Improvement of DNA Extraction Methods by ZnO and TiO_2 Nanoparticles

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***Abstract*—**The aim of this study is to investigate the quality and quantity of DNA isolated by three methods (boiling, alkali lysis and salting out) of DNA extraction in the presence of nanoparticles (ZnO and TiO_2). The result showed that ZnO nanoparticles of ~35 nm diameter with concentration of 0.4 mg/ml improved quality and quantity of extracted DNA from *E. coli* HB101 and *P. aeruginosa*. Furthermore, TiO_2 nanoparticles of~ 100 nm with concentration of 0.2 mg/ml lead to improve DNA quality that extracted from both bacteria by all DNA extraction methods. However, TiO_2 caused enhancement of the quantity of DNA when used with salting out method only.

***Keywords*—** DNA extraction methods, E. *coli*, *P. aeruginosa*, TiO_2, ZnO , Abbreviations Nps Nanoparticles.

I. Introduction

NANOSCIENCE is a branch of science grow rapidly in the last decade, allowing the manipulation of material at the nanoscal and allowing to control the fabrication of such systems and devices. The engineering nanoparticles offer potential applications in many areas beneficial for human kind, including sensors, medical imaging, drug delivery system and cosmetics (Birhanli, 2014).

It is clear that the metal based nanoparticles constitute an effective antimicrobial agent against common pathogenic microorganisms. Therefore, some of the nanoparticles such as silver, titanium dioxide and zinc oxide are receiving considerable attention as antimicrobials and additives in consumer, health-related and industrial products. Nanoparticles of titanium dioxide are used in cosmetics and filters that exhibit strong germicidal properties and remove odors and in conjunction with silver as an antimicrobial agent. Moreover, due to the photocatalytic activity, it has been used in wastewater treatment. Titanium dioxide nanoparticle is of increased of interest in wide applications such as a self-cleaning, self-disinfecting material for surface coatings and in food industries for disinfecting equipments (Wist et al. 2004). Zinc oxide (ZnO) and copper oxide nanomaterials due to their antimicrobial property are being incorporated into a variety of medical and skin coatings. ZnO nanoparticles was used in the wallpapers in hospitals as antimicrobials agent. ZnO powder is an active ingredient for dermatological applications in creams, lotions and ointments on account of its antibacterial properties (Martinez et al. 2003).

The process of genomic DNA isolation and purification has evolved considerably in the last decade. The advance of technology allows more efficiency for DNA processing (Pindi et al. 2013). The classic chemical methods for DNA extraction generally are toxic, time-consuming; multiple steps, and utilizes organic solvent extraction, alcohol precipitation as well as centrifugation. In the setting of bioseparation and purification, nanoparticles technology has become an increasingly popular tool for the separation of biomolecules (e.g. DNA, RNA and proteins) (Bandyopadhyay et al. 2011; Abdul khaliq et al. 2011). The present study aimed to study the effect of using nanoparticles (ZnO , TiO_2) in the extraction of DNA from gram negative bacteria in which the genomic DNA was isolated from bacteria by using a number of standard methods with some modifications by using a different concentrations of ZnO and TiO_2.

II. Materials and Methods

A. Bacterial Strains

Two strains were chosen for this study, *E. coli* HB101 (Bio Rad) and wild type *P. aeruginosa* obtained from Department of Biotechnology, Al-Nahrain University.

B. Nanoparticles

Two types of nanoparticles were used in this study. ZnO colloid Nps (20-50 nm, anatase, con. 50 % w/v, dissolved in water, Sigma) and TiO_2 (50-150 nm, Mix rutile and anatase con. 33.37 % w/v, dissolved in water, Sigma).

C. DNA Extraction

DNA was extracted from both bacteria according to the three standard methods by using nanoparticles as follow:

D. Salting out

Genomic DNA was extracted by using the salting out method as described by Pospiech and Neumann (1995), with some modification in which twenty ml of fresh culture for the selected isolate, grown in brain heart infusion broth at 37 ºC for 24hrs, pelleted by centrifugation at 6000 rpm for 15 min. The pellet was washed with 2 ml of nanoparticle solution [ZnO (0.4 mg/ml), or TiO_2 (0.2 mg/ml)], centrifuged and then the

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cells were resuspended in 1.5 ml of nanoparticles solution, mixed by inversion and incubated at 37ºC for 10 min. The mixture then sonicated for 4 min at 4 ºC using 60 KH_z. The sonication was performed for 2 cycles 2 min sonication then 2 min brake. The subsequent steps were done according to standard method.

