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Trace coliform in drinking water using polymerase chain reaction

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ABSTRACT

Microbial methods for identification of indicative coliforms of drinking water contamination have attracted the attention of scientific societies worldwide. Microbial methods, which lacks the defects and flaws of the previous methods, can be used as a suitable alternative in vitro. In this study, the PCR amplification of the LacZ gene was used to detect and identify coliforms in drinking water resources of villages of Kamyaran City. Results indicated that 5 purified bacteria contained the LacZ gene. It was also found that although microbial methods are highly sensitive and precise, they are not effective for smaller regions due to their shortage of facilities and equipment.

Keywords: LacZ gene, PCR, amplification, coliform

INTRODUCTION

Many problems in developing countries are related to shortage of healthy drinking water, and human health depends on water more than everything else[1]. Recent investigations suggest that the E.*coli* bacterium is the best indicator for assessment of fecal contamination and possible presence of intestinal pathogens[2]. Use of coliforms, as indicator of water contamination dates back to the twentieth century, when, MacConkey described the presence of lactose fermentative microorganisms[3]. In 1914, the United States Public Health Service introduced coliforms as a group of aerobic-anaerobic, Gram negative, bacillus (rod-shaped), and non-sporulating bacteria, which ferment lactose and produce acid and gas (Co_2) within 48 hours at a temperature of 37 to 35 $^{\circ}C$ [4]. The MPN method is currently used to detect coliforms in drinking water. However, due to the limitation of this method, including its long duration (24-96 hours), enzymatic methods were developed to overcome these constraints[2]. Advantages of the PCR method include its high sensitivity, specificity for target microorganisms, high speed, and ability to identify several bacteria simultaneously[5]. Primers used based on LacZ for detecting coliforms in drinking water are encoded by the Galactosidase protein[6]. Presence of the LacZ gene, which encodes Beta-Galactosidase,is the most prominent characteristic of coliforms[7]. The research objective was to identify coliform bacteria in drinking water resources of villages in Kamyaran County (Iran) through molecular analysis of the LacZ 16srRNA gene.

MATERIALS AND METHODS

Chemicals, Instruments, and Sterilization

DNA polymerase enzyme was obtained from SinaClon Company, and phenol, chloroform, boric acid, Bis-tris, isopropanol, ethidium bromide, sodium chloride, hydrochloric acid, and EDTA were obtained from Merck Company. Moreover, the DNA ladder mix marker was obtained from Fermentas Company, and yeast extract,

tryptone, agarose gel powder, sodium hydroxide, and ethanol were obtained from SinaClon Company. The media and solutions were sterilized in an autoclave at a temperature of 121 $^{\circ}C$ and a pressure of 1 atm for 20 minutes. Sterilization of solutions sensitive to autoclave was carried out using filters with 0.22- μm pores. To secure the sterilization process, mediums and solutions were stored at a temperature of 37 $^{\circ}C$ for 24 hours.

Primer

Using Oligo and DNASIS, the primer suitable for proliferation of the gene fragment was prepared. First, the sequence properties such as Tm and delta G loop (ΔG) were examined in Oligo. Primers were diluted with sterilized distilled water with a 1 to 5 ratio to obtain the 20 Pmol/ μL concentration, which is used in PCR reactions. A bacterial colony was inoculated in culture LB broth for the purpose of genome extraction, and it was stored for one night in an incubator. When the bacterium showed logarithmic growth, the medium was divided into 1.5-ml tubes, which were centrifuged for 5 minutes at 5000 rpm and a temperature of 4 $^{\circ}C$. Afterwards, the upper solution was removed and the rest of the genome purification process was carried out on the bacterial sediment using the CTAB-NaCl method[12].

To assess the quality of the extracted species, the agarose gel was used. In this method, the species were exposed to a 6 X loading buffer (1 μl of loading buffer per 5 macroliter of the species) and were put in 0.7% agarose gel wells (0.7 gram of agarose powder was solved in 100 ml of TBE buffer and was boiled). Afterwards, using an electrophoresis tank and electrical current (1-5 V per 1 cm of gel) the species moved along the gel. To observe the species on agarose gel, they were stained using ethidium bromide (with a concentration of 0.5 μ g/ml), and quality of species was examined using a device with UV radiation. To isolate and multiply the LacZ gene from the bacterium, the PCR method was used. The PCR reaction was carried out with 2 primer pairs and Taq polymerase enzyme. The product was exposed to electrophoresis on 1% agarose gel. The species for the PCR reaction were prepared with theTaq polymerase enzyme at a volume of 50 μl .

