Comunicazione Orale

DEVELOPMENT OF AN INTEGRATED LAMP-CRISPR/CAS12A SYSTEM FOR THE RAPID DETECTION OF SALMONELLA SPP. IN FOOD MATRICES

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Objective. Molecular methods have revolutionized the detection of foodborne pathogens, enabling rapid and reliable identification following a pre-enrichment step. Among the available techniques for the detection of Salmonella spp., Loop-Mediated Isothermal Amplification (LAMP) stands out for its speed, simplicity, and high specificity, owing to DNA amplification mediated by Bst DNA polymerase under isothermal conditions. However, the development of novel approaches capable of combining rapid amplification with enhanced target discrimination and simplified signal interpretation remains a key challenge in food pathogen diagnostics. In the present study, two Real-Time LAMP (RT-LAMP) protocols were optimized and applied for the rapid detection of Salmonella spp., assessing their sensitivity, specificity, inclusivity, and exclusivity through comparison with the reference method ISO 6579 and Real-Time PCR (AFNOR BRD 07/06-07/04). Furthermore, an innovative integrated LAMP-CRISPR/Cas12a platform was developed, combining isothermal amplification with CRISPR-mediated collateral cleavage activity for rapid and highly selective target detection, with the aim of exploring its potential as an alternative diagnostic tool for Salmonella identification.

Methods. To assess the inclusivity of the method, 50 target strains of Salmonella spp. isolated from food and human sources, representing different serotypes, were selected. In addition, a panel of 20 non-target strains was assembled to evaluate the exclusivity of the two LAMP systems. Subsequently, 19 samples belonging to different food matrices were analyzed using the ISO 6579 reference method, Real-Time PCR, and the two LAMP methods targeting the invA and ttr genes. In parallel, an integrated LAMP-CRISPR/-Cas12a diagnostic approach based on the invA gene was developed.

Results. The results demonstrated the high selectivity of both LAMP methods for the detection of Salmonella. Both primer sets successfully amplified all 50 target strains belonging to different serotypes, producing specific melting curves (Tm 86.5 ± 0.5 °C), while no amplification was observed in the 20 non-target strains, resulting in 100% selectivity. Analysis of the 19 food samples showed 100% agreement with both the ISO 6579 reference method and Real-Time PCR, confirming the effectiveness of the LAMP protocols in detecting Salmonella in food matrices. The development of the integrated LAMP-CRISPR/Cas12a strategy based on invA gene amplification enabled rapid and selective target identification. Following optimization, all Salmonella serotypes generated positive signals, whereas non-Salmonella strains showed no fluorescence. The system allowed the detection of Salmonella Typhimurium in less than 15 minutes, with a limit of detection of 2×10^2 CFU/mL.

Conclusions. The results obtained confirm the high sensitivity, specificity, and rapidity of both the LAMP and LAMP-CRISPR/Cas12a systems, highlighting their potential application in the field of food microbiological safety. This work was carried out within the framework of the Research Project IZSME 01/24, funded by the Italian Ministry of Health under its Veterinary Public Health and Food Safety Research Program.