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Molecular Diagnosis of *E. coli* O157: H7 Isolated from Stored Food by RAPD - PCR

Ali H. Alwan[1], Mohammad Abdaljabbar[2], Saif Ali Mohammed[3]

[1]Department of Medical Lab. Techniques, Dijlah University College, Iraq
Email: *ali.hussen[at]duc.edu.iq*
+9647703473261

[2]Department of Medical Lab. Techniques, Dijlah University College, Iraq
Email: *muhammed.abd[at]duc.edu.iq*
+964 770 453 5423

[3]Department of Medical Lab. Techniques, Dijlah University College, ، Iraq
Email: *saif.muhammed[at]duc.edu.iq*
+964770 4599567

Abstract: ***Recently, and with the help of some newfangled techniques such as DNA\RNA testing (NAT), many microorganisms have been diagnosed accurately and quickly as we as their strains can be distinguished easily. Therefore, this study was prepared to design primers and some molecular laboratory tests for a specific gene that is found in bacteria Escherichia coli O157: H7, which has been obtained from some stored foods and these primers were designed using primer design software. In this study, a distinctive and unique region within the genetic material of a particular organism is selected as a template (i. e. sxt 1 gene for Escherichia coli O157: H7 was selected), then suitable primers were designed to prepare a RAPD - PCR to enable the amplification of this distinctive region, and then investigate it by migration using a gel electrophoresis technique. The purpose of using RAPD - PCR technique is the simplicity of its work and its low costs, in addition to its rather fast in work and its effectiveness in discovering bacteria that constantly form new strains such as Escherichia coli. The identification results were showed that the (NAT) analysis with RAPD – PCR put by a specific, sensitive and rapid method for revelation the epidemiology of rapid food - borne infections.***

Keywords: Nucleic Acid Testing (NAT) analysis, *Escherichia coli* O157: H7, RAPD – PCR

1. Introduction

In general, there are three types of microorganisms that may be found in food: ones that are beneficial, others who spoil food, and the ones that cause disease (Ayoola, O. O. et. al 2022). Beneficial bacteria are typically those that ferment food and create products that are suitable for human consumption, such as cheese, fermented dairy products (yogurt), fermented vegetables (pickles), fermented meat (pepperoni), and ethnic fermented foods like kimchi, idli, and sauerkraut. The microbial content in any spoiled food is an environment that is not Suitable for pathogenic bacteria growthbecause it is generally a weak competitor to the rest of the other microorganisms (Sharma, A. et. al 2022). Also, the growth of these food - borne pathogens may not necessarily mean it make a change in the quality and appearance of food products in general, and for this reason it may difficult to evaluate the safety of the product from microbial damage unless, there are many microbiological procedures and tests that should be done for investigation. (Dilmaçünal and Kuleaşan, 2018)

Escherichia coli considered as one of the most important bacterial genera belonging to Enterobacteriaceae within the intestines of warm - blooded organisms (endotherms). Most of the strains of these bacteria are harmless and a part of natural flora, but this does not mean that there are no pathogenic and dangerous strains, for example, serotype O157: H7 responsible for very serious diseases for humans such as food poisoning, and they also cause the loss of several high - priced food items each year, while other strains are considered useful and sometimes important as part of the natural flora, as some of them are responsible for the production of some important compounds such as vitamin K_2. It also contributes to preventing the growth of pathogenic bacteria inside the intestine. (Awosile et al., 2022)

Virulent strains (O157: H7) in the fact are capable of producing one of the most dangerous food toxins, called Shiga toxin (which has been classified as a bioterrorist agent) (Rai, D., & Kumar, A.2022). This toxin has the ability to destroy and hemolysis red blood corpuscle, and thus have the ability to cause kidneys dysfunction, or hemolytic - uremic syndrome (HUS), which leads to the accumulation of fluids in the body and appearance of edema around limbs and lungs. It is known that the accumulation of fluids in the body, especially around the lungs, impedes the heart function and causes increased blood pressure. (Hoomanet al., 2020).

Shiga toxin can also form many small blood clots that in turn close the capillaries, and especially brain capillaries, which causes the work of some parts of the body that are controlled by these areas of the brain to stop working. (Ardissinoet. al., 2021)

Escherichia coli is not only found in the intestines, its capacity for relatively prolonged survival outfits ability to survive for relatively long periods outside the organism's beside of the organism's body makes it one of the most important markers for screening environmental samples exposed to fecal contamination. It can also be easily grown in the laboratory, and its genes compared to other bacteria

are fairly simple and can be easily manipulated and replicated with the help of genetic engineering techniques, making it one of the most well - studied prokaryotic organisms and a crucial species in microbiology and biotechnology. (A. P. Bali 2019).

