

Magnetic-silica nanoshell for extraction of fungal genomic DNA from *Rhizopus oryzae*Mousa Alghuthaymi<sup>1,\*</sup> <sup>1</sup>Department of Biology, Science and Humanities College, Alquwayiyah, Shaqra University, Saudi Arabia\*corresponding author e-mail address: [malghuthaymi@su.edu.sa](mailto:malghuthaymi@su.edu.sa) | Scopus ID [56431228000](https://orcid.org/0000-0001-9142-8000)

## ABSTRACT

The main objective of the present article describes an established genomic DNA purification technique improved in vitro by using magnetic nanoparticles as a stable phase adsorbent. Stöber technique was used to synthesis Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>3</sub> nanoshell, and described using transmission electron microscopy (TEM) and dynamic light scattering. The quality and quantity of purified DNA were verified by using agarose gel electrophoresis and some PCR strategies such as universal-primed UP-PCR, RAPD patterns. Up to 50 µg of DNA was obtained from a slight quantity of one hundred mg of fungal mycelium. Fungal DNA produced with the aid of this technique was applied as a fungal DNA template for PCR method to amplify UP-PCR, RAPD provided replicable profiles. DNA purified from fungal mats was 100 % pure enough to generally be used at high concentrations for PCR technique. The end result confirmed that Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>3</sub> nanoparticles of ~140 nm diameter with concentration of 0.4 mg/ml produced premium quality and amount of isolated DNA produced from fungal mats. The obtained DNA was free from any polluting proteins, polysaccharides and coloured dyes. Magnetic nanoparticles (MNP)-mediated genomic DNA extraction is pretty simple (loose from filtration and centrifugation), speedy (30 min), and ecofriendly (not contain any toxic chemical compounds).

**Keywords:** *Rhizopus*; DNA isolation; PCR enhancement; magnetic nanoparticles.

## 1. INTRODUCTION

*Rhizopus oryzae* is one of the greatest significant fungal species of Mucoromycotina [1]. Even though post-harvest disease through *Rhizopus* spp. is considerably more prevalent than pre-harvest contamination in the deficiency of harm or fruit damage, *Rhizopus* traces had been located, in a few cases, that should be associated with pre-harvest disease in numerous fruits [2-4]. Several fungal DNA isolation methods have been investigated within the published materials. On the other hand, these strategies are plenty of time, need an excessive standard of handling and may well not be appropriate for usage as a regular procedure's method [5]. Molecular investigation of nanoparticle interaction with DNA has began to discover the various types of nanomaterials including carbon nanotubes [7], copper nanoparticles silica nanoparticles [6], silver nanoparticles [8], iron/magnetic nanoparticles [9-13], and different nanoparticles and nanocomposites [4] may be used for DNA isolation. Newly, separation of DNA and RNA isolation from eukaryotic and prokaryotic cells by using graphene nanosheets [14]. An extensive analysis of the reviews concerning the applications magnetic nanoparticle for DNA adsorption was studied [15]. Nanoparticles with DNA adsorbent surfaces were substantially used for DNA isolation. Magnetic particles covered with silica or functionalized carboxyl corporations had been used to purify DNA from organic samples [4, 5, 16, 17]. Magnetic strategies utilizing magnetic

particles covered with unique biopolymers (e.g., agarose, silica) had been applied significantly for molecular detection functions [18]. Nevertheless, using simple (uncoated) magnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub>) permits them to make the most as well their belongings to retractably bind DNA below particular conditions. Adsorption techniques depending fully on MNPs may easily offer DNA with better quality to be used in enzymatic degradations of DNA, polymerase chain reaction, recognition of epigenetic markers, and genomic sequencing due to the fact the technique is straightforward and is almost free from poisonous chemicals or pressure-based purification methods as in centrifugation [17, 19, 20]. Molecular biologist now pays extra attention in the direction of fast DNA extraction using nanotechnology tools to study the excellent technique to purify DNA from various fungi. To obtain speedy cost powerful, delicate and non-unsafe technique for DNA isolation we employed magnetic nanoparticle coated both with amino group. Sodium dodecyl sulfate (SDS) is a sturdy anionic cleansing agent, which usually interferes with no n-covalent bonds within the proteins and denature. Also, SDS prompted the release of DNA from magnetic nanoparticles. The essential aim of the current research to purify fungal DNA from homogenized mycelium of eleven *Rhizopus oryzae* isolates by using combined lysis methods such as SDS and adsorb DNA via Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>3</sub> nanoparticles.

