Asian Journal of Biochemistry, Genetics and Molecular Biology

Volume 16, Issue 9, Page 21-39, 2024; Article no.AJBGMB.121351
ISSN: 2582-3698

Improving Food Safety in Africa Using Cutting-Edge Biotechnology & Molecular Biology Approaches

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Authors' contributions

This work was carried out in collaboration among all authors. Author YA conceptualized the review, designed the outline, drafted, reviewed and edited the manuscript and sourced for funding acquisition. Authors AOA, OGJ, AWW and TEO contributed equally by curating data and articles from online sources, reviewed the manuscript and funding acquisitions. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajbgmb/2024/v16i9403

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/121351

Review Article

Received: 11/06/2024
Accepted: 16/08/2024
Published: 23/08/2024

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Cite as: *Alhassan, Yusuf, Abdullahi Olanrewaju Ajadi, Oni Gbotemy Jerry, Ahmed Wopa Wurie, and Temitope Esther Olajide. 2024. "Improving Food Safety in Africa Using Cutting-Edge Biotechnology & Molecular Biology Approaches". Asian Journal of Biochemistry, Genetics and Molecular Biology 16 (9):21-39. https://doi.org/10.9734/ajbgmb/2024/v16i9403.*

ABSTRACT

Ensuring food safety in Africa is a critical challenge that requires innovative approaches. Food safety is a pressing concern in Africa, where the prevalence of foodborne diseases and contamination poses significant health and economic challenges. This review explores the potentials of advanced biotechnology and molecular biology approaches to enhance food safety across the continent. Key strategies include the use of whole genome sequencing, next-generation sequencing, foodomics, CRISPR systems, and other salient molecular diagnostics approaches and/or tools for improving food safety through rapid detection of contaminants, and the implementation of biotechnological methods to improve food processing and preservation. The integration of these cutting-edge techniques can mitigate the risks associated with foodborne pathogens, reduce post-harvest losses, and ensure the production of safe, nutritious food. By leveraging these innovations, Africa can build a robust food safety framework that aligns with global standards, ultimately contributing to public health, economic stability, and food security.

Keywords: Food safety; molecular diagnostics; next-generation sequencing; foodomics.

1. INTRODUCTION

1.1 Overview of Food Safety in Africa

Africa has long been recognized for its extensive food and agricultural production, a status largely attributed to the continent's vast land area and sizable population, which supports food self-sufficiency. Nonetheless, issues related to food safety, quality, and nutrition have historically received less attention, only becoming more prominent in recent years. According to the World Health Organization, approximately 91 million people in Africa suffer from foodborne illnesses annually, with 137,000 deaths, accounting for one-third of the global mortality associated with such diseases.

Foodborne diseases (FBDs) are widespread public health challenges that contribute to numerous outbreaks, adversely affecting both the health and economic well-being of populations worldwide [1]. These diseases often result from the consumption of food and water contaminated with microorganisms and toxins [2]. Moreover, inadequate practices in food processing, preparation, and storage further contribute to the spread of FBDs [1, 3]. The risks associated with foodborne diseases are present throughout the entire food production chain, from “farm to fork” [4, 2]. Once contaminated food reaches consumers, it can lead to severe health issues and, in some cases, death [2]. The impact of food hazards on public health is significant and often leads to considerable economic damage [5].

FBDs are particularly prevalent in low-income countries, where hygiene, sanitation, and safe food handling practices are often lacking [6,1]. It is estimated that 31 foodborne diseases result in approximately 600 million cases of illness and 420,000 deaths globally, with developing regions experiencing the highest risk [2,7]. Worldwide, millions of people fall ill due to food and waterborne diseases each year, with an estimated three million deaths, including 700,000 in Africa alone, caused by diarrhea linked to contaminated food and water. Such outbreaks can quickly escalate into food safety emergencies, negatively impacting national economies and livelihoods by reducing food availability for domestic consumption and leading to the closure of export markets. Although foodborne illnesses occur daily across the globe, particularly in developing regions like Africa, there is often inadequate or no reporting, making the true prevalence of these diseases largely unknown [8].

Information on food safety within the African region remains fragmented and insufficient. This deficiency is primarily due to the lack of effective surveillance, documentation, and reporting systems, which leads to inefficient resource allocation, duplicated efforts, and a lack of coordination among the countries in the region [9]. Food safety is not considered a high priority by most African governments, particularly when it comes to the needs of domestic populations, and is often viewed as separate from public health initiatives. The reality is that Africa faces numerous other challenges that frequently take precedence. For instance, in 2012, nearly 600,000 deaths from malaria—representing 90% of the global total—occurred in Africa. Addressing primary healthcare needs, providing education, treating HIV/AIDS, supporting

undernourished populations, and improving food security are all critical issues requiring substantial financial investment, which puts additional strain on already limited budgets.

According to the Food and Agriculture Organization (FAO), in 2010, Sub-Saharan Africa (SSA) had the highest proportion of undernourished people globally, with 239 million individuals, or 30% of the population [10]. While HIV is not typically considered a foodborne pathogen, it is relevant to food safety as it can potentially be transmitted through breast milk. Additionally, HIV infections increase susceptibility to other foodborne illnesses. SSA remains the region most affected by HIV/AIDS, with an estimated 23.5 million people living with the virus in 2011, accounting for 69% of the global HIV burden. Furthermore, 92% of pregnant women living with HIV/AIDS and 90% of children who acquired HIV in 2011 were found in SSA [11].

