

Genetic Diversity of *E. coli* O157:H7 Isolated from Aleppo River water samples using Random Amplified Polymorphic DNA (RAPD) marker

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In Syria, there is not a lot of research that studies *E. coli*, and its serotypes by PCR technology. *Escherichia coli* O157:H7 is a very important serotype, and one of the Shiga toxin-producing (classified as a bioterrorism agent). Aleppo River is highly polluted by the sewage water that transferred directly without processing. The farmers use the river water for irrigating vegetables and leafy greens directly. So, we detected *E. coli* and especially serotype *E. coli* O157:H7 in water samples that were taken from villages passed by the river. Sites of villages: Handarat (A), Industrial Zone (B), Souq Al-hal (C), Bustan Palace area (D), a huge area of vegetables. Al-sheikh Saeed (E), Al-wodaihi (F), Zietan (G), and Jezraya (H).

In this study, we have fulfilled a total count of bacteria, the census total coliform, and *Escherichia coli*, as well as the serotype *E. coli* O157:H7 in water samples by different Bacterial Media, Nutrient Agar, MacCONKEY Agar, Violet Red Bile Agar, Sorbitol-MacCONKEY Agar, and EMB agar [1].

For DNA Extraction we used Protocol for Preparation of Genomic DNA from Bacteria [2]. The molecular characterization was done for eight strains of *E. coli* isolated from collected samples from the sites of all villages. The samples were inoculated on blood agar and suspicious colonies, then transferred to EMB and MacConkey agar using a primer (COL-1) in RAPD technic. Molecular characterization also performed eight strains of serotype *E. coli* O157:H7 isolated in medium (Sorbitol-MacCONKEY Agar) from the following sites (Al-sheikh Saeed, Al-wodaihi, Zietan, and Jezraya), then by primers (OPA-03, OPA-13 OPC-12, OPE-20) in RAPD.

The results showed significant differences between collected samples. The total count of bacteria and total coliforms in the first site (Handarat) for water samples were the lowest, but in the seventh site (Jezraya) were the highest among all samples, at the beginning of village Jezraya the wastewaters from sewage treatment station and all villages flows to the main river, and it increased the bacteria. The results showed also the existence of *E. coli* in all sites, and *E. coli* O157:H7 in Al-sheikh Saeed, Al-wodaihi, Zietan, and Jezraya.

Extracted DNA from samples was amplified by RAPD. after electrophoresis, eight different bands were detected from isolated strains of *E. coli*. These results refer to the great genetic diversity of *Escherichia coli*. For serotype *E. coli* O157:H7 thirty-two different bands were detected, and primer OPA-13 was the best primers used, RAPD analysis had the high capacity of discriminatory for typing *E. coli* isolates. Because of its simplicity and rapidity.

References

- 1) MERCK. Microbiology Manual.1, 2000.
- 2) Jones, and Bartlet. Modified from Experimental Techniques in Bacterial Genetics, 1990.
<http://www.thelabrat.com/protocols/BacterialDNA.shtml>

Illustrations

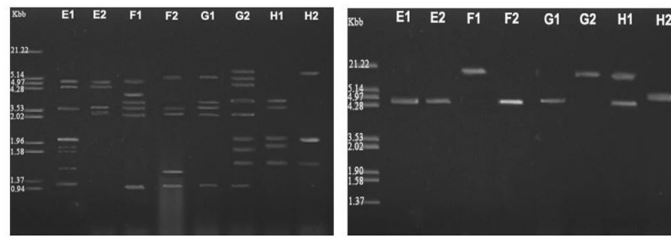


Рис. 1. RAPD-PCR test of *Escherichia coli* O157:H7 with primers OPA-13, OPE-20. M: lambda EcoRI-Hind III digested DNA marker. (E1-H2) *E. coli* O157:H7 isolated from water samples.