## 2. MATERIALS AND METHODS

**2.1. Magnetic nanoparticles synthesis.** Magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles were synthesized by employing precipitation from FeCl<sub>2</sub> and FeCl<sub>3</sub>(H<sub>2</sub>O) salts with the support of aqueous ammonia solution was discussed [21]. The SiO<sub>2</sub> shell modification was carried out on the produced Fe<sub>3</sub>O<sub>4</sub> nanoparticles by the Stöber technique [22]. The particles had been described for size via

transmission electron microscopy (TEM). Also, the mean nanoparticle length and size have been tested by dynamic light scattering (DLS) technique (Zetasizer, Malvern Instruments Ltd, UK).

**2.2. DNA purification method.** Half gram of *Rhizopus oryzae* mats collected from 3-day old fungal mats growing on the duplex

potato agar medium were grinding to a fine powder using liquid nitrogen [23]. One hundred milligram from homogenized mycelium was mixed with 300  $\mu\text{L}$  of lysis buffer (0.5% SDS and 0.2 M sodium hydroxide solution) for 2 min at 50 °C. The tubes were quietly mixed 3 times and centrifuged. The buffer combinations had been transferred to fresh Eppendorf tubes, and 100  $\mu\text{L}$  of  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  were used for DNA adsorption. The composition of binding buffer (1.25 mol  $\text{L}^{-1}$  NaCl, 10% polyethylene glycol-6000), also 5  $\mu\text{L}$  RNAse A (10 mg  $\text{mL}^{-1}$ ), had been delivered and Eppendorf tube was incubated for 12 min at 68°C to make sure goal DNA changed into absolutely attached to the surface of the magnetic nanoparticles. These multiplexes are then divided manually from the supernatant via magnet immobilization. The DNA pellet was purified in 200  $\mu\text{L}$  of 70% ethanol, after ethanol evaporates, DNA was eluted by using the addition of 60  $\mu\text{L}$  of TE elution buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.5).

**2.3. Assessments DNA Quantity.** The quantity and quality of isolated fungal DNA were assessed using numerous techniques. In the primary, DNA purity was checked by gel electrophoresis of the isolated fungal DNA on 1.7% agarose gel at 90 V for 100 min in 1X TAE. The agarose gel was visualized with 0.25  $\mu\text{g}/\text{mL}$  ethidium bromide solution for the fluorescence images of DNA profile. UVI-soft software (Gel Documentation and Analysis Systems, Uvitec, Cambridge, UK) was employed to grab the photographs and to assessment molecular weight size.

**2.4. DNA Digest for Purified DNA.** Ten microliter volume of the purified DNA solution was combined with 1  $\mu\text{L}$  the restriction enzyme reaction buffer, plus 8  $\mu\text{L}$  sterile deionized water and incubated with 10 units of the restriction endonuclease enzymes

including Eco R1/Hind III (Thermo Fisher Scientific) at 37°C for 3 hours. DNA digestion fragments were separated in 1% agarose gels and DNA stained with ethidium bromide solution (0.1 mg/mL) for 20 min.

