TITLE: RAPID DETECTION AND IDENTIFICATION OF YERSINIA ENTEROCOLITICA SEROTYPE O:3 USING A DUPLEX-PCR ASSAY

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ABSTRACT:

Yersinia enterocolitica, a member of the Enterobacteriaceae family, is a zoonotic agent that causes gastrointestinal diseases and some extraintestinal disorders in humans. Y. enterocolitica ssp palearctica bioserotype 4/O:3 is the primary pathogenic bioserotype in Europe, where it has a high public health relevance. The isolation and identification of Y. enterocolitica from various sources on selective media have been seldom successful for several reasons. Trying to avoid the problems associated with traditional culture-based methods, we developed a single duplex PCR assay for the detection and identification of Y. enterocolitica ssp palearctica bioserotype 4/O:3 using DNA extracted from a source. We combined the primer for tufA (elongation factor Tu) with the primer for rfbC (the biosynthesis of the O side chain) in one single reaction. A total of 79 Yersinia wild trains were selected to evaluate the duplex PCR assay. These strains were composed of 15 Y. enterocolitica serotype O:3 strains from human sources, and 64 Y. enterocolitica strains of various serotypes from human and animal sources. Nine Yersinia type strains were used as positive control for the PCR assay, comprising five Y. enterocolitica of several serotypes and four Yersinia other species, including Yersinia kristensenii, Yersinia frederiksenii, Yersinia ruckeri, and Yersinia pseudotuberculosis. For negative controls, five strains were used comprising Escherichia coli, Salmonella enterica ssp enterica, Citrobacter freundii, Enterobacter sakazakii, and Proteus vulgaris, all strains belonged to the Listeria collection (CLIST). All the Yersinia strains analyzed in this study showed the presence of the tufA gene, and the negative controls did not shown cross-reaction with this gene. The amplification of a 405 bp rfbC fragment only in the Y. enterocolitica serotype O:3 samples showed that this gene is an excellent serotype O:3 marker for diagnostic purposes. The results of multiple alignment of the tufA primers with the closest tufA sequenced fragments from NCBI database, in and out of the Yersinia genus, corroborate with the duplex PCR assay results. This assay could be a suitable screening method for the rapid detection and identification of Y. enterocolitica serogroup O:3, other serotypes, and other Yersinia species. We anticipate that this assay could be a useful tool for hospital and veterinary surveillance studies on Yersinia worldwide.

Keywords: Yersinia enterocolitica, detection, Identification, PCR, zoonosis.

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