J. Adv. Educ. Sci. Humanit. (July - December 2024) 2(2): 29-34
https://doi.org/10.5281/zenodo.14602192

JAESH
JOURNAL OF ADVANCES IN EDUCATION, SCIENCES AND HUMANITIES

REVIEW ARTICLE

Advances in rapid and traditional methods for microbiological control in food: a comprehensive approach to food safety

Avances en métodos rápidos y tradicionales para el control microbiológico en alimentos: un enfoque integral hacia la seguridad alimentaria

Daliannis Rodríguez

Received: 4 March 2024 / Accepted: 16 June 2024 / Published online: 31 July 2024

Abstract Food safety is a global challenge that demands effective methods for detecting and controlling pathogenic microorganisms in food. Traditional methods, while reliable, require extended time and significant resources. Rapid methods have emerged in response to these limitations, incorporating physicochemical, immunological, and molecular techniques and biosensors and miniaturized systems. This narrative review examines the key advances in rapid methods for microbiological control of food, highlighting their applications, advantages, and limitations. It concludes that implementing these techniques can optimize analytical processes, strengthening global food safety.

Keywords food safety, rapid methods, food microbiology, food security.

Resumen La inocuidad alimentaria es un desafío global que demanda métodos eficaces para la detección y control de microorganismos patógenos en alimentos. Los métodos tradicionales, aunque fiables, requieren tiempos prolongados y recursos significativos. En respuesta a estas limitaciones, han surgido métodos rápidos que incorporan técnicas físico-químicas, inmunológicas y moleculares, además de biosensores y sistemas miniaturizados. Esta revisión narrativa analiza los principales avances en métodos rápidos para el control microbiológico de los alimentos, destacando sus aplicaciones, ventajas y limitaciones. Se concluye que la implementación de estas técnicas puede optimizar los procesos analíticos, fortaleciendo la seguridad alimentaria global.

Palabras clave inocuidad alimentaria, métodos rápidos, microbiología de alimentos, seguridad alimentaria.

How to cite
Rodríguez, D. (2024). Advances in rapid and traditional methods for microbiological control in food: a comprehensive approach to food safety. *Journal of Advances in Education, Sciences and Humanities*, *2*(2), 29-34. https://doi.org/10.5281/zenodo.14602192

Daliannis Rodríguez
rcdaly92@gmail.com
Universidad UTE, campus Manabí, Montecristi, Ecuador.

PUBLIS EDITORIAL

Introduction

Foodborne diseases (FBD) constitute a global problem in the contemporary world; the vast majority are of biological origin (EFSA & ECDC, 2023). Tests to assess food safety worldwide are increasing for various reasons, including the growing public concern each time a food product is withdrawn due to food safety (Alonso & Poveda, 2008). Health regulations are also increasing in many countries and regions. Two examples are the Food Safety Modernization Act (FSMA) in the U.S. and China's food safety action plan (Mateos & Rodríguez, 2015).

There are numerous causative agents of FBD, including *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* (including enterotoxigenic strains producing Shiga toxin), among others (Puig et al., 2013). In recent years, the main causative agents of outbreaks in Cuba have been *Salmonella* spp. and coagulase-positive *Staphylococcus*. *Salmonella* is the most recurrent agent, causing a foodborne infection capable of hospitalization and even death (Puig et al., 2013b).

Currently, the analytical methods used are validated traditional methods, most of which are identical adoptions of ISO standards (Leyva et al., 2013). These require considerable time, human capital, equipment, culture media, and reagents for their development, making it necessary to have reliable rapid methods to obtain results in a shorter time with the required quality and reliability to meet customer needs.

Given the need for methods that expedite analysis results, several studies have been conducted at the Food Microbiology Laboratory of INHEM to determine the feasibility of using rapid kits from different brands, such as the Neogen Kit for *Listeria* spp. and *Salmonella* spp. (Jiménez, 2016), Rapid Test for Listeria from Oxoid (Martino et al., 2011), and the evaluation of a TECRA *Salmonella* Via Elisa Kit 3 M (Pereda, 2013).

In this context, microbiological analysis methods must guarantee the accuracy and reliability of the results and reduce the time and resources required for their execution. This review aims to analyze the methods used in food microbiological control, examining advances in both rapid and traditional techniques and their impact on food safety. The review seeks to provide a comprehensive perspective that contributes to the selection and implementation of more efficient analytical tools adapted to the current needs of the food industry.

