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Primer-immobilized Magnetic Nanoparticle for Bacterial DNA Separation

Venusrin Paripurana[1], Suthee Pinsaeng[2], Duangporn Polpanich[3], Pramuan Tangboriboonrat [4], Kulachart Jangpatarapongsa[5+]

[1, 2, 5] Center for Innovation Development and Technology Transfer, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand 10700

[1, 2, 5] Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand 10700

[3] National Nanotechnology Center, National Science and Technology Development Agency (NSTDA), Thailand Science Park, Pathumthani, Thailand 12120

[4] Department of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand 10400

Abstract. *Enterotoxigenic E.coli* (ETEC) is one of a common pathogen for diarrhea in children under 5 years old and traveler. Separation and collection of bacterial target DNA from stool samples before subjected to identify with molecular technique is found to be an effective way to increase the sensitivity of the technique. Magnetic nanoparticles (MNPs) have been extensively applied for DNA separation. In this study, ETEC was used as a model of the developed for detection of bacterial DNA in the stool sample. The carbodiimide method was used to bind the MNPs with forward primers (primer-MNPs). After heated ETEC sample and removed the cell debris, the primer-MNPs was mixed and incubated with the sample under the appropriate conditions which allowed the complementary of primer and target DNA. The obtained DNA bound MNPs were easily separated from the mixture with the help of a magnet and measured the DNA concentration by spectrophotometer. The results showed the binding of target DNA to primer-MNPs with specificity. This technique was possibly applied to early diagnose for diarrheagenic patients and useful for treatment in the proper time.

Keywords: Magnetic nanoparticle, ETEC, Bacterial detection

1. Introduction

Diarrheal disease is an important global problem, causes of high rates of the morbidity and mortality. It is an infection, causes by bacteria, virus, and parasite organisms, transmits via the oral-fecal route. Enterotoxigenic *E.coli* (ETEC) is one of the main type causes diarrhea among infants, travelers and people having impaired immunity, in which the developing countries are particularly at risk [1].

Generally, the conventional techniques for pathogenic bacteria diagnostic in stool sample consist of the culturing in selective media follow by the biochemical testing. However, they cannot be used for characterization at species and subspecies level. Nowadays, the molecular techniques have been applied for uncommon bacterial identification and for distinguishing the organisms at the strain or subspecies level. Using the stool samples, DNA extraction is challenging because the contaminating substances may come out with the target DNA such as human DNA, protein and lipid from food which can act as the inhibition factors on PCR step [2-5]. Moreover, the target DNA may be highly diluted with non-target DNA of both bacterial and human DNA [6].

+ Corresponding author. Tel.: + 662 441 4370; fax: + 662 441 4380.
E-mail address: kulachart.jan@mahidol.ac.th.

To solve these problems, this study aimed to develop a novel diagnostic technique for ETEC in the stool samples using MNPs as the DNA separator and concentrator before molecular step. We used the MNPs in combination with molecular techniques to separate the target DNA from the stool sample. The binding of a primer onto the surface of MNPs and its application has been recently reported [7-11], and complex between MNPs and the target DNA can be easily magnetized by an external magnetic field and immediately redispersed upon removal of the magnetic field as previously reported [12]. This technique was high sensitivity and specificity.

2. Materials and Methods

2.1. Preparation of Bacteria and the Extraction of DNA

ETEC was cultured at 37°C in tryptic soy agar (TSA) and colony was inoculated in tryptic soy broth (TSB) overnight at 37°C in shaker, then the bacteria was harvested by centrifugation and kept in 70% ethanol at -20°C for further experiment.

DNA from harvested ETEC was extracted by phenol-chloroform technique as manufacturer recommendation (TRIzol, Invitrogen, USA) and DNA concentration was measured by optical density (OD) at 260 nm before used.

2.2. Immobilization of Primer onto MNPs

LT specific primer was synthesized as previously reported [13,14] (Bio Basic Canada Inc., Canada.) and modified with amino group for covalent binding to the surface of MNPs containing surface carboxylic group (Chemicell Inc., Germany) by carbodiimide method [15]. In brief, MNPs were washed twice with 25 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0) and mixed with modified primer at 25°C, 900 rpm for 30 min. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was added to the mixture and incubated at 4°C, 900 rpm for overnight. Primer-MNPs were separated by applying magnetic field, then added Tris buffer (tris (hydroxymethyl) aminomethane) (pH 7.4) and incubated in thermomixer (Eppendorf, Germany) at 25°C, 900 rpm for 30 min. Primer-MNPs were separated and re-suspended with Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and stored at 4°C.