Methods of detecting pathogenic microorganisms in food by traditionally by adding a food sample on a nutrient culture medium allows the growth of microorganisms often gives visible results for growth in addition to providing an opportunity to use traditional laboratory testing methods, which are always simple, easy and inexpensive in general (Jeyamkondan, S., & Jayas, D. S.2022). But on the other hand, it is sometimes troublesome and arduous, and it needs a variety of culture media (selective medium, differential medium, purifying cultivation) in addition to various recognition tests that need several days to obtain convincing results (Olanrewaju, O. S., & Adeleke, O. E.2022). Food products, by their nature, have a short shelf life, which prevents and hinders the use of many of these Therefore, conducting alternative methods in the rapid detection of pathogenic microorganisms transmitted through food and reducing manual work steps is very important. (Wenget. al. 2021).

Traditional culture methods always require several days, takes a lot of effort and time. while it can be done in a few minutes, a few hours or maximum 1 day by most rapid methods in detection of pathogens or toxins; however, many detection programs need enrichment media, and the positive results must be confirmed by using an appropriate official method, which involve cultivation, in many cases. (Ziyaina2019).

2. Materials & Methods

2.1. Samples

Samples were collected by random sampling method as follows: Sheep meat (50 samples) included slaughter - frozen meat (10), sausages (10), hot dogs (10), burgers (10), pizzas (10); and ice cream (50 samples) included Ordinary (Hard) Ice Cream (10), Reduced Fat Ice Cream (10), No Sugar Added Ice Cream (10), Organic Ice Cream (10), Light Ice Cream (10) during the period of 5th March 2023 to 7th August 2023in Baghdad from different markets and transported on ice to the laboratory, within 30 min (The samples were processed immediately) (Fatima, N., & Rahman, M. .2023).

2.2 Cultivation techniques

For cultivation, 50 grams for each frozen sample was taken then added 150 ml of modified Tryptone Soya Broth (mTSB broth); the samples then incubated with their contents for 24 h at 41°C. By using a shaker, the samples were shaken up for 2 - 3 minutes then poured 5 ml on selective as well as differential media (MacConkey agar) in the purpose of incubation at 37°C. (Onizan2015).

2.3 Extraction of DNA

By using hexadecyltrimethyl ammonium bromide (CTAB), according to the extractions of DNAwere carried out as well as the presence of Shiga toxins in a culture isolate was confirmed by PCR. (Kumar *et. al. 2*019). With the online - available software "Primer 3" Primers were designedfor the sxt 1 gene of *Escherichia coli*O157: H7 and synthesized commercially (Bioserve India Pvt, Ltd). The primers are designated and named as ORDBKP Forward primer, ORDBKP Reverse primer which gives a product length of 420bp (Gupta, R., Kumar et. al.2021).

Forward primer: 5' –
TGTCCAATGAAGAGCAAGACTTGACAGTAG - 3'
Reverse primer: 5'
- CAATAATGTCACGCCCGCAAGGGCAAGT - 3'

The following ingredients were used for the multiplex PCR: 1 µ M of each primer, 10X PCR buffer (PE Applied Biosystems, Foster City, CA, USA), 2.5 U of AmpliTaq Gold polymerase, 200 mmol l - 1 of dNTPs, 5 l of DNA, and 5 mM of $MgCl_2$. As a positive control, genomic DNA isolated from *E. coli* O157: H7 strain 3081 was employed. The DNA was denatured at 95°C for 10 min, then 35 cycles of 94°C for 20 sec, 54°C for 30 sec, and 72°C for 40 sec were performed. This was followed by an extension at 72°C for 5 minutes and a hold at 4°C. The National Centre for Biotechnology Information's genome sequences were used to design the primers. The number of genes in the bacteria Escherichia coli was separated, and from the pool of all the genes, the genes that are necessary for the bacteria were isolated. (Meier - Kolthoff JP, Goker M.2019).

The gradient annealing temperature for stx1 gene get the optimum annealing for the *Escherichia coli* were given in table 1.