E. Alkali lysis

Plasmid DNA was extracted by alkali lysis method as described by Green and Sambrook (2012). The alkali lysis method was modified by adding 1ml of nanoparticle solution [ZnO (0.4 mg /ml) or TiO_2 (0.2 mg /ml)] in addition to 1 ml of solution I (50 mM Glucose, 10 mM EDTA and 25 mM Tris-HCl) in the step 1 of this method.

F. Boiling method

Boiling method (Green and Sambrook, 2012) was modified to extract plasmid DNA in presence of nanoparticles. The modification was carried out at step 2. In addition to STET (8 % Sucrose, 0.5 % Triton X- 100, 50 mM EDTA, 10 mM Tris-Cl) solution and lysozyme (10 mg/ ml), 100 µl of nanoparticles [ZnO (0.4 mg /ml) or TiO_2 (0.2 mg /ml)] were added and incubated at 37 ºC for 15 min.

III. Results

In this study, it was compared the quality and quantity of isolated genomic DNA with using nanoparticles (ZnO and TiO_2) and without using of these nanoparticles. Genomic DNA was extracted from *E. coli* HB 101 and *P. aeruginosa* by using a number of popular manual techniques (boiling method, alkali lysis and salting out methods).

Many attempts were made to examine different concentrations of nanoparticles ranging from 0.1 to 1 mg/ml in different steps of these methods. It was found that the best results were obtained when the nanoparticles used in the first steps and in concentration of 0.2 mg/ml for TiO_2 and 0.4 mg/ml for ZnO for all DNA extraction methods.

Boiling method

Results (table 1 and figure 1) pointed improvement of quality of DNA. The purity of extracted DNA from *E. coli* HB 101 and *P. aeruginosa* was increased from 1.6 to 1.7 - 1.8, when DNA extracted with the presence nanoparticles. Results showed also that the concentration of DNA was highly increased when DNA isolated with ZnO nanoparticles from both bacteria. The concentration increased from 104.8 to 355 ng/µl for *E. coli* DNA and from 88 to 278 ng/µl for *P. aeruginosa* DNA. While the concentration of DNA was not changed when isolated with TiO_2 nanoparticles for both bacteria (table 1).

TABLE I
CONCENTRATION AND PURITY OF DNA EXTRACTED FROM *E. COLI* AND *P. AERUGINOSA* BY USING BOILING METHOD

Bacteria	Concentration of DNA extracted Ng/µl			Purity of DNA extracted (260/280)		
	Without Nps	With ZnO	With TiO_2	Without Nps	With ZnO	With TiO_2
E. coli HB101	104.8	355	106	1.6	1.8	1.7
P. aeruginosa	88	278	90	1.6	1.7	1.7

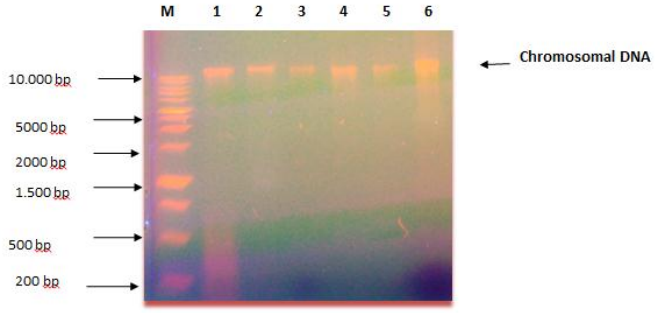

Fig 1 Gel electrophoreses of genomic DNA extracted by boiling method from the bacterial isolates. Electrophoreses was performed on agarose gel (0.7%) and run with 5 V/cm for 1 hr. Lane M is a (1 Kb) ladder, Lanes (1) Extraction of DNA from *E. coli* with ZnO (2) DNA of *E. coli* H 101 (3) Extraction of DNA from *E. coli* with TiO_2 (4) DNA of *P. aeruginosa* (5) Extraction of DNA from *p. aeruginosa* with TiO_2 (6) Extraction of DNA from *P. aeruginosa* with ZnO.

