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Isolation of genomic DNA from *Escherichia coli K12* strain by using Iron Oxide Nanoparticles

Ketkar Ketaki[1], Dr. Vora Dipak[2], Meghshree Deshmukh[3]
Department of Microbiology, Ramnarain Ruia College, Matunga, India

Abstract: *The nanoparticles which are synthesized by thermal reduction method are shelled having the core of Fe surrounded by Fe_2O_3. The synthesized nanoparticles are embedded inside as well as on the surface of activated carbon matrix. The nanoparticles are subjected to characterization using SEM and XRD. The particle size was found to be around 60-100nm. A very simple and inexpensive method for extracting bacterial genomic DNA was developed. A comparison was also made using conventional phenol-chloroform method and commercially available DNA extraction kit to check for the amount of DNA obtained. The developed method using nanoparticles took less time to extract DNA when compared to phenol-chloroform and kit method.*

Key Words: *Bacterial genomic DNA, Escherichia coli K12, Extraction of Genomic DNA, Iron oxide nanoparticles SEM.*

I. Introduction

Magnetic nanoparticles have immense potential in biomedical sciences and in engineering field. Magnetic nanoparticles can exist in stable colloidal suspension that will not aggregate allowing for their uniform distribution in a reaction mixture. Due to their ultrafine structure, magnetic properties and biocompatibility, iron oxide nanoparticles hold promise as contrast enhancing agents for MRI, targeted drug delivery, gene therapy, stem cell tracking, magnetic separation of bacteria[1] and isolation of genomic DNA.[2] Isolation of DNA is a prerequisite step for numerous molecular biology techniques. Methods for extraction of genomic DNA rely on the use of phenol-chloroform but the process is time consuming, tedious and utilizes toxic organic solvents [3], further it is inconvenient and impractical when dealing with either small amounts of DNA or numerous samples. [4]

II. Materials And Methods

2.1 Preparation of magnetic nanoparticles

Iron oxide nanoparticles were synthesized on the surface of activated carbon (AC) by the thermal reduction route. The precursor used for the synthesis of Fe was a modification of the process described by Sunkara B, Zhan J. [5] One gram of AC was added to an aqueous solution of 0.0369 M $FeCl_3.6H_2O$ in a beaker. The mixture was stirred for 12 h to impregnate iron (Fe^{3+}) species inside the pores of AC. The supernatant was removed by filtration. Thereafter, the solid material was placed in a quartz boat and heated in a tubular furnace to 850ºC (ramp rate - 3.5ºC/min) for 3 hr, under an atmosphere of nitrogen. The resultant product was removed from the furnace after cooling. [5]

2.2 Characterization of Iron Oxide nanoparticles

Particle morphology and composition was analyzed using a field emission scanning electron microscope (FE-SEM) (JSM-7600F), attached with energy dispersive X-ray (EDX). The SEM images of Activated carbon- Iron oxide Nanoparticles samples were taken at different magnifications as shown in Fig. 1 and 2. X-ray diffraction (XRD) was carried out using a Philips X'Pert Pro diffractometer, with CuKα radiation. The XRD spectrum of iron oxide nanoparticles is shown in Fig.3

2.3 Strains and culture conditions

Escherichia coli K12 was used throughout the study and was cultivated in Luria Bertani broth at 37^0C for 24 hrs under static conditions.

2.4 Isolation of bacterial genomic DNA

Phenol: Chloroform Method as described by A. Sebastianelli. [3]

2.5 Commercial Kit: Nucleo-pore gDNA Fungal Bacterial Mini Kit by Genetix Biotech Asia Pvt. Ltd.

2.6 Extraction by iron oxide nanoparticles

To separate genomic DNA from Escherichia coli K12 strain using Iron Nanoparticles, two ml of E. coli K 12 culture was grown overnight and centrifuged at 10,000 rpm for 10 min. Later the supernatant was

discarded and the pellet was resuspended in 500µl TE buffer. An equal volume of 1 % (w/v) sodium dodecyl sulfate solution was added to each of the samples to attain cell lysis. The solutions were allowed to stand at room temperature for 5 min after mixing by gentle inversion. [6] Binding buffer (1.0 ml) was added and 0.5 µg of iron oxide nanoparticles mixed and incubated for 5 min with intermittent gentle mixing. The FeNp pellets were immobilized using a magnetic stand, the supernatant discarded and the nanoparticles were washed first with 95% ethanol then 70% ethanol. The particles were then resuspended in 30 µl TE buffer and the DNA eluted from the FeNps by incubation at 65ºC. The supernatant was then transferred to a fresh tube by removing the FeNPs with the help of an external magnetic field. Purified DNA was resuspended in 25 µl TE buffer and finally electrophoresed on 1.0% agarose gel.

2.7 Elution of DNA from agarose gel

The extracted genomic DNA was electrophoresed on agarose gel in triplicate using EtBr (1µl). The gel was run and observed under a UV transilluminator. DNA was cut out from the corresponding wells and was resuspended in elution buffer. It was homogenized thoroughly and then this eluted DNA was used for further analysis. [7]

III. Result And Discussion

3.1 Chemical synthesis of iron oxide nanoparticles

Chemical synthesis of iron oxide nanoparticles was carried out by thermal reduction and characterized using SEM and XRD. Iron oxide nanoparticles are found embedded both on the external surface of the AC as well as inside the pores. It was also observed that the Zero Valent Iron (ZVI) particles were mostly in isolated state while a few aggregates were observed. The average ZVI particle size synthesized by the thermal route was in the range of 60-100 nm (Fig. 1, 2). The relative mass percentage of elements was deduced from low magnification SEM images. The percentage of iron was 5.7 % in the AC-ZVI sample. And the peak at 44.9° confirms their presence.