Globally, and particularly in Africa, food safety systems have not evolved in step with the growing complexity of food safety challenges. In Africa, these challenges are exacerbated by poor food safety management, lack of clear mandates, and minimal investment in sanitary and phytosanitary (SPS) infrastructure [12,13]. The situation is further complicated by a weak food safety culture across the continent. The prevalence of unsafe food has hindered the transformation of food systems in Africa, not only by harming public health but also by disrupting efforts to enhance trade in food and agricultural products. This results in reduced agricultural trade, loss of income, and economic setbacks [14,15].

Hunger remains a significant issue in Africa, primarily driven by food insecurity, which can only be addressed through improvements in food safety and security. Food safety plays a critical role in ensuring food security, as the primary cause of food insecurity is declining global food productivity, compounded by poverty, which negatively impacts the socio-economic well-being of citizens. Africa possesses abundant land resources, which, if properly harnessed for agricultural productivity, could significantly enhance food security and sustainability. Agriculture, which is 85 to 90 percent rain-fed in Sub-Saharan Africa, contributes 35 percent to the region's gross national product (GNP), 40 percent to exports, and provides 70 percent of employment. There is an urgent need to focus on agricultural innovations in Africa to boost food production. Ensuring the safety and availability of food must be prioritized by addressing the various challenges that hinder these goals, thereby stimulating economic growth and ensuring food security and safety on the continent [16].

The application of technological advancements is crucial for improving the detection of foodborne hazards and enhancing the diagnosis of foodborne illnesses. These technologies will play a key role in tackling food safety and food insecurity challenges. Emerging trends in food safety will be essential for effectively addressing food safety issues in African countries, enabling them to compete in both continental and global food markets.

The use of molecular biology and biotechnology approaches are among the major breakthroughs globally used in addressing food safety. Herein, we provide an overview of the rapidly advancing global biotechnology and molecular biology approaches that are currently trending, highlighting their role in the development and enhancement of food safety frameworks. Simultaneously, we offer a clearer understanding of novel molecular biology and biotechnology techniques used to address food safety challenges, specifically for African researchers, aiming to bridge the gap caused by the current lack of sophisticated molecular approaches in the region.

2. MOLECULAR BIOLOGY/ BIOTECHNO-LOGY AND FOOD SAFETY

A variety of methods and techniques have been utilized for detecting contaminants, pathogens, or microorganisms in food products. However, the growing demand for precise and rapid solutions to food safety challenges has recently led to the adoption of molecular biology techniques. These advanced approaches, which primarily involve the use of nucleic acids and antibodies for detecting foodborne pathogens, began emerging in the late 20th and early 21st centuries. For nearly a century, food analysts largely depended on conventional microbiological testing methods, which involved the use of culture media to grow and isolate bacterial pathogens in foods. Despite advancements, food diagnostics remain challenging due to the complexity of the food matrix and the heterogeneous nature of various food substrates [17].

Scientific progress has introduced several molecular biological diagnostic assays,

significantly impacting the methods used to detect foodborne pathogens and their associated toxins. Over the past two decades, there has been significant progress in developing and applying molecular techniques for detecting microorganisms in food products, driven by the increasing need for rapid results. These techniques typically target specific DNA, protein, or RNA sequences through processes such as polymerase chain reaction (PCR), real-time PCR, Western blot, and ELISA [18]. In many cases, these methods have replaced or supplemented traditional culture-based detection methods, though culture methods remain the gold standard for most bacterial foodborne pathogens. However, for certain foodborne viruses that cannot be cultured, nucleic acid-based assays are the only viable detection method. Microbial (bacterial or viral) nucleic acids may enter the food chain from the same sources as the pathogens themselves. While intact living cells contain intact DNA/RNA, even dead cells may retain intact nucleic acids. Additionally, the presence of fragmented extracellular nucleic acids from microbial or viral origins in food cannot be ruled out. For instance, adventitious viral nucleic acids have been identified in the porcine-derived trypsin enzyme [19].

2.1 Trending Molecular Biology & Biotechnology Techniques in Enhancing Food Safety

2.1.1 Whole genome sequencing

Recent advances in the application of molecular biology for enhancing food safety have been remarkable. The rapid adoption of data-intensive tools in food safety is driving the initial stages of a major transformation, anticipated to introduce a new era of high-precision research approaches. While various methodologies, such as Geographic Information Systems (GIS) technologies, play vital roles in precise food safety research, omics technologies are among the primary catalysts of this shift [20-22]. Notably, whole genome sequencing (WGS) enables highly sensitive "precision" subtyping, significantly improving the detection of foodborne disease outbreaks [23-25]. Moreover, WGS facilitates comprehensive characterization of foodborne pathogens, allowing for the identification of strains and clonal groups that differ in virulence and antimicrobial resistance [26-28]. The practical application of metagenomics and meta-transcriptomics for detecting foodborne and human pathogens is gaining momentum, while WGS continues to be increasingly employed in routine surveillance of foodborne pathogens [29-31].

The utilization of WGS in bacterial population genomics has greatly enhanced the understanding of genome evolution and the biology of bacterial pathogens [32,33]. Although genome sequencing was initially costly, the advent of next-generation sequencing technologies and more affordable small bench-top sequencers has substantially reduced overall sequencing expenses [34]. This reduction has brought the per-isolate cost of microbial WGS to a level comparable to or even below that of traditional subtyping methods, such as Pulsed Field Gel Electrophoresis (PFGE), making WGS an indispensable tool in contemporary outbreak investigations. One of the earliest reports of WGS in investigating a foodborne disease outbreak was by Gilmour et al. [35], detailing the genome sequences of two distinct *Listeria monocytogenes* strains involved in a multi-province outbreak in Canada in 2008. The first instance of WGS being used to infer the potential source of a foodborne outbreak was reported by Lienau et al. [36], which involved isolates from the multistate outbreak of *Salmonella Montevideo* that occurred between July 2009 and May 2010.