Historical background

The first person to appreciate and understand the presence and role of microorganisms in food was Pasteur. In 1837, he demonstrated that the acidification of milk was due to the growth of microorganisms. Before this date, other significant discoveries contributed to the development of microbiological studies. In 1659, Kircher demonstrated the presence of bacteria in milk, and in 1680, Leeuwenhoek observed yeast cells for the first time. These precedents led to the first study of mesophilic bacteria in 1888, and in 1895, the first records of bacterial counts in milk were made by Von Geuns. Subsequently, various studies were conducted for the microbiological analysis of food to determine the presence, type, and quantity of microorganisms in products (Jay et al., 2009).

Traditional methods for detecting pathogenic microorganisms

Basic methods to determine the number of microorganisms in food include standard plate count, in which viable microorganisms (Colony Forming Units, CFU) are quantified; the most probable number (MPN) method, which is a statistical determination of the number of viable cells in a sample; and direct microscopy counts, which include both viable and non-viable cells (Jay et al., 2009).

In the standard plate count technique, food samples are crushed and homogenized, serially diluted with an appropriate diluent, placed on a plate with an enriched agar medium, and incubated at a precise temperature for a specified time. Afterward, visible colonies are counted using a colony counter. In the MPN method, dilutions of the food to be analyzed are prepared similarly to the plate count. Three or five series of aliquots and dilutions are seeded into 9 or 15 tubes, respectively. The number of microorganisms in the food is determined using established MPN tables. The direct microscopy count involves preparing a smear of the food samples or cultures to be analyzed on a microscope slide, staining with appropriate substances, and observing and counting the cells using an oil immersion objective (Jay et al., 2009).

These microbiological analysis techniques in food are primarily aimed at detecting microorganisms that indicate the possible presence of pathogens or spoilage organisms. Conventional methods for microorganism detection, such as the MPN technique, membrane filtration, deep plating, or pour plating, require that the microorganism under analysis form a colony in an appropriate culture medium, which involves preparation, sterilization of materials, sufficient labor, relatively long incubation periods, the use of enrichment or recovery cultures, and necessary equipment such as incubators, refrigerators, autoclaves, and burners. It is also important to

note that foodborne microorganisms are constantly changing due to their inherent ability to evolve and their surprising ability to adapt to different forms of stress (Alonso & Poveda, 2008).

Rapid methods for pathogen detection in food

Some requirements that rapid and automated microbiological analysis methods for food must meet include accuracy in obtaining results according to established requirements (sensitivity, minimum detection limits, specificity of the analysis system, versatility, potential application, and comparison with reference methods), speed (in terms of the minimum time required to obtain results and the number of samples processed per assay, whether in hours or days), minimum cost (per analysis, reagents, labor), acceptability and reliability of the method by the scientific community and regulatory agencies of analytical systems, simplicity in sample preparation, operation of the analytical equipment, and data processing, as well as occupying minimal required space. They should be implemented as mechanisms for improving facilities' hygienic conditions and protecting food in pathogen detection (Mateos & Rodríguez, 2015).

Rapid methods are based on physicochemical techniques (dehydrated general or selective culture medium films, systems for determining the most probable number, chromogenic and fluorogenic media), immunological techniques (precipitation, agglutination, immunofluorescence, cytometry, immunoassay, enzyme-linked immunosorbent assay, immunochromatography, nephelometry, immunomicroscopy), and molecular techniques (hybridization, endpoint PCR, real-time PCR, ribotyping, microarrays, biochips) (Leotta, 2009).

Immunological tests

Immunological techniques are analytical procedures based on objectively visualizing the interaction between an antigen and its corresponding antibody. Due to their sensitivity, specificity, speed, and low cost, they are instrumental in the microbiological analysis of food. Three fundamental stages are identified in developing an immunological assay: 1) antigen preparation, 2) obtaining and evaluating the antibody, and 3) developing an appropriate immunoassay. The determining factor of these methods is the selection of an appropriate antibody. Positive results obtained through these methods are always considered presumptive positives, so they always require confirmation. The detection limit is between 10^4-10^5 cfu/mL. Once the antigen is selected, obtaining antibodies requires the use of experimental animals (Martínez, 2011).