## 2.5. Suitability DNA template for PCR Amplification.

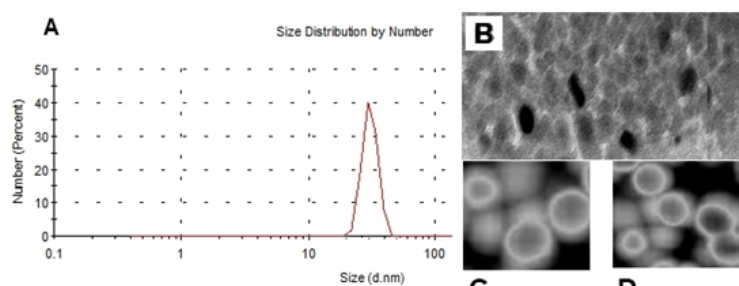
**2.5.1. UP-PCR reaction:** Polymerase chain reactions had been carried out in 200  $\mu\text{L}$  PCR tubes in a 20  $\mu\text{L}$  reaction volume level including 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.8 mM NaCl, 3.5 mM  $\text{MgCl}_2$ , 0.1% Triton X-100, 0.4 mM dNTPs, 20 pmol for AS4 universal primer (5'-TGT GGG CGC TCG ACA C-3'), 1.0 U Taq DNA polymerase (Jena-Bioscience, Germany) and 10 to 15 ng of the purified DNA. PCR amplification was completed in a Biometra Thermal Cycler with the following PCR parameters, 40 cycles of denaturation at 94°C for 30 s (first denaturation step at 94°C for 3 min), annealing at 56°C for 70 s and polymerization at 72°C for 60 s, with a last extension step of 72°C for 5 min. PCR reaction amplification tubes may be transferred and stored at 4 °C.

**2.5.2. RAPD analysis:** RAPD technique of the isolated DNA was done in a Biometra thermal cycler for 35 cycles of 35 s at 94 °C, 35 s at 40 °C and 1 min at 72 °C, and a final extension for 10 min cycle at 72 °C. PCR reactions were performed in a total volume of 25  $\mu\text{L}$  formulated with 0.2 M of dNTPs, 0.2 M of the OP1 RAPD primer (5'-TGCCGAGCTG-3'), 1x PCR amplification buffer, 0.3 mM of  $\text{MgCl}_2$ , 1U Taq DNA polymerase (Jena-Bioscience, Germany) and 25 ng of DNA amplicon and ultrapure water. PCR fragments for the above PCR methods were separated e on 1.5% agarose gel electrophoresis in 1X TAE buffer by loading 10  $\mu\text{L}$  into prepared wells. Agarose gels were stained with ethidium bromide solution.

## 3. RESULTS

### 3.1. Nanoparticles characterization.

Dynamic light scattering (DLS) technique assay was conducted on precipitated MNPs as it needs clear solution for laser to pass. the nanoparticles have a mean hydrodynamic diameter of 40 nm. Spherical morphology become detected in TEM photos of  $\text{Fe}_3\text{O}_4$  nanoparticles. From this image it can be visible that  $\text{Fe}_3\text{O}_4$  nanoparticles were shaped by way of 30–60 nm diameter of seed and the encompassing sheet has a thickness of approximately 2–5 nm (Fig. 1 A, B). It might be noticed that the  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  had spherical nanoparticles with an average size of about 140 nm and was monodispersed. The thickness of the  $\text{SiO}_2$  shell was approximately 8 nm, which can enhance the stability and biocompatibility of  $\text{Fe}_3\text{O}_4$  nanoparticles Figure 1 C, and D.

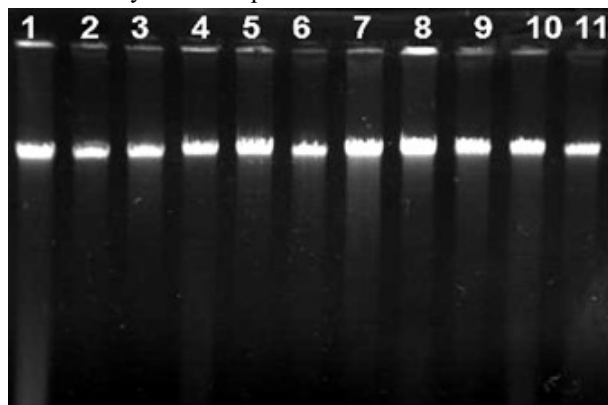


**Figure 1.** A) Dynamic light scattering curves of the dispersion of  $\text{Fe}_3\text{O}_4/\text{SiO}_2$  nanoparticles in pure water. B) TEM image of  $\text{Fe}_3\text{O}_4/\text{SiO}_2$  nanoparticles. Particle size (60-220 nm).