As an initial validation of WGS, the CDC employed it in 2010 to characterize *Vibrio cholerae* strains during the Haiti outbreak [37,38]. In 2013, a collaborative effort involving the FDA, USDA, NCBI, and a pilot group of ten states focused on using WGS for monitoring *Listeria monocytogenes* [39]. Following this, PulseNet integrated WGS routinely for characterizing outbreak-associated isolates, especially those of *Salmonella*, *E. coli*, *Campylobacter*, *Vibrio*, and *Shigella*. By 2019, WGS had become PulseNet's new gold standard for molecular subtyping, a transition facilitated by CDC funding to the District of Columbia, Puerto Rico, and all 50 states. Since 2013, USDA FSIS also developed WGS capabilities, sequencing all pathogenic isolates and submitting the data to NCBI in real time [40].

In 2013, the FDA's Center for Food Safety and Applied Nutrition launched the GenomeTrakr (GT) network, an integrated system of federal and state laboratories. In collaboration with NCBI, GT established a public database for WGS data from foodborne and environmental bacterial pathogens [41,42]. Additionally, the FDA has worked closely with the Office of

Regulatory Affairs to integrate the GT network into the Laboratory Flexible Funding Model (LFFM). The GT network has since expanded to include 54 federal, state health, and university laboratories in the U.S. and 21 laboratories in 10 other countries. The GT database now houses WGS data for over 752,000 isolates, with over 13,000 new entries each month. The FDA also developed GalaxyTrakr, a distributed analysis tool designed for non-bioinformaticians to process public health WGS data [43]. The implementation of HTS/WGS by governmental agencies has significantly enhanced the response time and quality during outbreaks [44,45].

Globally, WGS is gradually being approved for use in food manufacturing due to its potential benefits in improving nutritional quality and performance [46,47]. Although food processing safety research does not typically require the comprehensive microbial characterization needed by reference laboratories, WGS is increasingly used to trace the source of bacterial contamination [48]. With accumulating evidence of NGS's superiority over traditional molecular subtyping methods and its increasing cost-effectiveness, pressure has mounted to apply WGS for food safety. However, widespread adoption of WGS has been complicated by challenges such as the need for effective communication and multijurisdictional sharing of large-scale WGS data for disease surveillance. Fortunately, early engagement between the scientific community, public health sectors, industry, clinicians, and food regulatory bodies led to the creation of the Global Microbial Identifier (GMI) consortium [49]. This consortium envisions a global, interoperable analytical platform with standardized pathogen genome databases, typing systems, and bioinformatics tools for microbial and infectious disease identification and diagnostics, ultimately accessible to all nations with basic laboratory infrastructure [50].

2.1.2 Next generation sequencing (NGS)

Next-generation sequencing (NGS) represents a revolutionary advancement in sequencing technologies, enabling comprehensive analysis of DNA sequences at a reduced cost. NGS has facilitated the functional verification of probiotic strains, the isolation of foodborne pathogens, and the identification of food allergens, among other applications. Its use is widespread across various research domains, including the establishment of genomic databases crucial for identification purposes, as well as in medical and industrial sectors. The inception of DNA sequencing technology was driven by the scientific community's desire to understand all DNA sequences that constitute the human genome. DNA sequencing involves analyzing the order of the four bases—adenine (A), thymine (T), guanine (G), and cytosine (C)—within a DNA strand through biochemical methods. The origins of DNA sequencing trace back to 1977, with the method developed by British biochemist Frederick Sanger. Sanger sequencing, the earliest and most widely commercialized sequencing technique, relies on a DNA polymerase reaction during replication, utilizing a single-stranded DNA template for sequencing [51].

Sanger sequencing has been extensively validated over time, offering high technical reliability and a relatively simple analysis process. However, while it is efficient for analyzing short gene sequences, it presents significant limitations when applied to larger genomes, particularly in terms of cost and time. Additionally, due to enzyme efficiency constraints, the nucleotide sequence that can be obtained is typically less than 1 kilobase (kb) in length. To address these limitations, NGS was developed, enabling high-throughput sequencing. The first commercialized NGS platform, the 454 Pyrosequencer, was introduced in 2004, marking a significant leap in sequencing technology. NGS works by fragmenting a genome into numerous smaller pieces, which are then sequenced simultaneously. The resulting data are analyzed using bioinformatics techniques to assemble and interpret large volumes of genomic information rapidly. Following the release of the 454 Pyrosequencer, other companies like Roche, Illumina (Solexa), and Applied Biosystems introduced their NGS platforms. Unlike earlier sequencing devices that required electrophoresis equipment, NGS platforms can analyze thousands to billions of sequences simultaneously. With the advancement of these technologies, the time and cost associated with sequencing have significantly decreased. For instance, the cost of sequencing the human genome, which was approximately $100 million in 2001, had dropped to around $1,000 by 2017. Among the various NGS platforms, 454 Roche Pyrosequencing, Illumina sequencing, and PacBio SMRT are some of the most prominent [52].