The PCR program for the LacZ gene in the thermocycler for the Taq polymerase enzyme was arranged.

Assessment of Extracted DNA on Agarose Gel

To assess the quality of the extracted species, the agarose gel was used. In this method, the species were exposed to a 6 X loading buffer (1 μl of loading buffer per 5 macroliter of the species) (Table 2) and were put in 0.7% agarose gel wells (0.7 gram of agarose powder was solved in 100 ml of TBE buffer and was boiled). Afterwards, using an electrophoresis tank and electrical current (1-5 V per 1 cm of gel) the species moved along the gel. To observe the species on agarose gel, they were stained using ethidium bromide (with a concentration of 0.5 μ g/ml), and quality of species was examined using a Gel document device (BioRad Gel Doc 1000) and with UV radiation.

Polymerase Chain Reaction (PCR)

To isolate and multiply the LacZ gene from the bacterium, the PCR reaction was used. The PCR reaction was carried out with 2 primer pairs and Taq polymerase enzymes. The product was exposed to electrophoresis on 1% agarose gel. The species for the PCR reaction were prepared with the Taq polymerase enzyme at a volume of 50 μl . The PCR program for the LacZ gene in the thermocycler for the Taq polymerase enzyme was arranged.

Assessment of PCR Products Proliferated on Agarose Gel

To assess the quality of the extracted species, the agarose gel was used. The species were exposed to a 6 X loading buffer (1 μl of loading buffer per 5 macroliter of the species) and were put in 1% agarose gel wellsgram of agarose powder was solved in 100 ml of TBE buffer and was boiled). Afterwards, using an electrophoresis tank and electrical current (1-5 V per 1 cm of gel) the species moved along the gel. To observe the species on agarose gel, they were stained using ethidium bromide (with a concentration of 0.5 μ g/ml), and quality of species was examined using a Gel document device (BioRad Gel Doc 1000) and with UV radiation.

RESULTS

Analysis of the LacZ Forward Primer

Considering the LacZ gene sequence, the forward primer containing 20 nucleotides with the sequence presented in Table (1) was selected, and its properties were examined in Oligo.

Table (1): Sequence of primers selected for gene proliferation

Primer	Nucleotides containing	Product Size
lacZForward	5′ TTGAAAATGGTCTGCTGCTG 3′	234
lacZReverse	5′ TATTGGCTTCATCCACCACA 3′	

Figure (1) shows the characteristics of the LacZ forward primer. The primer connection temperature was 60.4 $^{\circ}C$ and the delta G (ΔG) of the primer connection to the target gene was -37.2 Kcal/mol. Moreover, the primer lacked inner loop. The possibility of formation of a primer dimer was also studied, and the delta G (ΔG) for the primer dimer reaction was -3.1 Kcal/mol. As seen, the primer characteristics suit the PCR reaction.