Results illustrated in table (1) had shown that extracted DNA by boiling method with ZnO nanoparticles at concentration of 0.4 mg/ml had increased the yield of isolated DNA about 3 to 3.4 folds.

Alkali lysis method

Results (table 2 and figure 2) had explained improvement of quality of extracted DNA. The purity of extracted DNA from *E. coli* HB 101 and *P. aeruginosa* was increased from 1.6 to 1.7 - 1.8, when DNA extracted with nanoparticles.

Additionally, the results had shown that the concentration of DNA was highly increased when DNA isolated with ZnO nanoparticles from both bacteria. The concentration increased from 187 to 466 ng/µl for *E. coli* DNA and from 205 to 398 ng/µl for *P. aeruginosa* DNA. However, extraction of DNA in the presence of TiO_2 nanoparticles did not have any effect (table 2).

TABLE II
CONCENTRATION AND PURITY OF DNA EXTRACTED FROM *E. COLI* AND *P. AERUGINOSA* BY USING ALKALI LYSIS METHOD.

Bacteria	Concentration of DNA extracted Ng/µl			Purity of DNA extracted (260/280)		
	Without Nps	With ZnO	With TiO_2	Without Nps	With ZnO	With TiO_2
E. coli HB101	187	466	196	1.6	1.7	1.75
P. aeruginosa	205	398	201	1.6	1.8	1.7

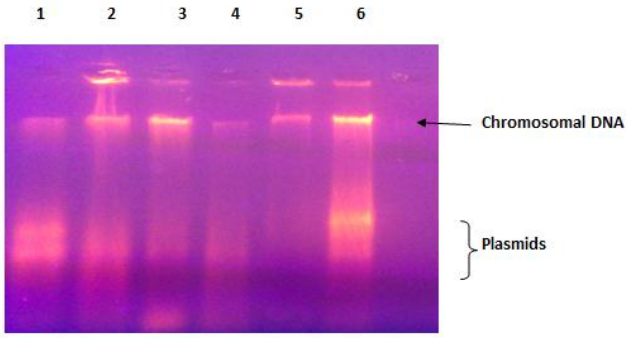

Fig. 2 Gel electrophoreses of genomic DNA extracted by alkali lysis method from bacterial isolates. Electrophoreses was performed on agarose gel (0.7%) and run with 5 V/cm) for 1.5 hrs. Lanes (1) Extraction of DNA from *E. coli* HB101 with TiO_2 (2) DNA of *E.coli* HB101(3) Extraction of DNA from *E .coli* with ZnO (4) Extraction of DNA from *P. aeruginosa* with TiO_2 (5) DNA of *P. aeruginosa* (6) Extraction of DNA from *P.aeruginosa* with ZnO.

From these results, it can be revealed that ZnO nanoparticles (0.4 mg/ml) had a good effect on DNA isolation and increased t he yield of DNA about 2 to 2.4 folds when extracted DNA by alkali lysis method in the presence of ZnO.

Salting out method

Results (table 3 and figure 3) declared that the quality of DNA was highly improved. The purity of extracted DNA from *E. coli* HB 101 and *P. aeruginosa* was 1.6 and 1.5 and became 1.8 to 1.7 respectively, when DNA extracted with nanoparticles.

Results had shown that the concentration of DNA was dramatically increased when DNA isolated with ZnO nanoparticles from both bacteria. The concentration increased from 220 to 788 ng/µl for *E. coli* DNA and from 302 to 556 ng/µl for *P. aeruginosa* DNA. While there was little increased in DNA concentration for both bacteria when DNA isolated with TiO_2 nanoparticles (table 3).

TABLE III
CONCENTRATION AND PURITY OF DNA EXTRACTED FROM *E. COLI* AND *P. AERUGINOSA* BY USING SALTING OUT METHOD.