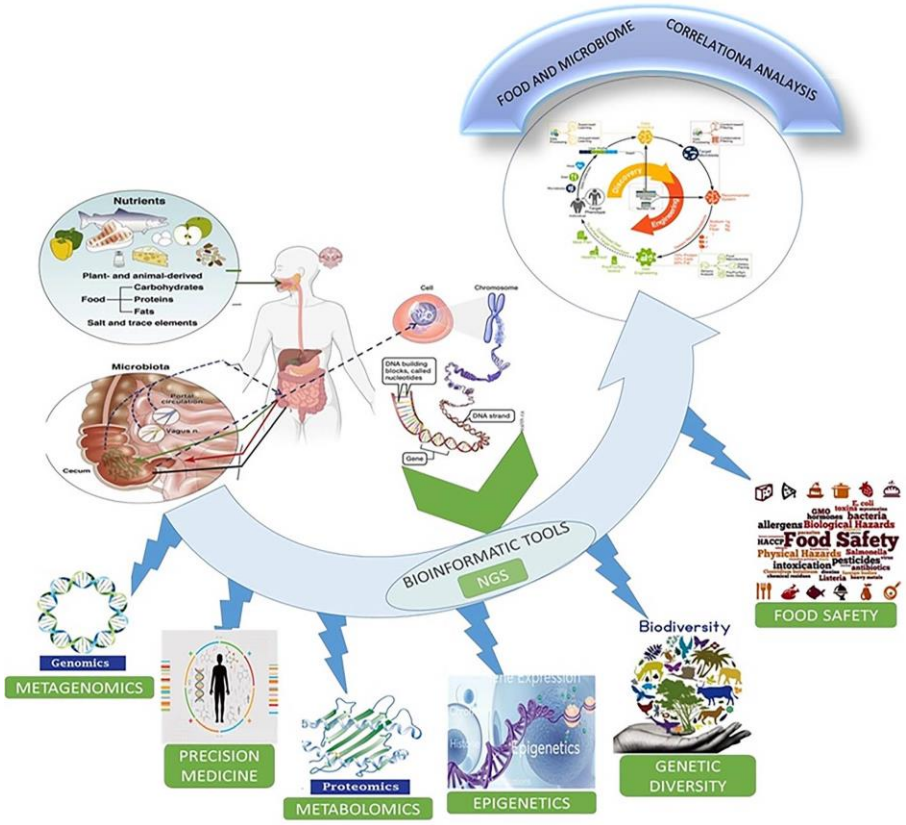

Fig. 1. NGS and other biotechnology techniques in application to food safety [54]

Next-generation sequencing (NGS) holds significant promise for research into the food microbiome, transforming traditional fermentation practices and uncovering minor genetic variations, among other uses. The advancement of NGS technologies has greatly benefited contemporary molecular biology techniques, offering valuable tools for both fundamental and applied research within the food and pharmaceutical sectors. With the swift progress of NGS technology, in-depth genetic research on microorganisms has become feasible. Prior to the advent of RNA-seq, gene expression studies relied on hybridization-based microarrays, which had limitations, such as difficulties in simultaneously analyzing multiple genes or accurately quantifying genes expressed at low levels. The development of NGS has overcome these issues by enabling large-scale analysis through RNA-seq. Currently, NGS is widely utilized in genomics, metagenomics, and transcriptomics. Its application to food microorganisms is categorized into three main areas: genome analysis of individual strains (e.g., probiotics and pathogenic bacteria), metagenomic studies to analyze strain composition during food fermentation or spoilage, and RNA-seq for verifying RNA expression and comparing gene expression patterns [53]. NGS techniques, grounded in metagenomics and transcriptomics, are used to investigate the functional activity of fermented foods, including microbial metabolites. This technology is instrumental in managing foodborne pathogens and addressing toxin-related hazards in food.

2.1.2.1 Foodomics

Foodomics explores the domains of food and nutrition by applying and integrating advanced omics technologies to enhance consumer well-being, health, and confidence. This field combines various related omics technologies, including transcriptomics (mRNA), nutrigenomics (nutrients), proteomics (proteins), metabolomics (metabolites), and genomics (gene detection) [55, 56]. The use of foodomics technologies has garnered significant interest in recent research focused on food, nutrition, and health [57]. These technologies are employed to analyze food composition, assess food quality, verify food authenticity, evaluate the activity of food proteins and peptides, identify allergens and toxins,

detect genetically modified organisms, and decode the human genome. They also help in understanding how food impacts genetics, leading to deeper insights into new food functions and processing technologies [58,59].

Proteomics and Metaproteomics: Proteomics, which focuses on the protein-coding regions of the genome, is extensively utilized in food technology. A subfield of proteomics, known as peptidomics, examines peptide sequences and their interactions. The human genome encodes approximately 20,000 proteins, which exceeds the 500 to 5,000 proteins typically detectable by proteomic methods [60]. Research often employs mass spectrometry (MS) coupled with chromatography to detect and identify numerous protein components in various food samples, including fingerprints used to spot food adulteration [61]. In proteomic studies, chromatographic techniques are frequently paired with MS-based methods. High-performance liquid chromatography (HPLC) coupled with tandem ion trap MS has identified a wide range of bioactive peptides from fermented milk and its hydrolysates, streamlining the traditionally time-consuming isolation and purification processes [62]. The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS technique is used for qualitative analyses and characterization of proteins and peptides [63]. MALDI-TOF MS has effectively determined the content and molecular weight of ginkgo seed proteins treated with high hydrostatic pressure [64]. Proteomic analyses using LC-MS and MALDI-TOF/TOF MS have provided valuable insights into foodborne parasites, offering promising data for the early detection, treatment, and diagnosis of specific parasitic infections [65].

