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ORIGINAL ARTICLE

Using metal nanoparticles (ZnO and Ag) to enhance DNA isolation and amplification

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Abstract

Recently, there have been significant improvements in the methods used to isolate and purify genomic DNA. The implementation of high-throughput facilities has accelerated the advancement of technology, enabling more rapid and effective DNA processing than ever before. The study's goal was to develop a cost-effective and efficient technique to enhance the characterization of isolated DNA that is suitable for subsequent molecular downstream applications. The DNA used as study object obtained from suspensions of pathogenic bacteria, *Klebsiella pneumoniae* (*K. pneumoniae*) and the *Enterobacter cloacae complex* (*E. cloacae)* using a manual salting out method. The method includes utilizing ZnO and Ag nanoparticles (Nps), to enhance isolation of genomic DNA. The study showed that using Nps at concentrations of 0.02 mg/ml for Ag and 0.004 mg/ml for ZnO led to better DNA quality and quantity compared to the control group without Nps, resulting in ideal outcomes. In addition, the presence of these Nps greatly increased the efficiency of PCR amplification for detecting the 16S ribosomal DNA (rDNA) of *K. pneumoniae and E. cloacae*. This resulted in the effective amplification of a 709 base pair segment in both type of Nps. The incorporation of nanoparticles technology into DNA extraction methods is in line with the current demands of the fast-progressing fields of molecular biology and biotechnology.

1. Introduction

Nanoscience and nanotechnology are disciplines specifically concentrating on manipulating and examining materials and phenomena at the nanoscale, encompassing dimensions ranging from one nanometer to several hundred nanometers. Scientists in these fields investigate materials' distinct characteristics and actions at a minuscule level [1]. This allows for developing and producing cutting-edge technologies that may be applied in diverse industries such as health, electronics, and materials science. The prefix "nano" denotes a scale of one billionth of a meter, emphasizing the remarkably minute dimensions of the structures and gadgets associated with these disciplines [2]. Nanoparticles known by their extraordinary multi-purpose in many fields and has physical function, chemical function, and the most important the biological feature [3]. Silver NPs possess antibacterial capabilities that effectively hinder the proliferation of bacteria, fungi, and other microbes. This functionality is especially beneficial when maintaining a sterile or antimicrobial environment is paramount. For example, silver nanoparticles can be incorporated into fabrics to generate antimicrobial apparel or utilized in medical devices to lower the risk of infections [4]. Silver Nps produce silver ions (Ag

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(I)), which have the ability for interaction with the charge-negative cellular membranes of bacteria. This contact interferes with cellular enzymes, impacts membrane permeability, and finally results in the lysis (rupture) and demise of the bacterial cells. Silver ions have antibacterial capabilities that render them highly effective in suppressing bacterial proliferation and thwarting the onset of illnesses [5]. Zinc oxide Nps, recognized for its semiconducting characteristics, is utilized in diverse domains such as photovoltaic (solar) cell, gas sensors devices, faience, catalysts, cosmetics, and varistors [6]. In order to adapt to the changing demands of the field, novel technologies have been implemented to improve some molecular biology processes overcoming the constraints of traditional approaches, the study used genomic DNA isolated from two pathogenic bacteria that are generally recognized as the main causes of hospital-acquired illnesses (nosocomial), which are *K. pneumoniae* and *the E. cloacae.* The bacterium *K. pneumoniae* is classified as Gram-negative, immobile, surrounded by a protective capsule, and capable of breaking down lactose through fermentation, it is typically found in the mouth, skin, and intestines. It can cause severe damage to the lungs if it is inhaled, specifically the alveoli, and eventually result in the development of bloody sputum [7]. *E. cloacae* is facultative anaerobic Gram-negative strains, these microbes have saprophytic behavior often found in nature, such as soil and sewage. Furthermore, they are a constituent of the commensal enteric flora found in the human gastrointestinal tract, clinically significant in recent years as nosocomial infections, especially in intensive care units [8]. In the field of bioseparation and purification, the use of nanoparticle technology has become increasingly popular as an efficient method for isolating biomolecules such as DNA, RNA, and proteins. This methodology signifies a substantial deviation from traditional procedures, providing improved effectiveness and accuracy in the extraction and purification of genetic material [9]. The objective is to enhance and optimize these procedures by using metal oxide Nps ZnO and Ag, with the ultimate aim of achieving improved outcomes in DNA isolation and it's consequently in PCR amplification results.

2. Materials and Methods

2.1 Nanoparticles

The Nps used in this study were supplied by Sigma, USA. Zinc oxide (ZnO) and silver (Ag) suspensions were prepared by diluting both with sterilized distilled deionized water to achieve precise concentrations of (0.4, 0.04, and 0.004 mg/ml) for Ag Nps and 0.2, 0.02, and 0.002 mg/ml for ZnO Nps as recommended in previous studies [10, 11].