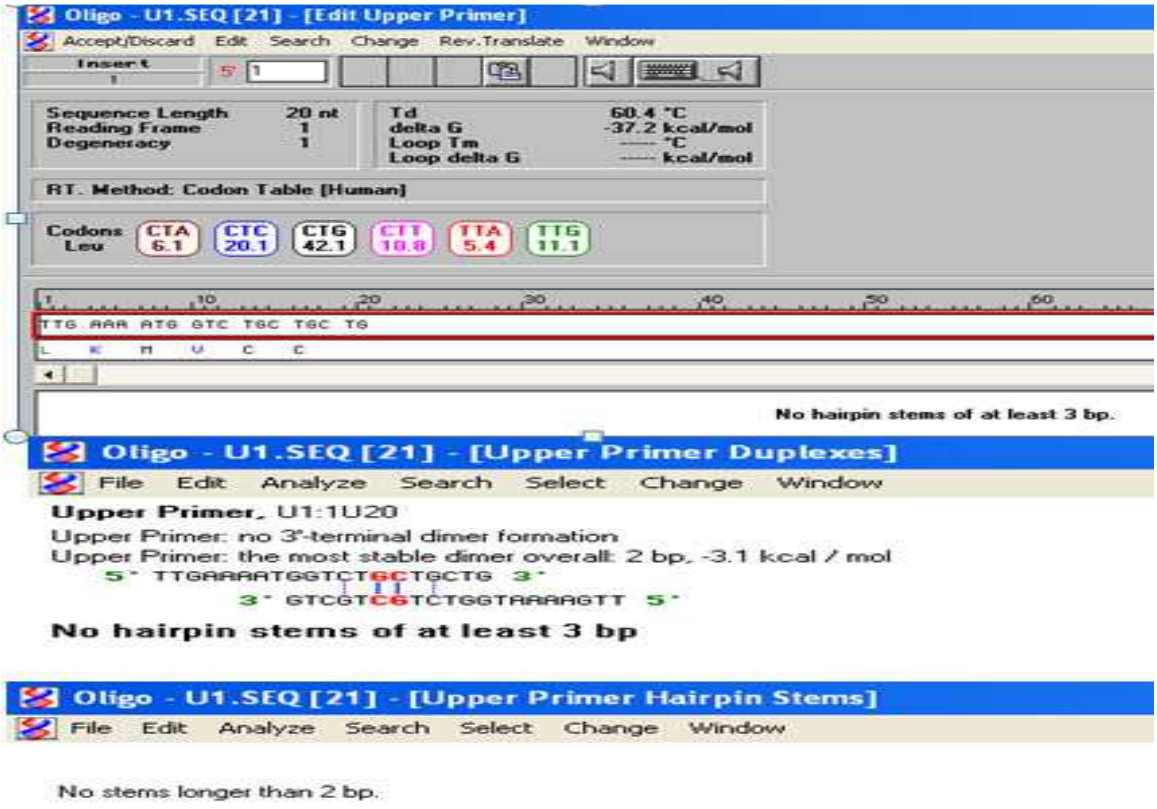

Figure (1): Analysis of characteristics of Lacz forward primer in Oligo

Considering the gene sequence, a 20-nucleotide lagging primer with the sequence was selected and its characteristics were examined in Oligo (Fig. 2). The primer connection temperature was 60.2 $^{\circ}C$ and the delta loop (ΔG) for the reaction of primer connection to the target gene was -37.1 Kcal/mol. The primer had an inner loop with delta G (ΔG) of -0.8 Kcal/mol and the maximum ΔG of primer dimer formation was -5.0. As seen, the primer suits the PCR reaction.