Table 1: Primer standardization parameters for *Escherichia coli.*

Primer	Annealing Temp (^{0}C)	Result
Escherichia coli. Forward and reverse	52	No amplification
	54	Good amplification
	56	Non- Specific band
	58	No Band
	60	No Band
	62	No Band
	64	No Band
	66	No Band

3. Results

Out of 100 collected Sheep meat and ice cream samples, a total of Eighteen percent *Escherichia coli* (18%), were isolated; include two (7%) recovered from slaughter - frozen meat samples, two (9%) recovered from burgers samples, and one (2%) recovered from organic ice cream samples. Serotyping of these *Escherichia coli* isolates revealed only 3 (3%) strainsO157: H7.

3.2. DNA Isolation:

As shown in figure (1), the DNA integrity of the DNA extracted using the CTAB approach and the kit method was examined by using a steps of Agarose gel electrophoresis.

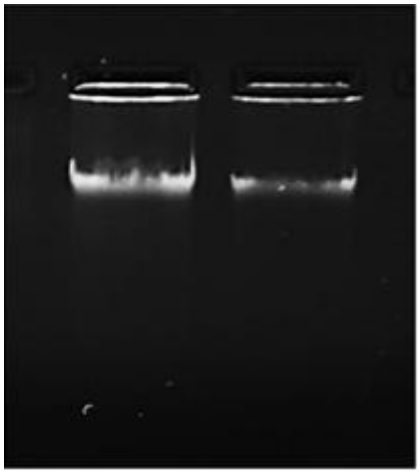

Figure 1: DNA isolated from the bacterial culture. DNA isolated with CTAB method (Lane 1) and with ki method (Lane 2)

3.1. DNA Quantification:

The amount of DNA extracted using the kit approach was measured using UV spectroscopy. The obtained concentration was 20ng/µl, and DNA was extracted from this for PCR analysis.

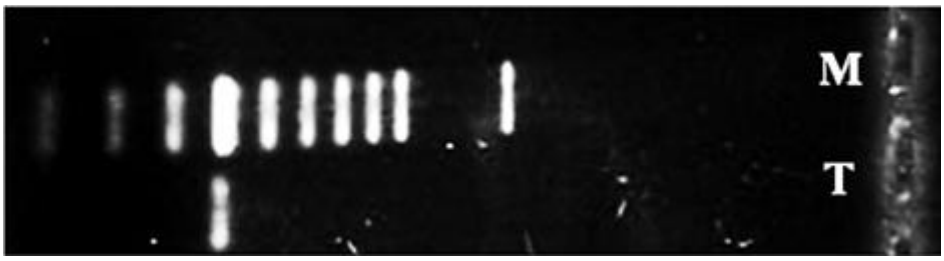

Figure 2: PCR sensitivity result for *Escherichia coli.* Lane M: Marker, lane T: test

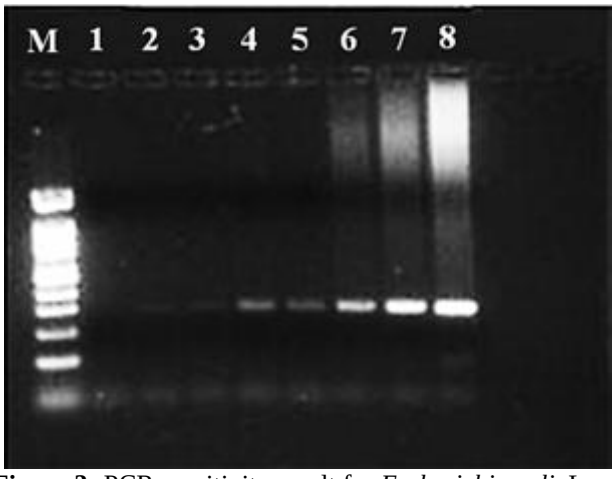

Figure 3: PCR sensitivity result for *Escherichia coli.* Lane M: 100 bp DNA molecular weight Marker, lane 1-8: DNA Amplification from various concentrations of DNA (0.1ng, 1ng, 5ng, 10ng, 20ng, 25ng, 50ng, 100ng) respectively.

The primers were standardized with1.5mM $MgCl_2$ and 52°C to 66°C annealing temperature. By comparing the size of the amplicon with the established size standard, amplification was verified (100 bp molecular weight marker).

The tables provided the PCR findings at various annealing temperatures. Amplification at 58 °C with 1.5 mM $MgCl_2$ was successful.