2.2 Collection of bacterial strains

The DNA was isolated from bacterial strains (*K. pneumoniae* and *E.cloacae*), which obtained from the Department of Biotechnology at Al-Nahrain University.

2.3 Biochemical identification of bacteria

The biochemical characters were identified using VITEK 2 (Biomeruixe, USA), a platform biochemical automated device commonly used in clinical laboratories for precise microbiological identification and to assess the sensitivity of *E. cloacae & K. pneumoniae* and yeast to antimicrobial agents [12].

2.4 Extraction of DNA

The genomic DNA extraction involved two steps: the first stage utilized the salting-out approach without Nps, while the second step incorporated Nps into a modified salting-out method [10]. The chosen bacterial strains were grown in brain-heart infusion broth at 37°C for 24 hours, then centrifuged at 6000 rpm for 15 minutes. Subsequently, 20 ml of the recovered sediment was combined with 1 ml of Ag and ZnO Nps concentrations. The sample was washed, mixed with 3 ml of SET buffer containing 25 mM EDTA, 75 mM NaCl, and 20 mM Tris-HCl, and adjusted to pH 8. After adding 1 mL of 10% SDS solution and incubating for 15 minutes at room temperature with inversion mixing, 2 mL of 5 M NaCl solution was added and mixed by inversion at room temperature. The mixture was centrifuged at 6000 rpm at 4°C for 15 minutes after introducing an equivalent amount of chloroform and mixing it by inversion for 15 minutes. The upper part of the liquid phase was transferred to a sterile 1.5-ml tube. Then, 0.6 volumes of isopropanol were added, mixed by inversion, and kept at room temperature for 5 minutes. The isopropanol layer was eliminated after centrifugation at 13000 rpm for 15 minutes at 4°C. The precipitated DNA was dissolved in 100 µl of TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8) and preserved at -20°C.

2.5 Nanoparticles and PCR

Universal sequences DNA segments common among clinical samples and bacterial pathogens were used to detect 16S ribosomal DNA (rDNA) in PCR amplification. The nucleic acids separated with Nps were used to evaluate DNA as a template for the PCR reaction. The amplification was performed using the green master mix (GoTaq Kit, Promega). The PCR reaction mixture consisted of the following components: 12.5 µl of master mix, 5 µl of purified DNA (100 ng) used as a template, 1 µl (10 pmol/µl) of each primer (forward primer: 5′GTGTAGCGGTGAAATGCG3′, reverse primer: 5′ACGGGCGGTGTGTACAA3′), and 5.5 µl of nuclease-free water to achieve a total reaction volume of 25 µl. The amplification procedure was conducted using a Veriti thermal cycler from applied biosystems under the following conditions: an initial step of 5 minutes at 94°C, followed by 30 cycles consisting of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C, and the final extension step for 5 minutes at 72°C. The PCR products underwent direct analysis in 1% agarose by gel electrophoresis.

3. Results and Discussion

The study aimed to assess and contrast the characteristics, quantity, and integrity of genomic DNA extracted using ZnO and Ag Nps with extraction methods that do not use these Nps. The genomic DNA from the *E. cloacae* and *K. pneumoniae* was extracted using the manual salting-out. During the initial phase of DNA extraction, various concentrations of Nps were employed: 0.4, 0.04, and 0.004 mg/ml for Ag Nps, and 0.2, 0.02, and 0.002 mg/ml for ZnO Nps. As displayed in Table 1 and Figure 1, the findings demonstrated an enhancement in the DNA's quality. More precisely, the DNA concentration experienced a substantial increase when it was separated with Ag Nps at a concentration of 0.04 mg/ml. This resulted in a 533 ng/µl rise for *E. cloacae* and 407 ng/µl for *K. pneumoniae*. Figure 1 exhibits good DNA integrity without any indications of degradation or smear bands across different concentrations of Nps. The mean A260/280 ratio of the DNA samples treated with Ag Nps was roughly 1.8, while the DNA samples without Nps had a ratio of 1.63, as demonstrated in Table 1. This suggests that the DNA was high purity and had little protein contamination.