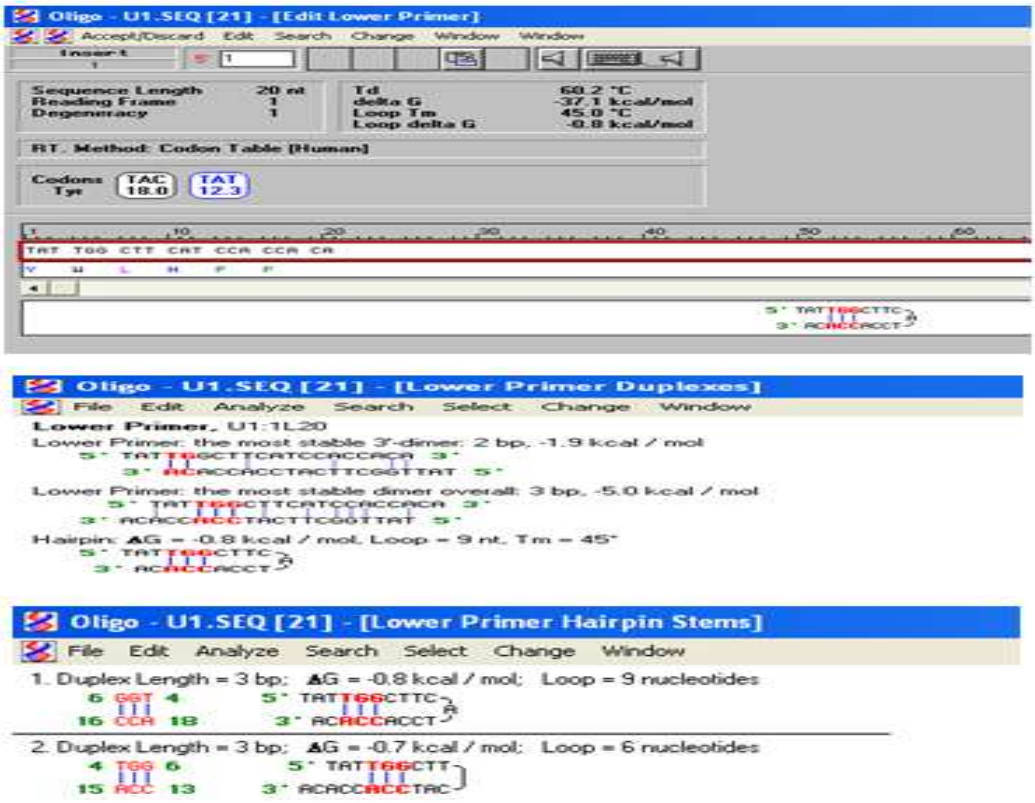

Figure (2): Analysis of LacZ lagging primer characteristics in Oligo

The possibility of formation of primer dimer was studied for the forward and lagging primer molecules. The ΔG of the primer-dimer formation was -6.3 Kcal/mol (Fig. 3). As seen, the primer suits the PCR reaction.

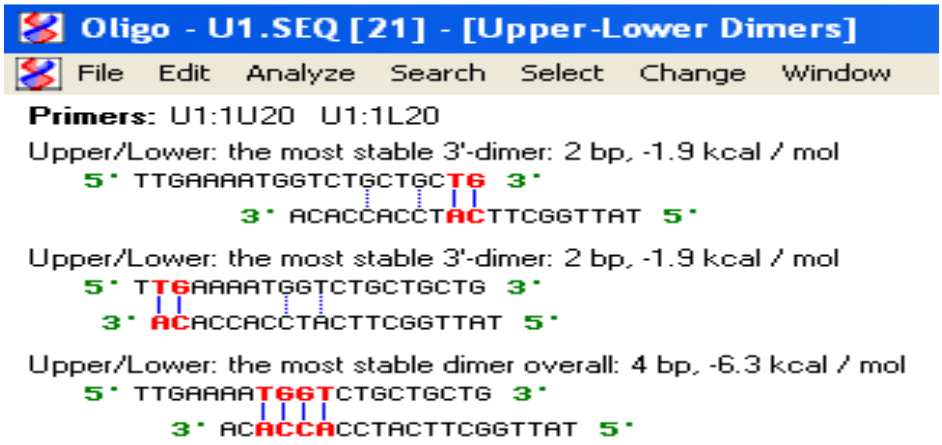

Figure (3): Assessment of possibility of formation of primer dimer related to the LacZ primers in Oligo

The designed primers were synthetized by SinaClon, and 2 μl of the primers were electrophoresed on 12% polyacrylamide gel with a concentration of 100 Pmol. The apparent quality of the synthesized primers was satisfactory (Fig. 4).

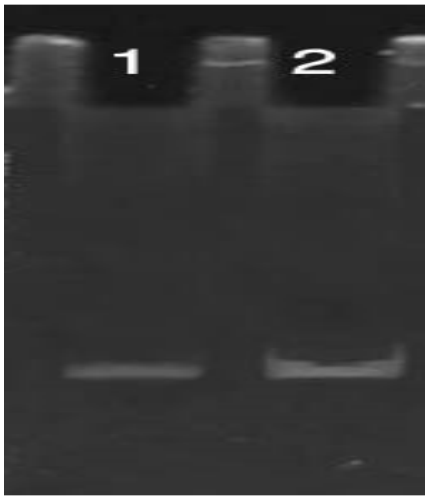

Figure (4): Analysis of LacZ primers on polyacrylamide gel (PAGE)

Column 1) 20-Nucleotide lagging primer; Column 2) 20-Nucleotide forward primer

Primers were examined to be used in the PCR reaction. During a reaction, the E.coli bacterial genome was used as positive control, the LacZ gene with a length of 243 base pairs was proliferated, and the product was electrophoresed on 1% agarose gel.The PCR product lacked non-specific bands, and its size was compared to the DNA molecular size marker and approved (Fig. 6). Throughout a reaction, the E.Coli bacterial genome was used as positive control, the LacZ gene with a length of 243 base pairs was proliferated, and the product was electrophoresed on 1% agarose gel. The PCR product lacked non-specific bands and its size was compared to the DNA molecular size marker and was approved (Fig. 5).