Metaproteomics, a relatively new term, refers to the application of proteomics at the microbial level. It is utilized in microbial research to uncover the total protein abundance of both beneficial and spoilage or pathogenic microorganisms within food systems, particularly under varying stress or growth conditions [66,67]. Foods are inherently biological systems where microorganisms facilitate metabolic transformations by degrading macromolecules through processes such as fermentation and ripening. For example, shotgun metaproteomics techniques identified 2,175 proteins in Chinese fermented fish, Siniperca chuatsi. Similarly, the detection of 63 amino acid degradation proteins in strains like Streptococcus sp., Bacillus sp., Escherichia sp., and Pseudoalteromonas sp. indicates that these microorganisms may contribute to aroma development in fermented fish [68]. De Angelis et al. [69] compiled extensive research elucidating the biotechnological properties, metabolic pathways, and environmental interactions of Lactobacillus sp., commonly used in fermented dairy, meat, sourdough, and vegetable products, through metaproteomics. To fully understand these interactions, integrating bioinformatics to reconstruct metabolic pathways has been recommended.

Metabolomics: Recently, food and nutrition scientists have shown increased interest in metabolomics, with significant advancements in metabolomics analyses over the past decades. This field has diverse applications in food and nutrition science, including physiological monitoring in dietary intervention or challenge studies, analysis of food components, assessment of food quality, evaluation of shelf life, and tracking the effects of food processing and consumption. The complexity of metabolomics is amplified by the intake of over 25,000 metabolites through food consumption, prompting extensive research across various food materials [70,71].

In metabolic profiling, several techniques are widely utilized, including gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), capillary electrophoresis/mass spectrometry (CE/MS), near-infrared spectrometry (NIR), Fourier transform infrared spectrometry (FTIR), direct infusion mass spectrometry (MS), and nuclear magnetic resonance (NMR) [72]. For instance, Ferri et al. [73] employed a metabolomics approach to analyze the flavor and antioxidant profiles of different Lactobacillus plantarum strains in sourdoughs made from durum wheat and KAMUT® Khorasan wheat, revealing that L. plantarum fermentation significantly influenced sensory and health-related compounds in both types of wheat flours. Similarly, Ochi and colleagues [74] applied metabolomics to profile Cheddar, Gouda, and Parmigiano-Reggiano cheeses, highlighting that Parmigiano-Reggiano cheese was distinct, with maturation significantly impacting its flavor.

HPLC/MS offers several advantages over GC/MS, including reduced sample preparation time and faster metabolite profile analysis.

Roullier-Gall and others [75] demonstrated that ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) effectively profiled red and white wines from the Burgundy region, providing accurate identification of the wines' chemical compositions. The combination of UHPLC and QTOF-MS also facilitates the identification of mass formulas and molecular structures of unknown compounds. Gil-Solsona et al. [76] successfully differentiated extra virgin Spanish olive oil samples from six regions using UHPLC-QTOF-MS, accurately identifying twelve compounds, although seven of them remained uncertain.

Metagenomics: Metagenomics, a high-throughput sequencing technology, is extensively utilized in the technology of fermented food products to monitor microbial dynamics throughout different stages of fermentation. This approach simplifies the identification of biomarkers for quality control or spoilage and enhances the management of the fermentation process [77]. Numerous studies have demonstrated the effectiveness of metagenomic analysis and data processing in examining the microbiota of various fermented foods. For instance, Xie et al. [78] identified the dominant microbial species in a traditional Chinese fermented soybean product as Enterobacter, Enterococcus, Leuconostoc, Lactobacillus, Citrobacter, and Leclercia. It has been suggested that combining metagenomics with metaproteomics can help determine key enzymes involved in soy fermentation and the functional genes associated with fermented products.

In studying the sourdough fermentation process and the microstructure of yeast and lactic acid bacteria, metagenomics has provided the most accurate and reliable data compared to other omics disciplines [79]. Additionally, metagenomics has unveiled significant new insights into "puer tea," a fermented Chinese tea. While it was previously known that Aspergillus, Fusarium, Penicillium, Rhizomucor, Trichoderma, Cladosporium, Mucor, and various yeasts play crucial roles in fermentation, metagenomics has revealed that bacteria are the predominant microorganisms, with yeast counts significantly higher than those of moulds. Nonetheless, researchers have noted that further work is needed to fully characterize the microbial community involved in puer tea pile fermentation using metagenomics [80].

Transcriptomics: Transcriptomics represents an advanced omics discipline that provides insights into how various factors can alter gene expression profiles [81]. While microarray-based techniques are more cost-effective compared to other transcriptomic technologies [53], RNA sequencing offers more comprehensive data due to its ability to directly characterize sequences, making it particularly useful for identifying the complete genomic sequences of microorganisms. Although transcriptomics, through RNA sequencing and microarrays, has a broad range of applications in biological research, its application in food microbiology is still in its early stages [82].

Proteomic and transcriptomic analyses have proven effective in characterizing the features and functionality of the probiotic Lactobacillus rhamnosus GG. These studies found that gene transcript levels varied significantly and identified 42 differentially abundant proteins, including both intracellular and surface-exposed proteins. These proteins appear to enhance the interactions between the probiotic bacterium and the host mucus when exposed to sublethal doses of bile [83]. Furthermore, transcriptomic analyses have enabled the distinction between starter and non-starter bacteria and the quantification of both live and dead cells. Integrating transcriptomics with proteomics and metabolomics can provide more detailed insights into cheese microflora and flavor development, which is crucial for optimizing processing parameters and reducing costs [84].