Table 1: DNA yield and purity following treatment with Ag Nps of E. cloacae & K. pneumoniae bacteria

Bacteria	Ag Nps mg/ml	DNA yield ng/µl	Purity (260-280)
E. cloacae	Without Nps	120.3	1.6
E. cloacae	0.4	180.9	1.81
E. cloacae	0.04	533	1.77
E. cloacae	0.004	208	1.67
K. pneumonia	Without Nps	123.2	1.65
K. pneumonia	0.4	343.3	1.88
K. pneumonia	0.04	407	1.72
K. pneumonia	0.004	397	1.7

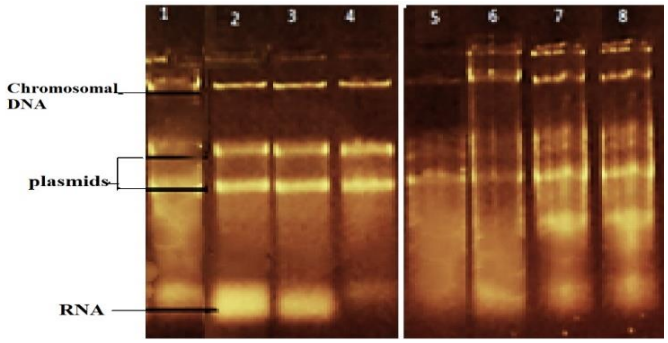

Figure1: Gel electrophoreses of *bacterial* genomic DNA extracted by salting out. Lanes (1) Extraction of DNA from *E. cloacae* without Ag Nps. (2) Extraction of DNA of *E. cloacae* with 0.4 mg/ml Ag (3) Extraction of DNA from *E. cloacae* with 0.04 mg/ml Ag (4) Extraction of DNA of *E. cloacae* with 0.004 mg/ml Ag . (5) Extraction of DNA from *K. pneumonia* without Ag. (6) Extraction of DNA of *K. pneumonia* with0.4 mg/ml Ag. (7) Extraction of DNA from *K. pneumonia* with 0.04 mg/ml Ag. (8) Extraction of DNA of *K. pneumonia* with 0.004 mg/ml Ag. (concentration of agarose gel was 0.8%, 5 V/cm, 1 hr)

Previous studies established that Ag Nps produced Ag (I) ions, which come into contact with a negatively charged bacterial cell wall; they can stop enzymes from working, change how permeable the membrane is, and strongly attract DNA [14, 15].

The results showed in Table 2 and Figure 2 show a significant increase in DNA yield when extracted using ZnO Nps for both bacteria at a dosage of 0.002 mg/ml; particularly, the concentration increased to 312.9 ng/µl for *E. cloacae* and 370 ng/µl for *K. pneumoniae.* The average A260/280 ratio for DNA samples treated with ZnO Nps treatment was 1.81. Whereas the DNA samples without Nps showed a ratio of 1.6, as stated in Table 2. This suggests the DNA had a high purity level and little protein contamination.

Table 2: DNA yield and purity following treatment with ZnO Nps in E. cloacae and K. pneumoniae Bacteria

Bacteria	ZnO Nps mg/ml	DNA yield ng/µl	Purity (260-280)
E. cloacae	Without Nps	95	1.64
E. cloacae	0.2	160	1.76
E. cloacae	0.02	152.8	1.8
E. cloacae	0.002	312.9	1.78
K. pneumonia	Without Nps	101	1.63
K. pneumonia	0.2	205	1.75
K. pneumonia	0.02	247	1.9
K. pneumonia	0.002	370	1.88

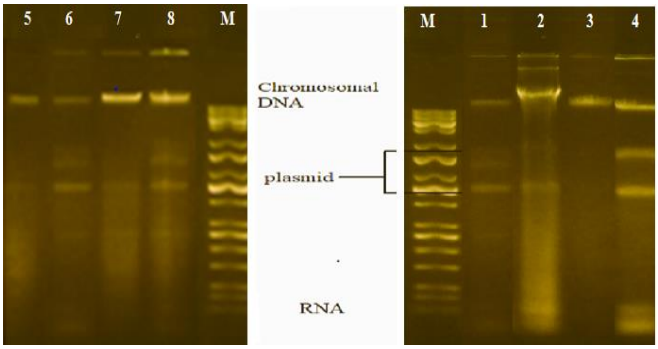

Figure 2: Gel electrophoreses of *bacterial* genomic DNA extracted by salting out. Lanes (1) Extraction of DNA from *E. cloacae* without ZnO nanoparticles. (2) Extraction of DNA of *E. cloacae* with 0.2 mg/ml ZnO (3) Extraction of DNA from *E. cloacae* with 0.02 mg/ml (4) Extraction of DNA of *E. cloacae* with 0.002 mg/ml. (5) Extraction of DNA from *K. pneumonia* without ZnO. (6) Extraction of DNA of *K. pneumonia* with 0.2 mg/ml ZnO. (7) Extraction of DNA from *K. pneumonia* with 0.02 mg/ml ZnO. (8) Extraction of DNA of *K. pneumonia* with 0.002 mg/ml ZnO. (concentration of agarose gel was 0.8%, 5 V/cm, 1 hr)