The success of immunological techniques in the microbiological analysis of food has been enhanced by the development of monoclonal antibody technology, which provides clones of hybrid cells that continuously and inexhaustibly produce antibodies with known biological activity and constant specificity (Mateos & Rodríguez, 2015).

In recent years, most commercial kits for the specific identification of microorganisms and/or their toxins or metabolites have progressively replaced polyclonal antibodies with monoclonal ones. Furthermore, numerous studies have demonstrated that the reproducibility of commercial kits using monoclonal antibodies is superior (Mateos & Rodríguez, 2015).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA (Enzyme-Linked Immunosorbent Assay) is one of the most commonly used antibody-based formats for pathogen analysis in food. It involves using an antibody bound to a solid matrix that captures the antigens present in the enriched culture. A second antibody conjugated with an enzyme is used for detection. In the presence of a substrate, the enzyme catalyzes a colorimetric reaction. The walls of the wells in microtitration plates are the most commonly used solid support in this type of assay (Martínez, 2011).

Flow cytometry

Flow cytometry is a rapid and sensitive optical technique that allows for the detection of individual cells in complex matrices and the measurement of various physiological characteristics of these cells. In this method, cells are passed one by one through a cytometer, where a laser light beam is directed onto them. When this occurs, the light is scattered and absorbed by the microorganisms. The degree and nature of the light scattering caused by the intrinsic properties of the cells can be recorded and analyzed with a system of lenses and photoelectric cells. In this way, the number, size, and shape of the microorganisms are estimated. Specific microbial groups can also be detected (Marie et al., 1999). For this purpose, flow cytometry can be combined with specific antibodies labeled with fluorescence or with specific oligonucleotide probes (Barbosa et al., 2008).

Molecular biology

In molecular methods, selecting a specific DNA sequence and appropriate amplification conditions are essential. These are the determining factors for the specificity of molecular methods. Among the methods based on molecular biology, we can mention PCR, a molecular technique based on the

polymerase chain reaction (PCR), which has revolutionized the molecular diagnosis of infectious diseases. Unlike traditional methods, which require 6 or 7 days to provide a definitive result, PCR achieves the same in just 1 to 3 days, depending on various modifications to the work protocol. This technique detects specific DNA sequences and is not altered by phenotypic variations that can be evidenced by biochemical patterns (Pérez et al., 2008).

Currently, PCR techniques, which develop in multiple steps—from the amplification of genetic material to the analysis of the final products—are evolving toward faster and more automated single-tube procedures. These advances in PCR techniques are based on fluorescent compounds and offer numerous advantages in routine food analysis. For example, the time required to obtain results is reduced, as it does not require the subsequent electrophoretic analysis of PCR products (Foley & Grant, 2007).

The advantages of these assays, along with their ease of use and susceptibility to automation, make them very attractive for application in food to overcome the long enrichment culture stage. Research and development in this field may grow and lead to rapid, specific, and sensitive detection assays that can be performed directly on food samples shortly (Foley & Grant, 2007).

Biosensor

A biosensor is a compact analytical device that integrates a signal transduction system with a biological recognition element (enzyme, organelle, tissue, cell, biological receptor, antibody, or nucleic acid) or biomimetic (molecularly imprinted polymers). This system allows the signal (electrical, optical, piezoelectric, thermal, or nanomechanical) produced by the interaction between the recognition element and the substance or organism being detected (analyte) to be processed using software with appropriate algorithms. Biosensors are characterized by their high specificity, sensitivity, reliability, and multiplexing capability.

These devices can detect biotoxins (bacterial toxins, mycotoxins, and marine toxins), spoilage microorganisms, and pathogens (bacteria, molds, yeasts, viruses, and parasites) present in food. While the primary applications of biosensors are in genomic research and medicine, specific devices (based on nucleic acid hybridization and antigen-antibody interactions) are already available for detecting *Salmonella* spp., *L. monocytogenes*, *E. coli*, *S. aureus*, *Clostridium botulinum*, and other microorganisms (Mateos & Rodríguez, 2015).

Miniaturized systems and diagnostic kits

Miniaturized systems arise from the concept of microtiter plates (96 wells, 8x12 format), which allow the reduction of reagent and medium volumes required for assays. Additionally, it is possible to study, in a manageable format, the effect of a compound on many isolates or the effect of a series of compounds on a specific isolate.