2.3. DNA Separation

The extracted DNA from ETEC was separated by mixing with primer-MNPs (10 mM/µl). Primer-MNPs (2 µl) was incubated with the various concentration of DNA sample (1.0, 0.5 and 0.25 µg/µl, 20 µl) at 94°C for 3 min and 50°C for 5 min, respectively. The target DNA bound to Primer-MNPs was then separated by magnetic field. The obtained DNA was measured OD at 260 nm using Nanodrop (Thermo Fisher Scientific Inc., USA).

2.4. PCR Reaction

LT forward and reverse primer [13,14] were used for the specificity testing in PCR reaction using 94°C, 54°C and 72°C for denaturation, annealing and elongation, respectively. The PCR products were analyzed by agarose gel electrophoresis with ethidium bromide (EtBr) under UV light.

3. Results

Specificity of the primers used in the experiment was tested by PCR and agarose gel electrophoresis. A known concentration of ETEC DNA and DEPC-treated water were used as positive and negative controls, respectively. Fig. 1 showed that the specific band at the molecular size of 450 base pairs was presented in lane 2 which referred to ETEC samples, whereas DEPC-treated water did not present any bands on the gel. This suggested the specificity of primers used in this technique.

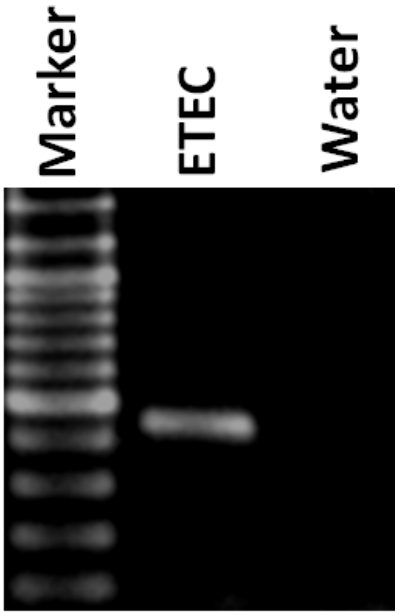

Fig. 1: Specificity of LT primers. Lane 1 showed 100 bps marker, Lane 2 showed the ETEC sample, Lane 3 showed DEPC-treated water, respectively.

3.1. DNA Separation Using Primer-MNP

The extracted DNA from ETEC was isolated by primer-MNPs. The amount of the target DNA bound to the primer-MNPs was measured and Primer-MNPs alone without the incubation with extracted DNA were used as negative control and used to set blank before measurement. Our results showed that the concentration of the separated DNA was increased with increasing the concentration of initial DNA from 0.25 to 1.0 µg/µl, respectively (Fig. 2). This suggests that the separated DNA by the using primer-MNPs was dose dependence manner.

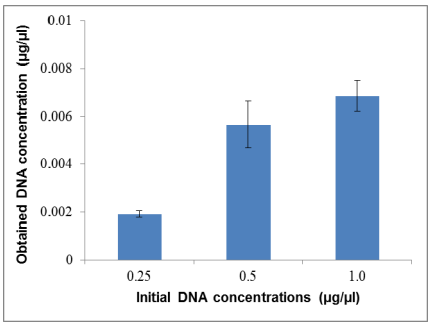

Fig. 2: The amount of obtained DNA concentration (Y-axis) separated by primer-MNPs using initial DNA at various concentration (X-axis).

4. Discussion and Conclusion

The high specificity of LT primers used in the experiment can be suggested their use as the probe for DNA separation or the conventional PCR reaction for bacterial diagnostic. In this work, the primer-MNPs can be used directly to separate the target DNA from the samples. This technique was easily to perform, cost effective, save time and avoided the contamination of the target DNA with others chemical substance. This technique can be applied for the DNA separation in the complicated samples such as the samples collected from the patients or environment such as stool, soil, water sample etc. However, this technique is still ongoing to develop and improve to increase the efficiency.

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