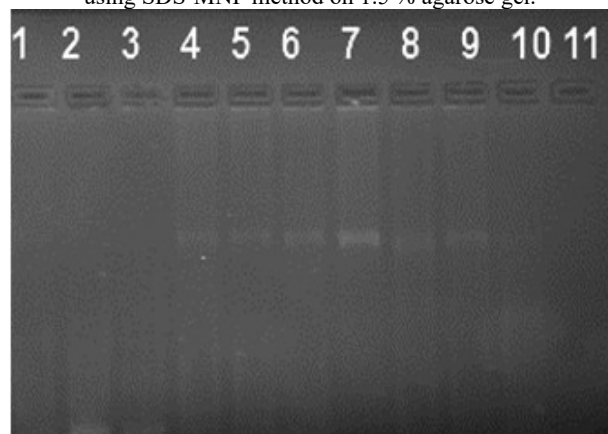
### 3.2. DNA Yield.

To confirm the reliability of the current method, DNA isolated from eleven *Rhizopus* isolates associated with apple were subjected to four molecular evaluation methods. DNA quantity and quality were examined through direct electrophoresis, one single main band was envisioned on the agarose gel, displaying the absence of polyphenols, no detectable RNA contamination, no smearing DNA and no signs of degradation (Fig. 2). The suggested approach could acquire a high DNA quantity about 500 ng was observed (overall DNA)/100 mg of fungal mats. The purity of genomic DNA was further shown by degradation of the genomic DNA via the usage of limit enzyme Eco R1/Hind III mix. Then, image became displaying the banding sample of digestible DNA in conjunction with genomic DNA in Fig. 3. The satisfactory of the purified DNA was evaluated by employing various PCR methods such as, universally primed-PCR and random amplified polymorphic DNA. The extracted DNA used for PCR amplification with AS4 universally primed and RAPD primer OP1 (Fig. 4, 5). The purified DNA formed good banding profiles representing the good quality of the obtained DNA. However, the amount in addition to the quality of the purified genomic DNA has been high sufficient to carry out loads of PCR-based totally reactions.  $\text{Fe}_3\text{O}_4@\text{SiO}_2$  NPs produced by Stöber method investigated distinguished physicochemical characters that may be applied for molecular applications such as genomic DNA

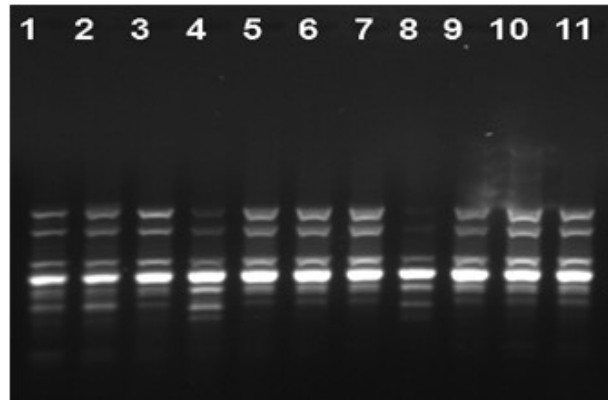
isolation with a separation high quantity and quality combined with traditional lysis technique.



**Figure 2.** DNA isolated from different *Rhizopus oryzae* isolates, extracted using SDS-MNP method on 1.5 % agarose gel.



**Figure 3.** Fungal DNA digested with mixture of two restriction endonucleases Eco R1/Hind III. Lane 1.



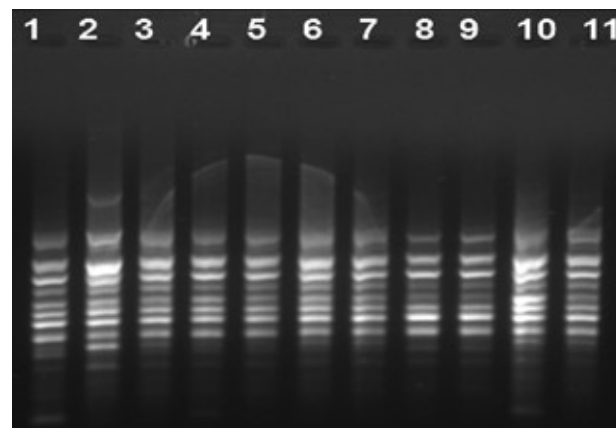
**Figure 4.** Quality evaluation of extracted DNA through UP-PCR amplification of *Rhizopus* isolates using DNA extracted following the present method.