This line of research has enabled the development of some of the selective culture media available in the market today. Among the miniaturized microbial identification systems currently available, based on the metabolism of specific substrates by microorganisms and their detection through various indicator systems, the following stand out: disposable cards for the simple identification of suspicious colonies through rapid biochemical tests like OBIS (Oxoid Biochemical Identification System, Cambridge, UK); galleries that allow identification of over 800 species of bacteria and yeasts, such as the API system (BioMérieux Hazelwood, Mo, USA); plastic tubes with compartments containing agar with various substrates and a needle inside for quick and easy inoculation from a single colony, like the BBL Enterotube and Oxi/Ferm Tube (BD Becton, Dickinson and Company, NY, USA); and plastic supports with easily inoculated wells containing chromogenic and/or fluorogenic substrates in dehydrated form, which are rehydrated upon contact with the sample (BBL Crystal, BD Becton, Dickinson and Company, USA; RapID systems and MicroID, Remel KS, USA; Biochemical ID systems, Microgen Bioproducts, Surrey, UK) (Leotta, 2009).

One of the most well-known miniaturized and automated systems is the VITEK system (BioMérieux Hazelwood, Mo, USA), which, based on color changes in substrates or gas production from cultures inoculated into wells of a plastic card containing dehydrated biochemical substrates, can identify *E. coli* in 2-4 hours. A similar speed in obtaining results can be achieved with the Biolog system (AES Chemunex, Rennes, France), which detects the ability of microorganisms to oxidize 95 carbon sources. The possible metabolic patterns allow, in addition to identification, the establishment of phylogenetic relationships among different isolates. Using a single redox chromogen, tetrazolium violet, which irreversibly reduces to formazan (purple color) due to bacterial metabolic activity, facilitates the visual reading of results.

In any case, most of the systems mentioned in this section offer the possibility of automated result reading and interpretation. For the automation of the MPN method, plastic cards have been developed that contain three groups of 16 wells, with a logarithmic volume difference for each well group, and culture media with fluorescent indicators (Tem-

po, BioMérieux, Hazelwood, Mo, USA). This system significantly reduces the need for reagents, space, and time compared to the conventional MPN method (Leotta, 2009).

Food System is a 24-well panel containing culture media with dried biochemical substrates for the presumptive search and identification of microorganisms from meat, dairy, and other food products. The panel allows the search and identification of *Salmonella* spp., *Citrobacter* spp., *S. aureus*, *E. coli*, *Bacillus cereus*, *Listeria* spp., and fungi, among others; it is validated according to ISO 16140 (2003) for the detection of *Salmonella* spp. and *Listeria* spp.

The situation of miniaturized systems and diagnostic kits worldwide

According to a study published by BCC Research LLC, the market volume for testing devices or methods for global food safety reached $10.5 billion in 2014 and is expected to reach approximately $13.6 billion by 2019, representing a 5.3% annual growth over five years until 2019 (Mateos & Rodríguez, 2015).

Conclusions

Foodborne diseases pose a risk to public health and require the development of efficient methods for their control. Rapid and automated methods enable the detection of microorganisms with greater accuracy, shorter processing times, and reduced costs compared to traditional methods. Advanced technologies such as PCR, biosensors, and flow cytometry have transformed food microbiology by providing more reliable and faster results. The validation and standardization of these technologies support their global implementation, contributing to improved food safety.

References

Alonso, L.X., & Poveda, J.A. (2008). *Estudio comparativo en técnicas de recuento rápido en el mercado y placas PETRIFILM 3M para el análisis de alimentos*. Pontifica Universidad Javeriana. https://repository.javeriana.edu.co/bitstream/handle/10554/8238/tesis230.pdf?sequence=1&isAllowed=y

Barbosa, J., Costa-de-Oliveira, S., Gonçalves, A., & Pina-Vaz, C. (2008). Optimization of a flow cytometry protocol for detection and viability assessment of Giardia lamblia. *Travel Medical Infectious Diseases*, *6*(4), 234-239. http://www.doi.org/10.1016/j.tmaid.2008.01.004

European Food Safety Authority (EFSA), & European Centre for Disease Prevention and Control (ECDC). (2023). The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2020/2021. *EFSA Journal*, *21*(3), 7867. https://doi.org/10.2903/j.efsa.2023.7867

Foley, S.L., & Grant, K. (2007). *Molecular Techniques of Detection and Discrimination of Foodborne Pathogens and Their Toxins*. In: Simjee, S. (eds.) Foodborne Diseases. Infectious Disease. Humana Press. https://doi.org/10.1007/978-1-59745-501-5_20

ISO 16140. (2003). Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods. Switzerland: ISO.

