Proceedings of the 10th World Congress on Recent Advances in Nanotechnology (RAN 2025)
Barcelona, Spain - April, 2025
Paper No. ICNNFC 110
DOI: 10.11159/icnnfc25.110

Optimization of Iron Nanoparticle Biosynthesis Using Bacterial Isolates from Natural Environments

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Iron nanoparticles (FeNPs) have a wide range of applications in various industries due to their unique properties, such as high surface area, superparamagnetism, and favorable surface-to-volume ratio [1]. Key applications include the electromagnetic industry (battery development), bioremediation, wastewater treatment, pharmaceuticals (as vesicles for targeted drug delivery), and nanomedicine for diagnostics like magnetic resonance imaging [2]. However, conventional physical and chemical synthesis methods are expensive, especially for biological applications requiring special biocompatible coatings [3]. These methods also consume significant energy and use toxic reagents, posing environmental risks [4]. Therefore, biological synthesis using bacteria that reduce iron extracellularly or intracellularly presents a sustainable alternative. This method aligns with global sustainability goals, as it relies on bacterial metabolic reactions. It is also simple and economical, given the widespread availability of bacteria in various environments, the ease of laboratory cultivation, and their short generation time. The FeNPs have an inherent biocompatible coating and reduced toxicity [5]. However, two major challenges hindering the industrial success of this approach are inconsistent size uniformity and low yield compared to conventional methods [6].

The main objective of our ongoing study is to optimize iron bionanoparticles production using bacteria from natural environments by comparing different conditions for FeNP synthesis. We aim to isolate and identify iron-reducing and magnetotactic bacteria, test various synthesis conditions (effects of strain selection, iron salt type and concentration, pH, and temperature), and isolate, purify, and characterize FeNPs using physical methods (UV-Vis, FTIR, SEM). The goal is to develop a protocol with yields and uniformity comparable to physicochemical methods at an industrial scale.

Here, we present results from the first step of optimization, focusing on the isolation and selection of bacteria that demonstrated intracellular and extracellular synthesis of FeNPs. Bacteria were isolated from samples collected from seven locations in Serbia, including river waters and mud, rusty surfaces, rocks, soil, and mining sites. Pure strains were identified via 16S rRNA sequencing and tested for FeNP synthesis.

A total of 62 isolates were obtained. Four isolates (two *Stenotrophomonas* sp. and two *Bacillus megaterium*) exhibited magnetosomes and synthesized intracellular magnetite nanoparticles, confirmed by movement towards a magnetic field under a microscope. Extracellular synthesis was observed in 36 isolates (22 from $FeCl_3 \times 6\ H_2O$ and 14 from $FeCl_2 \times 4\ H_2O$) from 11 genera, based on yellow-orange precipitates indicating iron reduction. Notably, two *B. megaterium* isolates exhibited both intracellular and extracellular synthesis, while eight isolates synthesized iron from both salts. The most common genera in FeNP synthesis were *Bacillus* and *Pseudomonas*.

This part of the study confirmed extracellular FeNP synthesis by known nanoparticle-producing genera, such as *Enterobacter* sp., *Achromobacter* sp., and *Stenotrophomonas* sp. It also demonstrated FeNP synthesis by strains from genera previously known for silver and gold nanoparticle synthesis, including *Lysinibacillus* sp., *Geobacillus* sp., *Serratia* sp., and *Arthrobacter* sp. Additionally, FeNP synthesis was observed in *Buttiauxella* sp. and *Pseudoarthrobacter* sp., for which nanoparticle synthesis had not been previously studied, opening new research opportunities.

In conclusion, this study presents a first step toward optimizing bacterial FeNP synthesis by selecting strains for both intracellular and extracellular production. The potential for FeNP synthesis in these bacteria is promising, especially for sustainable nanotechnology development.

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