### 3.3. Discussion.

It was first discovered that a couple of nanoparticles almost like the following metals, MNP, ZnO, TiO<sub>2</sub>, CuO, and Ag<sub>2</sub>O is having a tendency to precipitate the proteins and confirmed DNA with great quality [4, 24]. Magnetic nanoparticles offer an elevating multitude of a common approach for the purification of biomolecules, and DNA isolation from bacteria and fungi respectively [25]. Magnetic nanoparticles (MNPs) containing Fe<sub>3</sub>O<sub>4</sub> (magnetite) are talented as diagnosis strategies in plant protection especially purification for DNA from several air-borne fungi including *Cladosporium* [26].

## 4. CONCLUSIONS

In the current method, silica-layered with iron oxide nanoparticles conjugated with DNA lysis technique evolved may



**Figure 5.** Quality evaluation of extracted DNA through RAPD-PCR amplification of *Rhizopus* isolates using DNA extracted following the present method.

To be able to isolate DNA from fungal mycelium, fungal cell wall must be homogenized well. The recent work explains a fungal genomic DNA isolation process improved in vitro utilizing magnetic nanoparticles as a stable adsorbent. The quantity of the purified DNA with magnetic approach comparative to the classic techniques. The purified DNA has grown to be responsive to restriction digestion using restrict endonuclease Eco R1/Hind III mix. Silica-coated Iron oxide nanoparticles present only a few basic safeties against DNase cleavage of DNA and probably reduced the restrict enzymes functions [27]. The quantity of DNA obtained was usually pertaining to the preliminary amount of and level of homogenization of fungal mycelium applied for DNA extraction. Fungal cell wall dysfunction by liquid nitrogen and Eppendorf micro pestle was presented with the ideal DNA quantity when compared to the cell wall degradation via ultrasound technique [28]. The advancements DNA purification method simply by employ of all these nanoparticles may possibly be linked to their particular function in fungal cell membrane lyses, precipitating of proteins and inhibited restriction enzymes. Magnetic separation technology, utilizing magnetic nanoparticles offers many positive aspects [29]. DNA adsorption mechanism of magnetic mesoporous silica nanoparticles recommends that the DNA adsorption into mesoporous might produce more intermolecular hydrogen bonds than patients engineered on the exterior surface [30]. For identification of DNA-binding mechanisms on nanoparticles is significant for different applications which include molecular detection of various fungal pathogens, gene delivery and gene editing [31, 32]. Here, the DNA can be released in the buffer due to its chemical action. A different approach to induce DNA release in the final buffer without any chemical substance action on the specific DNA is to increase the buffer chamber temperature so as to weaken the forces binding the DNA to the Fe<sub>3</sub>O<sub>4</sub>@SiO<sub>2</sub> nanoparticles. This technique is rapid and convenient to conduct. Additionally, the analyst in which we are interested, subjects to a very tiny quantity of chemicals in comparison with other strategies.

easily significantly make the easier procedure of immediate DNA amplicon separation for PCR considering that enrichment of the



cell population with the help of MNP concentrated on and separation. The purification of DNA was checked through agarose gel electrophoresis, restriction enzymes, and two PCR methods. DNA template 50 µg was provided and the PCR amplification was good for RAPD and UP-PCR. The full amount procedure of DNA isolation is usually completed soon after only 30 min. The

improved isolation method was adjusted based on various features, to improve the DNA yield supplement, decrease the time period, and prevent the application of expensive reagents in isolation methods, and DNA needs to be amenable to a few downstream enzymatic functions, such as molecular characterization of fungal diversity.

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## 6. ACKNOWLEDGEMENTS

Author thank Department of Biology, Science and Humanities College, Shaqra University, Saudi Arabia, for providing partial funds to carry out this research work.



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