Bandyopadhyay *et al*. (2011) observed that specific nanoparticles, such as ZnO and silver oxide, tend to cause protein precipitation, leading to a high yield of DNA. The enhanced DNA yield and quality observed while utilizing nanoparticles can be attributed to the heightened vulnerability of the cell membrane. The increased vulnerability made possible by Nps aids in the breakdown of the cell membrane

and causes the formation of protein Nps [16]. Prior studies showed that binding of Nps to DNA changed the normal conformation of DNA molecules [17, 18]. Nps that bind to DNA with high affinity could inhibit the normal functions of some critical DNA-binding proteins, such as RNA or DNA polymerases, which could result in competitive inhibition of genetic functions [19, 20].

Thus, the impact of ZnO and Ag Nps on PCR inhibitory effects was also examined in this study. The optimal concentration of nanomaterial, which resulted in the maximum yield of separated DNA using the modified salting-out approach, was utilized as the DNA template for the PCR amplification process.

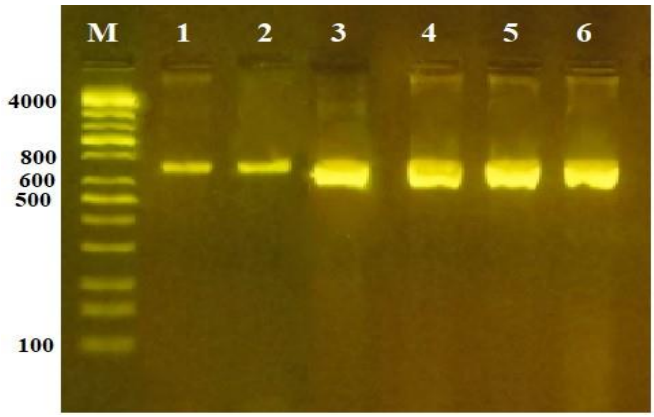

Figure 3: Gel electrophoreses of 709 base pair amplicons of *bacterial* 16S rDNA gene fragment. Lanes (M) DNA molecular marker. Lanes (1) PCR product of genomic DNA isolated from *E. cloacae* without nanoparticles. (2) PCR product of genomic DNA isolated from *K. pneumonia* without Nps. (3) PCR product of DNA isolated from *E. cloacae* with 0.04 mg/ml Ag. (4) PCR product of DNA isolated from *K. pneumonia* with 0.04 mg/ml Ag. (5) PCR product of DNA isolated from *E. cloacae* with 0.002 mg/ml ZnO. (6) PCR product of DNA isolated from *K. pneumonia* with 0.002 mg/ml ZnO. (concentration of agarose gel was 1.5%, 5 V/cm, 1 hr)

Electrophoresis of the amplified PCR products on 1.5% agarose gels revealed the presence of DNA bands for their predicted positions. Amplification of 709 bp of *bacterial* 16S rDNA gene fragment yielded successful results from both *E. cloacae & K. pneumoniae* (Fig .3). Although Ag Nps yield an increased quantity of DNA compared to ZnO Nps, the PCR amplification results, as shown in Figure 3, demonstrate that both ZnO and Ag Nps improve the specificity and efficiency of the amplification reaction as demonstrated by many research [21]. In order to overcome the issues associated with metal oxide nanoparticles, such as their high cost, limited availability of highly purified materials for molecular genetics applications, and their potential cytotoxic and genotoxic effects, it is necessary to minimize their usage and prevent their dispersion in the environment [22]. The current study minimizes the concentrations of used Nps recommended in previous studies [10, 11]. It determines concentrations fair enough to gain appropriate DNA results that can be relied upon in subsequent studies. Thus, this study resulted in an easy and simple method and materials. It is available and does not require complex equipment. It is possible to obtain quantitative, qualitative, and pure results from DNA. It can be relied upon, especially in samples that are expected to contain a small amount of DNA, such as tissue, hair, and even samples associated with the forensic field [23].

4. Conclusions

The salting-out extraction method was used to extract DNA from *E. cloacae* and *K. pneumoniae* pathogens in the presence of ZnO Nps (0.002 mg/ml) and Ag Nps (0.04 mg/ml), resulting in improvements in both the quality and quantity of the DNA. The effectiveness of this procedure was demonstrated in successfully eliminating RNA and protein contaminants, exhibiting results comparable to those of commercially available kits. Furthermore, the approach exhibited adequate PCR amplification, suggesting a lack of enzyme inhibitors. Consequently, the purified DNA acquired has shown satisfactory properties and can be confidently used for subsequent molecular applications.

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