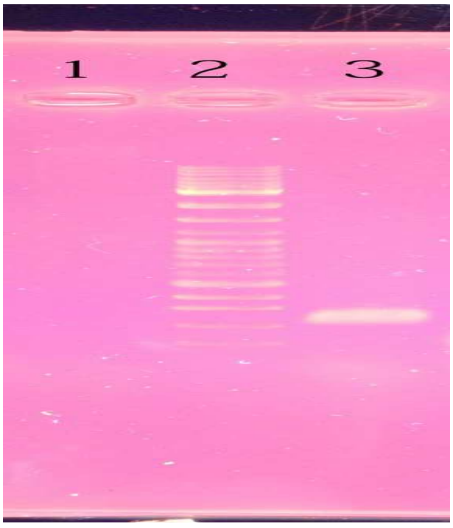

Figure (5): PCR product of LacZ gene on 1% agarose gel

The LacZ gene was amplified with a length of 243 base pairs. The PCR product lacked non-specific bands and its size was compared to DNA molecular size marker and was confirmed. Results indicated that species 1, 2, 3, 4 and 5 contained the LacZ gene (Fig. 6).

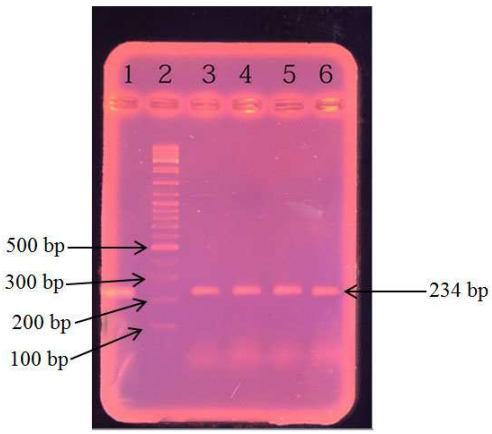

Figure (6): Analysis of purified genomic DNA from bacterial strains on 0.7% agarose gel

Identification of isolated bacteria using the LacZgene primers
Lane 1) Amplified PCR product of LacZ gene from
Lane 2) DNA ladder mix molecular size markerLane
Lane 3) AmplifiedPCR product of LacZ gene from bacterium no. 6
Lane 4) Amplified PCT product of LacZ gene from bacterium no. 7
Lane 5)AmplifiedPCR product of LacZ gene from
bacterium no. 8
Lane 6)AmplifiedPCR product of LacZ gene from
bacterium no. 9

Column 2) DNA ladder mix marker
Columns 3, 4, 5, 6, and 7) Purified genomic DNA species from bacteria under study

DISCUSSION

In this research, PCR amplification of LacZ gene (Beta-Galactosidase gene) was used to identify coliform bacteria. The PCR technique is a simple, sensitive, specific and cost-effective method for identifying different genera of genes and biologic factors. It is simple but lacks the limitations of former techniques and thus it is widely used in laboratories to identify pathogens. This technique was for the first time described by Kary and Mayer, which included a primer pair from the protected Stx1 and Stx2 areas in homologous genes [14]. Actual estimation of indicative bacteria in public drinking water resources with the PCR technique was proved by previous studies by other researchers [2]. For instance, Bej*et al*. [15] used LamB and LacZ gene amplification (encoder of beta-galactosidase) for the first time to prove presence or absence of coliform bacteria and *E.Coli*in drinking water.Tantawiwat*et al*. [16] used the LacZ gene to control presence of E. Coli and stated that this method could be used to identify bacteria in drinking water due to its high speed and sensitivity.

In the present research, after designing the primer, its accuracy was examined in BLAST and it showed the highest specificity with the LacZ genes of coliforms in the gene bank. Of all of the species under study, 5 species contained LacZ gene, which proves their coliform nature. However, presence of the LacZ gene, which encodes beta-galactosidase) is adequate for identifying the coliforms [7]. In the PCR technique, amplification of the gene (beta-galactosidase) was used to identify coliforms and the Beta Duiidaglucuronidase gene is used to specifically detect *E.Coli*[16]. Today, a combination of three different PCR methods (including WecG, LacZ and 16S rRNA genes) is used to identify all coliforms (TC) in 100 ml of a water sample [17].

CONCLUSION

In this study, samples were obtained from 50 rural drinking water resources in Kamyaran County. Using Oligo and DNASIS, the primer suitable for proliferation of the gene fragment was designed, and the genome purification process was conducted on bacterial sediments using CTAB-NaCl. Among the purified bacteria, five bacteria contained the LacZ gene, which proves they are coliforms. To determine the genera of coliforms in subsequent studies, it is recommended to use the Yaio gene, which is a proper alternative to Uida. Although this method is highly sensitive and precise, it cannot be employed in small cities as it requires laboratory equipment, instruments, and substances.

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