in fermented foods, yogurt and supplements. The cytoplasmic extract of Lactobacillus acidophilus was prepared by free-throw method. Iron chloride solution was added and incubated for 3 weeks at 37°C in the presence of 5% carbon dioxide. The change in color of solution to black indicates formation of iron oxide nanoparticles. The synthesized nanoparticles were characterized by XRD analysis for phase confirmation. XRD shows that the crystallite size of particles was of size about 25 nm. M-H curve of synthesized nanoparticles show superparamagnetic nature. Lactobacillus acidophilus can be used for biosynthesis of iron oxide nanoparticles for biomedical applications owing to their superparamagnetic property and biocompatibility.
Keywords: *Iron oxide nanoparticles, Lactobacillus acidophilus, Biosynthesis, Probiotic, Biomedical applications.*

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INTRODUCTION

Nanotechnology is a promising and widely thriving branch of science that has received major success in the era of current technology. Nanoparticles are materials that show distinctiveness in size (1-100 nm), structure, and mechanical, physio-chemical, catalytic, magnetic, electric, thermal, optical scattering properties and shape [1]. The characteristics and reactivity of nanoparticles is principally defined by their ultra-small size and a large surface area [2]. Out of these, one of the greatest biocompatible nanoparticles are iron oxides as they have magnificent characteristics like superparamagnetism, colloidal stability, long blood half-lives, low susceptibility to oxidation and flexible surface chemistry. They have a wide range of applications in environmental regulation (like adsorption of dyes, antibiotic degradation etc.), food related processes, biomedical (magnetic cell sorting, drug delivery, magnetic particle imaging, magnetic resonance imaging, tissue engineering, immunoassays, bioengineering, stem cell tracking, hyperthermia therapy of cancer, bio sensing and cosmetics along with antimicrobial activity against various pathogens like bacteria, fungi and ROS [3, 4]. Preparation of nanoparticles can be done by various ways in terms of cost, environmental pollution and energy consumption which are not affordable. Hence, the importance of green chemistry to produce nanoparticles is growing day by day. For this purpose, various plants, algae and microorganisms are used to prepare nanoparticles.

At the present study, iron oxide nanoparticles were successfully synthesized by cytoplasmic extract of *Lactobacillus acidophilus* without using any hazardous chemical. It is an efficient, cost-effective and ecofriendly way for producing metal nanoparticles. It seems that the reduction-oxidation enzymes and other reducing compounds in the cytoplasmic extract of *Lactobacillus acidophilus* cause the metal ion reduction and production of iron oxide nanoparticles. The synthesized nanoparticles were further subjected to study their usefulness for biomedical applications.

MATERIAL AND METHODS

a) Preparing *Lactobacillus acidophilus* cytoplasmic extract

Lactobacillus acidophilus was grown in a MRS Broth medium was incubated for 24 hr at 37°C. After incubation, the MRS Broth medium containing the bacteria was centrifuged for 15 minutes at 3000 rpm. Then supernatant was discarded and the sediment taken. Phosphate buffered saline was added in the sediment and centrifuged for 10 min at 3000 rpm. The washing step was repeated three times. Then it was placed inside the nitrogen tank (liquid nitrogen -196°C) for 15 minutes and further in steam bath for 15 minutes (37°C). At the last, after centrifugation for 30 minutes at 12000 rpm, the supernatant was collected as the cytoplasmic extract of *Lactobacillus acidophilus*.

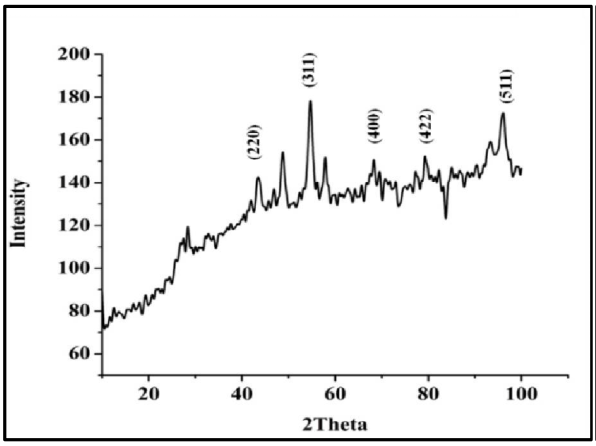
b) Nanoparticles synthesis from cytoplasmic extract of *Lactobacillus acidophilus*

