عنوان مقاله:

Optimization for Rapid Detection of E. coli O۱۵۷:H۷ Using Real-Time Loop-Mediated Isothermal Amplification

محل انتشار:

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خلاصه مقاله:

Background: E. coli O۱۵۷:H۷-related food poisoning is one of the most well-known causes of bloody diarrhea illness around the world. Therefore, devising a rapid, highly sensitive, and convenient detection technique for this species is crucial. In this work, we optimized a colorimetric Loop-mediated isothermal amplification (LAMP) for detecting the intimin gene from E. coli O۱۵۷:H۷. Methods: In this study, eae (intimin), one of the virulence factors of E. coli O۱۵۷:H۷, was selected as the target gene, and specific primers were designed for the sequence of this gene using the Primer Explorer V۵ software. The LAMP reaction was optimized with three variable factors of MgSO۴ concentration, temperature, and incubation time, in a traditional (separate) way and by Taguchi experimental design. Finally, the LAMP products were visualized by ۲% agarose gel electrophoresis stained with ethidium bromide or green fluorescence (SYBR green I) and the pink fluorescence (KBC power load), which can be observed using the naked eye. Results: The LAMP reaction was optimized at ۶۳°C and ۸ mM MgSO۴ for ۴۰ min. Also, the naked eye can readily visualize the LAMP products within ۴۰ minutes and have a detection limit of ۳.۲×۱۰۴ CFU/mL according to ۰.۳۸ fg from the genome. Designed primers based on the gene sequence proved their specificity by testing ۴ species of other common foodborne pathogenic microorganisms. Conclusion: The rapid, sensitive, one-step-visually developed LAMP assay could be of interest for screening functions in food analytical laboratories without special equipment or trained personnel.

کلمات کلیدی:

Colorimetric LAMP assay, Food poisoning, E. coli O۱۵۷:H۷, Detection, Optimization.