2.1.3 Clustered regularly interspaced short palindromic repeats (CRISPR) system

This method requires only two essential components, a Cas enzyme and a guide RNA (gRNA), making it simpler and more affordable than previous approaches. It also offers versatility, since it can be tailored for different organisms by simply changing the gRNA to fit the new target organism, and it does away with the requirement for costly equipment. Since early diagnosis and intervention can stop disease outbreaks, CRISPR-based detection techniques are especially useful for public health surveillance and response [86].

Microbiological immune defenses include CRISPR systems, which are essential for identifying foreign nucleic acids based on their sequences and eradicating invasive pathogens through endonuclease activity linked to the

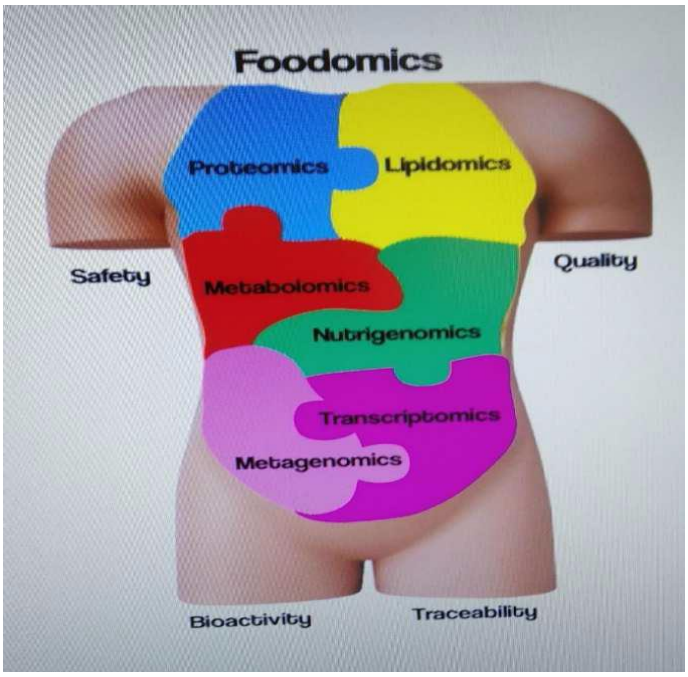

Fig. 2. Omics disciplines which make up the Foodomics [85]

CRISPR-associated (Cas) enzyme [87]. The Cas enzyme (a CRISPR-associated protein) and guide RNA (gRNA) are the two main components of the CRISPR system. In order to direct the Cas enzyme to cut the DNA at the specified site, the gRNA is designed to attach to a particular DNA sequence inside the target genome. Effective and accurate DNA editing is made possible by this exact targeting [88].

In recent years, the CRISPR-Cas system has expanded its uses beyond genome and RNA editing to include nucleic acid detection. Technologies based on CRISPR/Cas have emerged as revolutionary tools for pathogen identification in a variety of sample types [89]. The most widely used CRISPR systems for nucleic acid detection are those in class 2, which comprise Cas9, Cas12, Cas13, and Cas14 [90]. For example, CRISPR-based assays have been created by researchers to quickly identify Salmonella species in food, water, and clinical samples. Because of their great sensitivity and specificity, these assays are essential for monitoring illness and guaranteeing food safety [91].

A CRISPR-Cas13a (CCB) bacterial detection platform was used in a study by Zhou et al. [92] to identify the pathogen Staphylococcus aureus in food samples. Excellent selectivity for S. aureus was demonstrated by the CCB-detection approach, with little interference from other bacterial species. Moreover, this technique performed similarly to conventional culture-based approaches but had faster findings and higher sensitivity for identifying both spiked and non-spiked food samples.

The CRISPR / Cas9-triggered isothermal exponential amplification reaction (CAS-EXPAR), which Huang et al. [93] demonstrated, is another noteworthy advancement in the detection of Listeria monocytogenes. A highly pathogenic foodborne bacterium, Listeria monocytogenes is present in a variety of foods, such as milk, dairy products, eggs, poultry, and meat [94,95]. Targeting the hemolysin (hly) gene of L. monocytogenes is the CAS-EXPAR technique. This method makes use of both nicking endonuclease (NEase)-mediated amplification and the unique nicking activity of Cas9. RNA is taken out of the bacteria, changed into cDNA, and then Cas9 cleaves it with the help of particular sgRNA and PAMmers. Without the need of exogenous primers, the cleaved products are amplified via EXPAR-mediated amplification utilizing EXPAR templates. Finally, SYBR green fluorescence is used to identify the amplified products.

Recently, scientists have created more comprehensive and reliable techniques for using CRISPR/Cas to identify microorganisms in food

and other materials. For example, Shen et al. [96] created a novel allosteric probe (AP) with CRISPR/Cas13a (APCCas) for the detection of Salmonella enteritidis, employing entire bacteria as the target. Ma et al. [97] have also created a CRISPR/Cas12a-powered dual-mode biosensor that is based on gold nanoparticles (AuNPs). The Salmonella virulence gene Invasion gene A (invA) was the target DNA. Sun et al. [98] created a CRISPR/Cas9 induced SDA−RCA technique on the UiO66 platform to identify Escherichia coli O157:H7. A technique based on CRISPR/Cas and loop mediated Isothermal Amplification (CIA) detection of P. aeruginosa has been developed by Mukama et al. [99]. Wang et al. established a CRISPR/Cas system for A. baumannii detection [100]. The CRISPR-mediated DNA-FISH was recently introduced by colleagues Kyeonghye Guk et al. [101]. By focusing on the gene mecA, this CRISPR-mediated DNA-FISH was created to identify methicillin-resistant Staphylococcus aureus (MRSA).