For synthesis of iron oxide nanoparticles, aqueous iron chloride (III) solution [10^{-3}M] was added to the *Lactobacillus acidophilus* cytoplasmic extract in 1:10 (v/v). The pH was adjusted to 5. Then the solution was incubated for 3 weeks at 37°C in the darkness and in presence of 5% carbon dioxide. After incubation, extracellular accumulation of metal nanoparticles with ambient color change was observed by change in color from colorless to black. After the color change, the solution was poured into sterile Falcon tubes for centrifugation for 10 minutes at 2500 rpm. The supernatant was discarded and the sediment was washed thrice with sterile deionized water and once with acetone. The sediment was then dried in oven for 24 hr at 40°C. It was then powdered using a porcelain mortar. The magnetic property of biosynthesized iron oxide nanoparticles was observed with a magnet. XRD analysis was done for phase confirmation and crystallite size determination. Magnetic studies of nanoparticles were done using Vibrating Sample Magnetometer to obtain M-H curve.

RESULTS AND DISCUSSIONS

Fig. 1 shows the powder XRD pattern for Fe_3O_4 nanoparticles. The main characteristic peaks were obtained with the (hkl) values of (220), (311), (400), (422) and (511). These peaks were matched with the JCPDS file number 82-1533, which corresponds to Fe_3O_4 phase. The nanoparticles show inverse spinel structure. The crystallite size of nanoparticles found to be 25nm, which was calculated from FWHM of the most intense peaks using the De-bye–Scherrer formula.

Magnetic properties of the nanoparticles were studied using their M–H curves. M–H curve of nanoparticles at 300K is shown in Fig. 2. The Saturation Magnetization (Ms), Coercivity (Ce) and Remenance (Mr) values were negligible for the nanoparticles as seen in the M–H curves. The graph clearly shows superparamagnetic nature of nanoparticles at 300K as Ce and Mr values were negligible. Superparamagnetic behavior of nanoparticles at room temperature is very useful in *in vivo* applications as they do not retain magnetization before and after exposure to an external magnetic field, reducing the probability of particle aggregation due to magnetic dipole attraction [5].



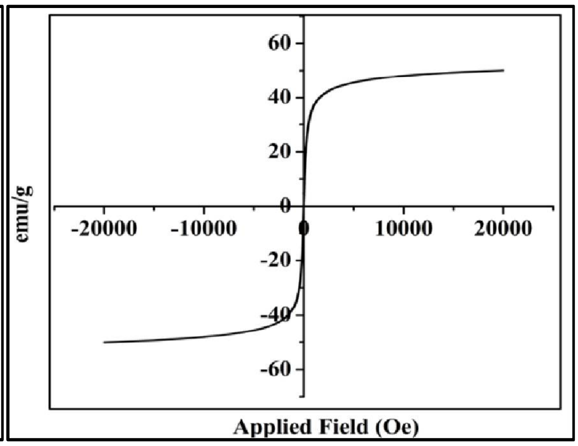


Fig. 1: XRD patterns obtained from biosynthesized Fe_3O_4 nanoparticles.

Fig. 2: M-H curve obtained from biosynthesized Fe_3O_4 nanoparticles

CONCLUSION

The cytoplasmic extract of *Lactobacillus acidophilus* can be used to synthesize magnetic iron oxide nanoparticles when incubated with iron chloride (III) solution. The synthesized nanoparticles showed superparamagnetic nature owing to negligible coercivity and remanence values. The nanoparticles showed crystallite size about 25 nm with inverse spinel structure as confirmed by from XRD studies.

CONFLICT OF INTEREST
Nil

ETHICS OF HUMAN AND ANIMAL EXPERIMENTATION
The Authors ensure that the study does not involve any type of experiments on humans or animals.

AUTHOR'S CONTRIBUTION
Dr. Prajakta B. Shete did all the laboratory work in the present study and prepared the manuscript. Dr. Girish R. Pathade made the proof reading of the manuscript.

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