Molecular vs. Microbiological Detection of *Escherichia coli* O157: A Comparison in the Context of Food Safety

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Abstract:

Problem Statement: *Escherichia coli* O157 is a highly virulent foodborne pathogen responsible for serious diseases such as hemorrhagic colitis and Hemolytic Uremic Syndrome. Detection of this bacterium in food matrices, such as ground beef, is essential to ensure food safety. However, classical microbiological methods, although reliable, are slow and can be affected by the native microbiota of foods, making accurate identification of *E. coli* O157 difficult. The need for faster and more sensitive methods has led to the development of molecular techniques such as PCR and qPCR, which promise to offer a more efficient and specific alternative.

Methodology: In this study, molecular techniques (qPCR-SYBR Green) were compared with classical microbiological methods for the detection of *E. coli* O157 in artificially contaminated ground beef. Four qPCR protocols targeting the *rfbE, stx1 and stx2* genes were validated, and the sensitivity, specificity, and robustness parameters were evaluated. Additionally, microbiological cultures and biochemical tests were performed to compare the times, precision, and reliability of each method.

Results: The qPCR-SYBR Green protocols demonstrated efficiencies between 77 % and 97 %, with high linearity (R^2 of 0.99) and low detection limits for each primer sequence (3.1667×10^{-2} ng μL^{-1} for *rfbE* (primers rfbE and O157); 1.7228×10^{-3} ng μL^{-1} for *stx1* and 3.5185 x 10-3 ng µL-1 for *stx2*). Both inclusivity and exclusivity were 100 %, as well as analytical precision, positive and negative predictive value. In addition, they were quite robust processes. These protocols were able to identify up to 4 CFU/mL of *E. coli* O157 in ground beef. In comparison, traditional microbiological techniques such as culture and biochemical identification required between 24 and 48 hours to confirm the presence of the pathogen, while qPCR provided results within hours. In addition, molecular analyses were shown to be less susceptible to interference from the native microbiota of the meat matrix, improving the accuracy in the detection of *E. coli* O157.

Conclusion: Molecular methods, such as qPCR, offer a significant advantage over classical microbiological methods, in terms of speed, sensitivity and specificity. Although traditional cultures remain a gold standard in microbiological diagnosis, qPCR allows for faster and more efficient detection of *E. coli* O157, overcoming the limitations imposed by the native microbiota of foods. However, the combination of both techniques can provide a complementary approach, ensuring greater reliability and accuracy in pathogen identification.

The integration of molecular techniques such as qPCR into food safety analyses can significantly transform the turnaround time for pathogen detection, allowing earlier interventions to prevent outbreaks. Even though classical microbiological techniques remain important for confirming results, qPCR represents a valuable tool for routine monitoring of *E. coli* O157, thus improving the safety and quality of food products.

Biography

María Yepes-Pérez, Karent Carrero-Contreras, Amanda Lucía Mora Martínez and Neil Vásquez Araque are part of the Probiom research group at the National University of Colombia, Medellín. This group specializes in key areas of food research, including toxicology and the production of functional foods. In the field of food toxicology, they have investigated diseases caused by *E. coli* O157:H7 and other Shiga toxin-producing strains (STEC). Yepes-Pérez and Carrero-Contreras have focused their PhD and Master's theses, respectively, on the validation of molecular techniques such as conventional PCR, qPCR and PCR-ELISA, comparing them with traditional culture methods. The goal of their studies is to provide faster and more reliable methods to improve food safety and pathogen detection in clinical and food diagnostic laboratories.

About Research Topic

Foodborne diarrheal diseases represent a serious public health problem, affecting nearly half of the world's population and causing morbidity and mortality. *Escherichia coli* O157:H7 is one of the most pathogenic zoonotic agents, capable of contaminating various foods, especially beef, as well as water and beverages. A minimal number of these bacteria (less than 100) can cause serious health problems, such as hemolytic uremic syndrome (HUS). Early detection is therefore essential to prevent infections from consuming contaminated food, which requires the optimization of analytical methods to identify pathogenic microorganisms such as STEC.

Traditional methods to detect *E. coli* O157:H7 are based on cultivation in specific media, but their effectiveness is limited in samples with a high microbial load, which complicates bacterial characterization and often requires prior enrichment that can take days or even weeks. In this context, PCR techniques emerge as rapid and reliable tools, constituting a valuable alternative to improve food safety.

About Institution:

The National University of Colombia, located in Medellín, is distinguished by its numerous research groups focused on various areas of knowledge, with the aim of addressing environmental problems and improving the living conditions of the country's population. Training in the field of food is especially significant, as it prepares students to work in quality control laboratories in different companies nationwide. In addition, the university provides training in food safety, helping to standardize the measures necessary to guarantee quality and safety in food production.