PHENOTYPIC AND MOLECULAR DETECTION OF ENTEROPATHOGENIC *E. coli* IN FROZEN BURGER MEAT

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Introduction. One of the most common causes of diarrhea and even death in less than two years of age infants in developing countries is Enteropathogenic *E.coli* (EPEC), a facultative Gram-negative rod-shaped bacterium, member of the *Enterobacteriaceae* family. Its detection in foods is mainly performed by traditional culture and serological methods, which lasts more than a week. (1, 2). The aim of this work was to develop the specific detection of an EPEC strain by amplifying both *bfp* and *eae* genes coding for the bundle-forming pili and the intimine proteic subunits, respectively, with multiplex-PCR (plex-PCR) (3, 4), of bacterial DNA extracted from commercial frozen burger meat in order to reduce its detection time compared with traditional culture techniques.

Methods. Primers for PCR amplification of *eae* and *bfp* genes from EPEC were designed using Genbank sequences, then were PCR amplified and sequenced. Resultant sequences were identified by comparing against the non-redundant GenBank database with the BLAST tool of the NCBI homepage. Bacterial chromosomal DNA was extracted from a pure culture of the EPEC strain inoculated in Luria Broth (37°C/24h), and from the inoculated frozen burger meat (SuKarne) enrichment culture of the strain (final concentration 1 CFU/10g), according to BAM recommended technique (5). Extracted chromosomal DNA was used as template for the 50 µL plex-PCR reaction. Reagents proportions were according to suppliers recommendation (Fermentas),the final primers concentration were 0.1 µM for *eae* gene and 0.2 µM for *bfp* gene, respectively. Plex-PCR conditions were: 95°C, 5 min, 1X; 95°C/1min, 56°C/1min, 72°C,1.5min, 30X; 72°C/5min, 1X; 4°C/∞. Amplicons were visualized with an agarose gel electrophoresis.

Results. Sequence analysis of EPEC *bfp* and *eae* correspond genes matched with some EPEC sequences in the GenBank database (data not shown). The plex-PCR products for genes *bfp* and *eae,* amplified from extracted bacterial DNA from freeze burger meat inoculated with EPEC strain and sampled at 0, 3 and 24 h resulted in two amplified bands of 2500 bp and 900 bp respectively, and were similar to those obtained with the pure strain chromosomal DNA plex-PCR (data not shown). It was observed and increase in band intensity according with incubation time during the enrichment process (Figure 1; lines 4, 5 and 6).

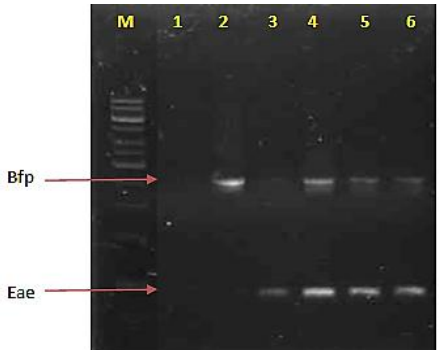

Figure 1. Agarose gel electrophoresis for plex-PCR from the bacterial chromosomal DNA of frozen burger meat inoculated with EPEC 100V. Line M, 1 Kb ladder (Fermentas); Line 1, *E. coli* ATCC 11229; Line 2, EPEC *bfp* gene; Line 3, EPEC *eae* gene; Line 4, plex-PCR 0h; Line 5, plex-PCR 3 h; Line 6, plex-PCR 24 h.

Conclusions. A plex-PCR technique for the specific detection of an EPEC strain present in commercial frozen burger meat by amplifying the *bfp* and *eae* genes were developed. It was possible to reduce the detection time for this strain from more than a week in the traditional culture technique to less than 30 hours. EPEC strains were not detected by using this method, among the not-inoculated frozen hamburger samples analyzed,

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