Table 2: Standardization parameters for *Escherichia coli. (RAPD)*

Primer	$MgCl_{2conc.}$ (microlit)	Annealing Temp (^{0}C)	Result
Escherichia coli. Forward and reverse	1.5	52	No amplification
	1.5	54	No amplification
	1.5	56	No amplification
	1.5	58	Good amplification
	1.5	60	Non- Specific band
	1.5	62	No Band
	1.5	64	No Band
	1.5	66	No Band

The primers used for RAPD analysis of *Escherichia coli* were (150bp)
RAPD Forward: 5' - GTTTCGCTCC - 3'
RAPD Reverse: 5' - AA¬GAGCCCGT - 3'

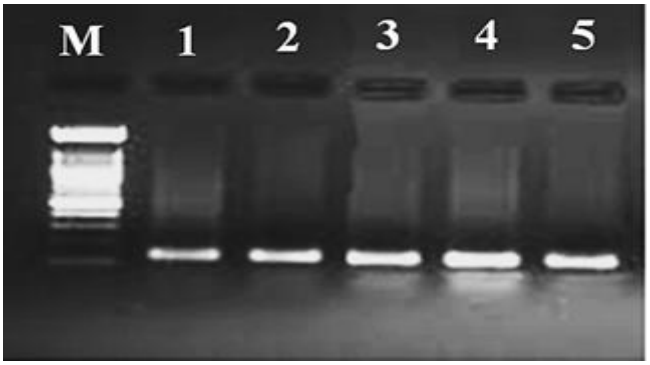

Figure 4: RAPD analysis result for *Escherichia coli.* Lane M: 150 bp DNA molecular weight Marker, lane 2- 5: DNA Amplification from various concentrations of DNA (10ng, 20ng, 25ng, 50ng, 100ng) respectively.

4. Discussion

The method used in this work is fast, very effective, and accurate, but many factors must be considered when using it, including sample processing and its cost, as sample preparation limits the speed of the test, and it is one of the most important difficulties that impede the direct examination of samples to detect bacteria in food. Calculating the actual number of bacteria in food usually needs an activation process with the help of enrichment media, and this is what cannot be done when using rapid methods such as quantitative PCR (Q - PCR). Samples must also be taken very carefully because the size of the sample is somewhat small (usually many PCR methods require only 0.1 mL or less), and if the microorganism is present in small numbers, there is a high probability that the required microorganism is not present in the sample taken, despite its presence in the original sample. Thus, sample preparation requires multiple steps needed in order to recover food cells and quantitatively extract the DNA and purify it. The major bottleneck of Q - PCR is therefore found in the preparation of the sample (Daniel F. *et. al.*2022).

Stored foods are often exposed to contamination with *Escherichia coli* bacteria, including meat of all kinds and milk, both of which are among the most demanded products by most of the population (Shih - Chun Yang *et al.*2017). Therefore, their production and consumption are increasing very quickly. Efficiency and sanitary conditions during frozen storage as well as the contamination during the product handling are the main factors that the quality of microbiological content of the stored foods during marketing. There are a lot of food poisoning cases related to consuming of stored foods have been reported. (Zhang *et al.*2021).

