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Simultaneous detection of foodborn pathogenes *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* spp. By Multiplex PCR in ready–to–eat vegetables

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Introduction: Foodborne pathogens are a major public health problem. Despite the increase in management and consumer interest in food safety, the number of food poisoning cases has been continuously increased in recent years. For example in 2010, human salmonellosis cases were reported in Europe and in 2011, a multistate foodborne *E.coli* O157:H7 outbreak affected 8 people and also a larger multistate *Listeria monocytogenes* outbreak caused 146 infections and 29 deaths. Traditional culture methods for detection of microorganisms in food are laborious and time consuming. Due to Economic impact of foodborne contamination and diseases, there are great efforts to develop more sensitive methods for rapid and accurate pathogen detection and identification in foodstuffs. Recently, molecular techniques such as PCR based DNA amplification have been employed that offer several advantages over the classical microbiological methods, such as shortening time of analysis, lowering detection limits, increasing specificity and having potential for automation. In this study we used multiplex PCR system, for the simultaneous detection of the three pathogens (*salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7) in artificially inoculated lettuce.

Materials and methods: Bacterial strains used in this study, Salmonella entrica serovar Typhimurium PTCC1709, Listeria monocytogenes PTCC1298 and E. coli O157:H7 NCTC12900, were grown in Trypticase Soy Broth at 37°C and then serial dilutions of strains from 100 to 106 cfu/ml (1 to 1000000 cfu/ml) were prepared using normal saline (0.85 g/l). Lettuce samples were cut into pieces of 5 ± 0.2 grams. To sterilize, the pieces overwhelm in 3% sodium hypochlorite solution for 15 min and in order to eliminate extra chlorine ions, the samples moved to sterile distilled water containing some drops of 1% sodium thiosulfate for 2 min. Bacterial strains were inoculated on lettuce as pure and mixed cultures separately. For the mixed cultures 100 µl of each bacterial strain inoculated on lettuce (in total 300 µl). The eluted bacteria were re-suspended in 100 µl of sterile normal saline and used for DNA extraction. DNA extraction was performed on each bacterial strain before and after inoculation to lettuce. The target genes were the rfb gene (antigen O157 producer) for E. coli O157:H7, the invA gene (invasion protein A) for salmonella spp., and the prfA gene (transcriptional activator of the virulence factor) for L. monocytogenes. The genes described here have been used as the most specific and reliable genetic targets for the above microorganisms. As an internal control gene, the 16S rRNA gene was targeted in the presence of bacterial DNA.

Monoplex PCR reactions were performed in a final volume of 25 µl. Master mix composition was as follows: PCR buffer 10X, 2.5 µl; MgCl2 25mM, 2.5 µl; Taq DNA Polymerase 5 U/µl, 0.2 µl; dNTPs 10 mM, 0.4 µl; F/R primers 10 pmol, 1µl; extracted DNA as template, 2 µl and distilled water, 15.4 µl. Thermal cycler conditions were as follows: predenaturation at 94°C for 5min; 35 cycles consisting of denaturation at 94°C for 30s, annealing at 55°C for 30s and extension at 72°C for 60s; and final elongation at 72°C for 7 min. Multiplex PCR reactions were performed in a final volume of 25µl using 4 µl of total extracted DNA from three pathogens as template (mixture of three bacteria). Master mix composition was as follows: PCR buffer 10X, 2.5 µl; MgCl2 25 mM, 2.5 µl; Taq DNA Polymerase 5U/µl, 0.5 µl; dNTPs 10mM, 1 µl; EC-F/R, 1 µl; SAL-F/R, 0.8 µl and LIS-F/R, 1 µl (concentration of each primer was 10 pmol) and distilled water 8.9 µl. Thermal cycler conditions were as follows: pre-denaturation at 94°C for 3 min; 35 cycles consisting of denaturation at 94°C for 30s, annealing at 57°C for 30s and extension at 72°C for 90s; and final elongation at 72°C for 10 min. PCR products were visualized via gel electrophoresis, with 1% agarose gels.

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Results and Discussion: The main focus of this work was on the use of rapid method instead of culturing stages for detection of foodborne pathogens from ready-to-eat vegetables. Lettuce was used as a model for ready-to-eat vegetables and inoculated with *E. coli* O157:H7*, salmonella* spp. and *L. monocytogenes*.

Multiplex PCR was performed after elution of bacteria from lettuce and the test provided a detection limit of 1 cfu/1g for three foodborne pathogenic bacteria

The primer selection and multiplex PCR optimization allowed the setting of a robust method with performances comparable to a duplex PCR system, when tested on a complex food system. The sensitivity and robustness of the method proposed, together with its ability to perform well on a complex food matrix, make it a suitable method to be implemented in control laboratories for the detection of the target pathogens in food samples.

Key words: Multiplex PCR, Foodborne Pathogens, Ready-To-Eat Vegetables, Rapid Detection