2.1.4 Other Molecular Biology/Biotechnology Techniques

Molecular biology techniques, including Polymerase Chain Reaction (PCR), expression cloning, microarrays, biosensors, gel electrophoresis, macromolecule blotting, and probing, have profoundly impacted novel food development, traceability, food authentication, and genetic modification [102].

PCR and its derivative, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), are reliable methods for detecting adulterations in various meat products [103]. Identifying the animal species used in meat production is crucial for both sanitary and economic reasons. For example, PCR-RFLP assays have been employed to analyze the presence of equine and ruminant species in Egyptian sausage and minced meat.

Real-Time PCR has gained significant attention due to its precision, speed, and reproducibility, making it a valuable tool in the food safety sector for quality control and analysis [104,105]. Kabacaoğlu and Karakaş [106] demonstrated the effectiveness of Real-Time PCR in detecting adulteration in starch-based products, revealing precise DNA measurements. Similarly, Sobrino-Gregorio et al. [107] utilized Real-Time PCR to assess the inclusion of sugars from various plant sources in honey. Villa et al. [108] developed a Real-Time PCR-based method to identify adulterations in saffron plant products.

For accurate analysis using Real-Time PCR, it is essential to extract sufficient quantities of the target gene region from the nucleic acids. Quantitative PCR methods are typically used for analyzing genetically modified organisms (GMOs) and pathogen microbes in food quality control laboratories. In contrast, qualitative approaches are effective for identifying meat types, milk origins, and allergens. Increasing the use of Real-Time PCR methods is likely to enhance the detection and prevention of food adulteration.

Food control agencies and related industries widely utilize the ELISA (Enzyme-Linked Immunosorbent Assay) method to assess the presence and concentration of allergenic proteins in food products [109]. Another frequently employed technique for protein identification and allergen detection is Western blotting [110]. This method involves separating proteins using gel electrophoresis, followed by the detection of specific proteins or antigen-antibody interactions within blood or tissue samples [111].

In the realm of food safety, detecting food pathogens is of paramount importance. The lateral flow assay (LFA) is an advanced technique increasingly used for pathogen detection. It offers high sensitivity, rapid detection times, and straightforward operation, making it ideal for on-site testing [112]. The LFA method has gained popularity due to its ability to quickly and cost-effectively quantify and detect pathogens and proteins.

3. BIOTECHNOLOGY RESEARCH POTENTIALS AND INITIATIVES FOCUSED ON FOOD SAFETY IN AFRICA

Recent theories have sought to explain why African countries, despite their rich agricultural biodiversity, continue to be net importers of plant and animal products [114-116]. As of 2017, Africa's staggering import bill for food and meat, amounting to $82 billion, highlights a significant issue. This situation may be attributed to the underutilization of advanced molecular biology and biotechnology techniques that could optimize the use of plant and animal genetic resources [117]. The previous section has outlined some sophisticated methods that could help address this gap.

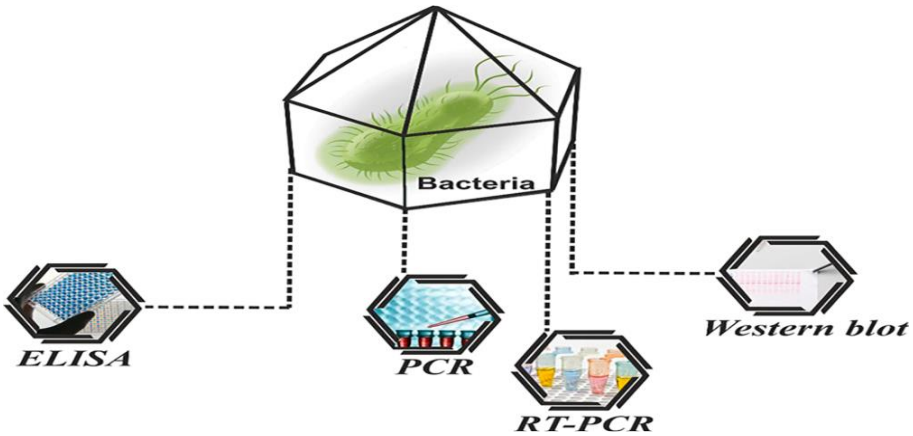

Fig. 3. Common biotechnology methods for detecting pathogenic bacteria [113]

Despite the substantial challenges faced by Africa—including technological, political, economic, developmental, and social constraints—the concerted efforts of agricultural researchers, food safety experts, farmers, and industry professionals have led to increased food production in response to the continent's growing food demands [118]. Furthermore, experts in development, social sciences, and politics recognize that there is considerable untapped potential in Africa's food production sector [119].

Although there is a strong call for enhancing technological approaches to tackle pressing food safety issues in Africa, global researchers remain optimistic that current molecular biology, genomics, and biotechnology techniques used in the region can be further improved to address food insecurity [120]. Below are some key examples of current strategies and innovations being implemented or explored in Africa to enhance food safety and agriculture.