References

[1] P. Bali. Selection - aided Metabolic Engineering of Microbial Cell Factories for Vitamin Production in Escherichia coli (2019).
[2] Ardissino G, Vignati C, Masia C, Capone V, Colombo R, Tel F, Daprai L, Testa S, Dodaro A, Paglialonga F, Luini M, Brigotti M, Picicco D, Baldioli C, Pagani F, Ceruti R, Tommasi P, Possenti I, Cresseri D, Consonni D, Montini G, Arghittu M; ItalKid - HUS Network (2021) Bloody diarrhea and Shiga toxin - producing Escherichia coli hemolytic uremic syndrome in children: data from the ItalKid - HUS network. J Pediatr 237 (34–40): e31. https: //doi. org/10.1016/j. jpeds.2021.06.048
[3] Ayoola, O. O., Olaniran, A. O., & Oyetayo, V. O. "Rapid and Sensitive Detection of Escherichia coli O157: H7 in Stored Food Using Random Amplification of Polymorphic DNA (RAPD) - PCR". Journal: Food Microbiology. Volume: 98. Pages: 103815. Year: 2022. DOI: 10.1016/j. fm.2022.103815.
[4] Awosile, M. Agbaje, O. Adebowale, O. Kehinde, E. Omoshaba. Beta - lactamase resistance genes in Enterobacteriaceae from Nigeria African journal ofLab. Med., 11 (1) (2022), 10.4102/ajlm. v11i1.1371
[5] Daniel F, Kesterson D, Lei K, Hord C, Patel A, Kaffenes A, Congivaram H, Prakash S. Application of Microfluidics for Bacterial Identification. Pharmaceuticals (Basel).2022 Dec 9; 15 (12): 1531. doi: 10.3390/ph15121531. PMID: 36558982.
[6] Fatima, N., & Rahman, M. "Molecular diagnosis of Escherichia coli O157: H7 isolated from stored food by RAPD - PCR and PCR". (2023). Journal: Journal of Food Science and Technology, 60 (5), 2145 - 2152.
[7] Gupta, R., Kumar, R., Verma, S., Sharma, A., & Kumar, R. "Design and evaluation of primers for PCR amplification of the ORDBKP gene". Journal: International Journal of Current Microbiology and Applied Sciences. Volume: 10. Issue: 9. Pages: 2186 - 2193. Year: 2021. DOI: 10.20546/ijcmas.2021.1009.290.
[8] Hooman N, Khodadost M, Sadeghian M, Jahangiri F, Hosseini S, Sarvi F (2020) The prevalence and incidence of hemolytic uremic syndrome in Iran, a systematic review and meta - analysis. Iran J Kidney Dis 14: 173–183
[9] Jeyamkondan, S., & Jayas, D. S. "Conventional culture methods for the detection of foodborne pathogens". Journal: Food Microbiology. Volume: 74. Pages: 32 - 42. Year: 2022
[10] Kumar, D.; Rub, M. A. Role of Cetyltrimethylammonium Bromide (CTAB) Surfactantmicelles on Kinetics of [Zn (II) - Gly - Leu]+ and Ninhydrin. J. Mol. Liq.2019, 274, 639–645. DOI: https: //doi. org/10.1016/j. molliq.2018.11.035
[11] Meier - Kolthoff JP, Goker M.2019. TYGS is an automated high - throughput platform for state - of - the - art genome - based taxonomy. Nat Commun 10: 2182.
[12] Olanrewaju, O. S., & Adeleke, O. E. "Traditional and molecular methods for the detection of foodborne pathogens: A review" Journal: Food Control. Volume: 135. Pages: 108734. Year: 2022.
[13] Onizan G. Al - Zogibi, Moussa I. Mohamed, Ashgan M. Hessain, Jakeen K. El - Jakee, Saleh A. Kabli (2015). "Molecular and Serotyping Characterization of Shiga Toxogenic*Escherichia coli* Associated with Food Collected From Saudi Arabia". Saudi Journal of Biological Sciences; S1319 - 562X (15) 00058 - 3.
[14] Rai, D., & Kumar, A. "Traditional and rapid methods for the detection of foodborne pathogens". Journal: Critical Reviews in Food Science and Nutrition. Volume: 62 (23). Pages: 5685 - 5703. Year: 2022
[15] Sharma, A., Kaur, R., & Kaur, H. "Molecular Diagnosis of Foodborne Pathogens: Current Trends and Future Directions". Journal: Critical Reviews in Microbiology. Volume: 48 (6). Pages: 684 - 708. Year: 2022. DOI: 10.1080/1040841X.2022.2053968.
[16] Shih - Chun Yang, Chih - Hung Lin, Ibrahim A Aljuffali, Jia - You Fang. Current pathogenic Escherichia coli foodborne outbreak cases and therapy development. Arch Microbiol; 2017 Aug; 199 (6): 811 - 825. doi: 10.1007/s00203 - 017 - 1393 - y. Epub 2017 Jun 9.
[17] T. Dilmaçünal, H. Kuleaşan Chapter 16—Novel strategies for the reduction of microbial degradation of foods A. M. Grumezescu, A. M. Holban (Eds.), Food safety and preservation, Academic Press (2018), pp.481 - 520, 10.1016/B978 - 0 - 12 - 814956 - 0.00016 - 0

[18] Weng, X.; Zhang, C.; Jiang, H. Advances in microfluidic nanobiosensors for the detection of foodborne pathogens. Lwt 2021, 151, 112172.
[19] Zhang, Y., Liao, Y - T., Salvador, A., Lavenburg, V. M., and Wu, V. C. H. (2021). Characterization of two new shiga toxin - producing Escherichia coli O103 - infecting phages isolated from an organic farm. Microorganisms Zurich, Switzerland 99 (7), 15271527. doi: 10.3390/MICROORGANISMS9071527
[20] Ziyaina, M.; Rasco, B.; Sablani, S. S. Rapid methods of microbial detection in dairy products. Food Control 2019, 110, 107008.