Jay, J.M., Loessner, M.J., & Golden, D.A. (2009). *Microbiología moderna de los alimentos*. España: Editorial Acribia, S.A.

Jiménez, L. (2016). *Evaluación de la calidad sanitaria en quesos frescos artesanales mediante métodos rápidos y tradicionales*. Universidad de La Habana.

Leotta, G.A. (2009). Métodos rápidos: una herramienta útil y práctica para el análisis microbiológico de los alimentos. *Revista Argentina de Microbiología*, *41*(2), 63-64. https://www.redalyc.org/pdf/2130/213016783001.pdf

Leyva, V., Pereda, G., Martino, T.K., Aportela, N., Puig, Y., Ferrer, Y., Martínez, Y., Camejo, A., Pérez, Y., de los Reyes, M., Carrera, J.A., Valdés, O.M., Dueñas, O., Castillo, A.I., Pérez, D.R., & Luna, M.V. (2013). Implementación de Sistema de Gestión de Calidad en laboratorio de Microbiología de Alimentos.: Editorial Lazo Adentro. https://www.isbncuba.ccl.cerlalc.org/catalogo.php?mode=detalle&nt=24098

Marie, D., Brussaard, C.P.D., Thyrhaug, R., Bratbak, G., & Vaulot, D. (1999). Enumeration of marine viruses in culture and natural samples by flow cytometry. *Applied and Environmental Microbiology*, *65*(1), 45-52. https://doi.org/10.1128/AEM.65.1.45-52.1999

Martínez, I. (2011). *Desarrollo de métodos de detección de Salmonella basados en la reacción en cadena de la polimerasa y su validación en muestras alimentarias*. Universidad del País Vasco. https://dialnet.unirioja.es/servlet/tesis?codigo=97548

Martino, T., Leyva, V., Peña, Y., Lamela, G., López, N., & Almaral, O. (2011). Alimentación, Nutrición y Salud. Cap. VIII. La Habana: MINSAP. https://revalnutricion.sld.cu/index.php/rcan/article/view/1185

Mateos, F., & Rodríguez, S. (2015). Tendencias sobre Seguridad Alimentaria. Informe de Vigilancia Tecnológica, 46-70. https://www.eoi.es/sites/default/files/savia/docu-

ments/EOI_SeguridadAlimentaria_2015.pdf

Pereda, G. (2013). *Evaluación de un kit TECRA Salmonella kits Elisa 3 M*. Instituto Nacional de Higiene, Epidemiología y Microbiología.

Pérez, C.M., Sánchez, M.M., Henao, S., & Cardona-Castro, N.M. (2008). Estandarización y evaluación de dos pruebas de Reacción en Cadena de la Polimerasa para el diagnóstico de Salmonella enterica subespecie enterica en huevos. *Archivos de Medicina Veterinaria*, *40*(3), 235-242. https://dx.doi.org/10.4067/S0301-732X2008000300003

Puig, Y., Leyva, V., Robert, M., Brady, A., & Pérez, Y. (2013a). Agentes bacterianos asociados a brotes de enfermedades transmitidas por alimentos en La Habana, 2006-2010. *Revista Cubana de Higiene y Epidemiología*, *51*(1), 74-83. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1561-30032013000100008&lng=es&tlng=es

Puig, Y., Robert, M., Brady, A., & Leyva, V. (2013b). Factores epidemiológicos de interés en brotes de enfermedades transmitidas por alimentos en La Habana. *Revista Cubana de Higiene y Epidemiología*, *51*(3), 262-268. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1561-30032013000300004&lng=es&tlng=es

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Daliannis Rodríguez: Conceptualization, research, methodology, visualization, writing the original draft, writing, review and editing.

Data availability statement

Not applicable.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

Disclaimer/Editor's note

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and not of Journal of Advances in Education, Sciences and Humanities.

Journal of Advances in Education, Sciences and Humanities and/or the editors disclaim any responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products mentioned in the content.