Bacteria	Concentration of DNA extracted Ng/µl			Purity of DNA extracted (260/280)		
	Without Nps	With ZnO	With TiO_2	Without Nps	With ZnO	With TiO_2
E. coli HB101	220	788	476	1.6	1.7	1.8
P. aeruginosa	302	556	466	1.5	1.7	1.7

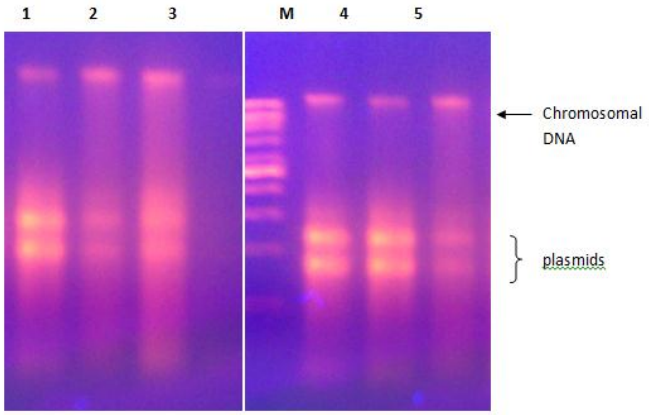

Fig. 3 Gel electrophoreses of genomic DNA extracted by salting out from the bacterial isolates. Electrophoreses was performed on agarose gel (0.7%) and run with 5 V/cm for 1-2 hrs. Lane (M) is (1 Kb) ladder Lanes: (1) DNA of *P. aeruginosa* (2) Extraction of DNA from *P. aeruginosa* with TiO_2 (3) Extraction of DNA from *P.aeruginosa* with ZnO (4) DNA of *E. coli* HB 101 (5) Extraction of DNA from *E. coli* with TiO_2 (6) Extraction of DNA from *E. coli* with ZnO.

The above results showed that ZnO nanoparticles (at concentration 0.4 mg/ml) had a good effect on DNA isolation and increased the yield of isolated DNA about 2 to 3.6 folds when DNA extracted by salting out method in presence of ZnO nanoparticles. While presence of TiO_2 increased the yield of isolated DNA about 1.54 to 2.16 folds.

IV. DISCUSSION

One of the major problems associated with studying genes and their expressions is the difficulty to obtain adequate and pure nucleic acid samples. Some of the most difficult contaminants DNA are humic acids and proteins. Other difficulties are enzymes, consuming time for extraction and using different organic materials (Constance, 2013).

Results indicated that modified DNA extraction methods (Boiling, Alkali lysis and Salting out) in presence of ZnO nanoparticles gave a good enhancement of quality and quantity of the DNA extracted from *E. coli* HB 101 and *P. aeruginosa*. While presence of TiO_2 lead to enhancement of DNA quality extracted from both bacteria by all DNA extraction methods. However, TiO_2 caused enhancement of DNA quantity extracted from both bacteria when used with salting out method only.

To sum up, the enhancement of DNA quantity and quality by using nanoparticles may be attributed to increase the fragility of cells membrane, help to hydrolyze it, and aid to precipitate the proteins.

Bandyopadhyay et al. (2011) referred that some nanoparticles when used with commercial kit for DNA extraction, gave a high quantity of genomic DNA. They explained also that extraction method by using nanoparticles can be performed in any laboratory without the requirements of sophisticated equipments. The procedure yields an ultrapure quality and at least equal

quantity of DNA compared with the conventional (classic methods). Using nanoparticles proved to be powerful and decreasing the time, the number of chemicals that's need for DNA purification, also it's not toxic and cheep.

Bioneer used nanoparticles in mini plasmid kit, they referred that the nanoparticles solution effectively bound to the protein aggregate and increase the total weight of the complex. Also it is used as a lysate buffer in which it help the cell membrane to hydrolyses and exit all the contents [Bioneer/USA (WWW.bioneer.com)].

It was found that some nanoparticles like ZnO, TiO_2 , CuO, and silver oxide is tend to precipitate the proteins and obtained DNA with high purity (Yeates et al. 1998; Bandyopadhyay et al. 2011).

Leng et al. (2006) referred that ZnO nanoparticles provide some protection against deoxyribonuclease (DNase) cleavage of DNA and may be inhibited the restriction enzymes.

V. Conclusions

In conclusions, the improvement of DNA quality and quantity were obtained when DNA extracted from Gram negative bacteria by extraction methods (boiling, alkali lysis and salting out) in the presence of ZnO (0.4 mg/ml).While in the presence of TiO_2 (0.2 mg/ml), improvement in DNA quantity extraction from both bacteria was obtained when it had been used the salting out method only. Improvements caused by these nanoparticles could be attributed to their role in cell membrane lyses, precipitating of proteins and inhibited restriction enzymes.

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