Advancements in molecular biology for food safety in Africa are evident through various successful projects and international collaborations. Notably, South Africa and Argentina have pioneered molecular farming, focusing on the development of experimental therapeutics and vaccines for both livestock and human diseases [121]. Additionally, Africa has explored genetically modified (GM) crops, emphasizing the importance of biosafety processes and policies [122]. The potential of genomics in promoting sustainable agricultural research for food security in sub-Saharan Africa has been recognized, with a call for leveraging local resources and building capacity. However, challenges such as funding limitations, inadequate practical applications, and varying attitudes towards biosafety regulations persist [123]. These challenges highlight the ongoing need for investment and collaboration in molecular biology to enhance food safety in Africa.

Arthur and Yobo [122] introduced a decision-making tool designed to address the debate over whether to implement GM crop cultivation in certain sub-Saharan African countries. This tool offers a structured, reasoned approach that helps identify potential adverse effects of genetically modified organisms and assesses the seriousness and likelihood of such impacts [124]. Johnson et al. [125] proposed two additional decision-making tools to assist policymakers in reaching a consensus: scientific risk assessment and risk analysis methods. These tools are crucial for regulatory decisions regarding GM crops, with risk assessment forming the basis for determining whether to authorize the environmental release of GM organisms [124]. Furthermore, scientific decision-making tools such as environmental impact assessments and Life Cycle Assessments (LCAs), also known as “cradle-to-grave” analyses, if applied in sub-Saharan African countries, can help evaluate the environmental impacts of GM crops throughout their life cycle [126].

Bio-fortification, while not a panacea, has emerged as a highly effective strategy to address malnutrition. Organizations such as CIMMYT, IITA, and various national partners across Africa, Asia, and Latin America have successfully used both conventional breeding and molecular techniques to develop and release several nutritious maize cultivars. These cultivars have achieved high levels of nutrition without sacrificing grain yield or other critical agronomic

and adaptive traits. Many of these bio-fortified maize varieties are now cultivated by farmers and widely accepted by consumers in numerous countries [127]. The integration of advanced phenotyping methods with molecular breeding has enabled the attainment of breeding goals for various nutrients in maize.

The introduction of genomic technologies, such as molecular marker-assisted selection, has been shown to significantly enhance productivity, particularly in developed regions [128]. Agricultural production in Africa faces numerous challenges, including drought, disease, and heat stress, which contribute to low yields. However, genomic selection has demonstrated its efficacy in improving traits related to heat and drought tolerance. For example, Cerrudo et al. [129] reported that genomic selection increased genetic gains for these traits in maize by 4.4 to 19.4%. This improvement underscores the potential of genomic marker-assisted selection to substantially boost the production of maize—a staple crop and major source of carbohydrates for both humans and animals in Africa—thus meeting the increasing demand.

Furthermore, recent re-sequencing of the entire genomes of four upland NERICA rice varieties has identified potential causal genes linked to key agronomic traits such as salinity tolerance, susceptibility to bacterial leaf blight, grain shattering, and awnness. This highlights the significant potential of genomics in enhancing plant cultivars that were originally developed through traditional selective breeding.

4. FUTURE DIRECTIONS AND RECOMMENDATIONS

To further enhance food safety in Africa using molecular biology approaches, it is essential to expand the implementation of advanced molecular techniques like those highlighted in section 2 of this work. These techniques should become integral parts of routine monitoring and surveillance systems for foodborne pathogens, providing comprehensive and real-time data on microbial communities and their dynamics in various food matrices. Additionally, developing local capacities through extensive training programs and upgrading laboratory infrastructure are vital. Investment in modern equipment and consistent supply of necessary reagents and consumables will ensure high-throughput and accurate molecular analyses.

Collaboration and networking among African countries are crucial for sharing resources, expertise, and data. Establishing regional centers of excellence in molecular food safety can help standardize protocols and coordinate efforts across the continent. Public-private partnerships should also be encouraged to drive innovation and facilitate the practical application of molecular techniques in food safety practices. Integrating molecular data with traditional microbiological methods can enhance the robustness of food safety diagnostics, improving both sensitivity and specificity in pathogen detection.

Policymakers should focus on developing harmonized food safety standards and regulations across African countries, incorporating molecular biology techniques for pathogen detection and monitoring. Establishing clear regulatory frameworks for the use of genetically modified organisms (GMOs) and other biotechnological advancements in food safety is also necessary. Funding focused research programs that address local food safety challenges and develop tailored molecular solutions should be a priority. Innovations in detection methods, such as the development of novel molecular assays and portable diagnostic tools for rapid on-site testing, should be encouraged to reduce reliance on centralized laboratory facilities.

Public awareness campaigns should be launched to educate consumers about the benefits of molecular techniques in ensuring food safety and highlight the importance of safe food handling practices. Engaging various stakeholders, including policymakers, food industry representatives, and community leaders, in discussions about the role of molecular biology in food safety can garner support and collaboration. Implementing molecular techniques for environmental monitoring of food production areas and promoting sustainable agricultural practices, such as breeding disease-resistant crops and optimizing pesticide use through precision agriculture, are essential for maintaining long-term food safety and security in Africa.

By focusing on these future directions and recommendations, African nations can significantly enhance their food safety frameworks, reduce the burden of foodborne diseases, and improve public health outcomes across the continent. The integration of advanced molecular techniques, capacity

building, collaborative networks, and supportive policies will collectively contribute to a more secure and sustainable food supply.

5. CONCLUSION

As scientific discoveries and technologies advance, molecular biology approaches will find more in-depth applications in ensuring safer foods for all. Advances in novel detection approaches for food-borne allergens and pathogens are particularly relevant for the African scientific community as they serve as potential alternatives.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. ChatGPT (Version 0.12.0)
2. Quillbot

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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