3.2 Comparison of extracted DNA by different methods

DNA extraction is usually affected by factors such as incomplete cell lysis, DNA adsorption to a particular material, co-extraction of enzymatic inhibitors and degradation or damage of DNA. DNA extraction has therefore been highlighted as a limitation of culture-independent methods where the quality and yield of DNA plays a crucial role. In this study the quality and quantity of the extracted DNA obtained by FeNps was compared to the conventional phenol–chloroform method and also kit method. In the case of FeNps extraction method DNA yield was around 2.54g, in phenol–chloroform and by using kit method 1.91g and 1.66g respectively of DNA was obtained. A simple, rapid method was designed for DNA extraction using FeNps from E coli K12. This method is easy and rapid compared to conventional methods. It is possible to isolate a good quality of DNA using iron oxide nanoparticles without any enzymatic treatment.

3.3 Elution of DNA from agarose gel

The extracted genomic DNA was run on agarose gels in triplicates, of which the first sample was run using EtBr (1µl). The gel was run and observed under UV transilluminator. DNA was cut from the corresponding wells using scalpel and was resuspended in elution buffer. It was homogenized properly and then the eluted DNA was used to check for the amount of DNA obtained. Fig. 4

IV. Figures

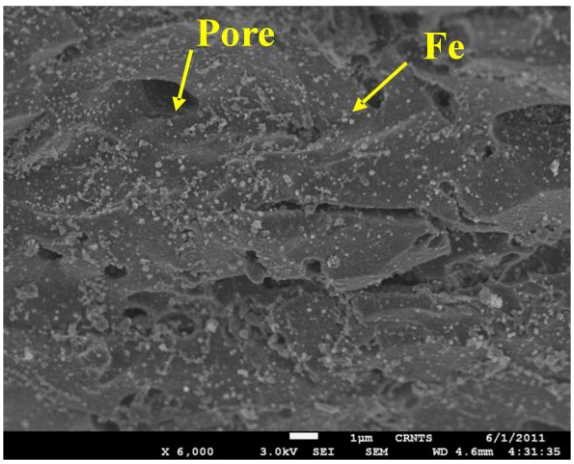

Fig 1 SEM image of activated Carbon embedded nanoparticles

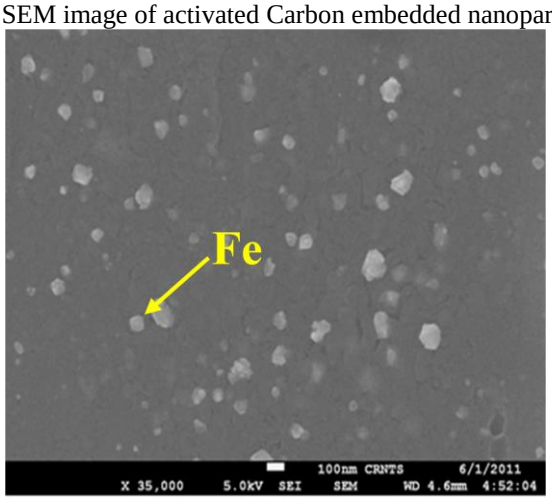

Fig 2 SEM images of Fe oxide nanoparticles. Size 60-100nm

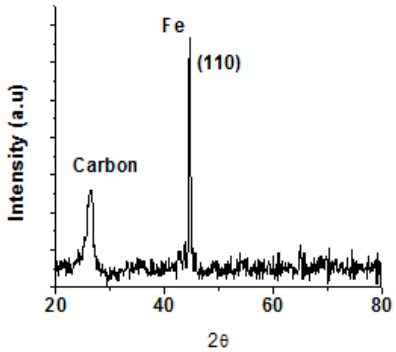

Fig 3 XRD image of Iron Oxide Nanoparticles. Peak at 44.9° confirms their presence

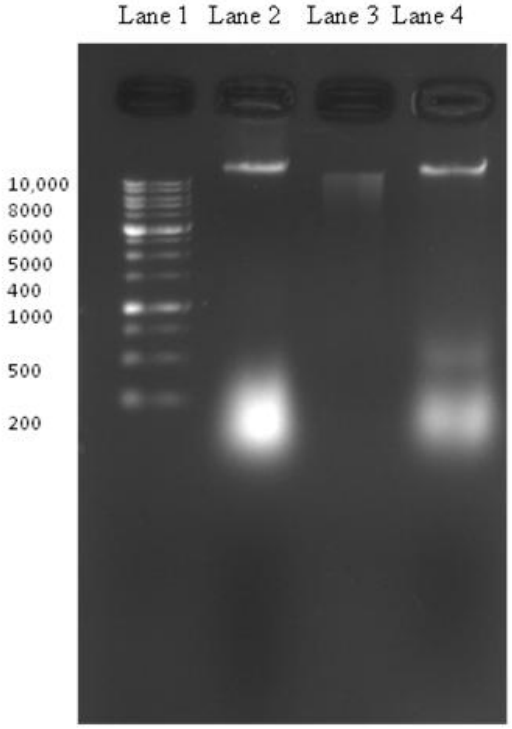

Figure 4 AGE image. Lane 1: 1 kb ladder. Lane 2: Extraction of Genomic DNA by manual method. Lane 3: Extraction of Genomic DNA by Kit method. Lane 4: Extraction of Genomic DNA by using 1 µg of iron oxide nanoparticles

V. Conclusions

A new method for extraction of genomic DNA from Escherichia coli K12 strain using FeNPs has been successfully developed and its performance has been compared against conventional methods and also using commercial kit. The procedure developed has several advantages. It is quick, inexpensive and does not require use of organic solvents. There is no need for sophisticated instruments, only a magnet is needed.

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