

REVIEW ARTICLE

MEAT QUALITY & SAFETY ASSESSMENT

Recent Advances on Harnessing Multi-Omic Approaches for Assessing Meat Quality and Safety

Rajendran Thomas 1* , Devarshi Bharadwaj¹, Sangeeta Singha², Dolly Sharma¹

¹ Food Quality Control Laboratory, ICAR-National Research Centre on Pig, Rani, Guwahati 781131, Assam, India 2 Faculty of Science, Assam down town University, Panikhaiti, Guwahati, Assam-781026, India Corresponding author: Rajendran Thomas, Email: thomasrlpt@gmail.com

Article Chronicle: Received: 22/05/24 Accepted: 25/06/24 Published: 30/06/24

Abstract

Effective methods for identifying, averting, and reducing foodborne diseases are necessary to ensure the safety of meat products, which is crucial for maintaining public health. Omics technologies have become such effective instruments for tackling the intricate problems related to meat safety in recent times. In order to improve meat safety, this review offers a comprehensive account of recent developments in the fields of genomics, metagenomics, proteomics, transcriptomics, and metabolomics. Omics technologies are indispensable for ensuring meat safety as their holistic approach provides a deep understanding of meat composition and microbial dynamics, enabling targeted interventions to minimize food safety hazards. Continued research and innovation in omics hold promise for further enhancing meat safety and safeguarding public health. By overcoming the identified obstacles, omics technologies have the potential to revolutionize meat safety assessment and ensure the delivery of safe and high-quality meat products to consumers. In order to promote the continued application of these methods, scientific training programs that bridge the gaps in omics technologies, bioinformatics, and food and public health microbiology are advocated.

Keywords: Meat quality and safety, Multi-Omic, Omics technology, Public health food microbiology

1 Introduction

The world's population is still expanding at a rate that has never been seen before, which presents serious problems for food security and productivity. According to projections, there will be over 9 billion people on the planet by 2050, which will put tremendous strain on agricultural systems to feed this growing population in a sustainable manner. Although plant-based diets typically require fewer resources to produce compared to animal-based diets, meat among other animal products has been a part of human diet since time immemorial. Historically, meat consumption has been higher per capita in developed nations, but emerging economies, especially those in Asia, have also seen notable increases in meat consumption as meat and its products possess nutritional properties that are generally hard to obtain from other animal products. Moreover, meat products provide distinctive flavours, shelf-life stability, and convenience while helping to value-add parts of the carcass that are less ideal for fresh consumption. Meat has also been as-

sociated to reports of it causing foodborne illnesses and as a problem for public health. Effective control is thus required and having a thorough understanding of meat safety from production to consumption is necessary(1). Since the 1990s, meat safety is based on the knowledge or awareness of risk, and attempts are made to manage a risk by enhancing or improving a process, or imposing arbitrary standards at a particular point in the chain(2). The physical and chemical characteristics of meat, such as its neutral pH, high water activity, and quantity of nutrients, might facilitate bacterial growth during storage, leading to the formation of undesirable colors, textures, or odours that worsen spoilage. Meat is hence, a complex environment with regard to its microbial load and composition, containing a wide variety of microorganisms that can originate from the animal itself or the environment they were processed, like slaughterhouses. This is crucial because, depending on whether a food is fermenting or spoiling, the microbiota may have a positive or negative impact on its quality and safety. Moreover, the processing and storage conditions have a significant impact

on the microbial shaping in both situations(3). Understanding national variations in foodborne disease epidemiology and the relative contribution of meat from different species as vehicles for human infection have improved as a result of the process of calculating the worldwide incidence of foodborne disease, which has required the application of new techniques. As per the Global Burden of Foodborne Disease Study by (World Health Organization, 2015)(4) , which assessed the burden of foodborne illness in 14 areas encompassing 195 nations worldwide. An estimated 600 million foodborne disease cases, including 230,000 instances of diarrheal disorders, were linked to 31 foodborne dangers in 2010. These cases resulted in a total of 420,000 reported deaths. An amazing range of functional diversity is provided by several biotic parameters (e.g., the microbiota presents in the raw food, their components, and their relative abundance) and environmental parameters (e.g., temperature, gas mixtures used for packaging, storage conditions), such as the addition of preservative compounds. Depending on these characteristics, distinct microbial assemblages between the different groups can be seen. As a result, there are far too many difficult-to-handle spoilage scenarios, which restricts our ability to generate basic information in this field of study. Traditional scientific methods were largely used in the 20th century to gain an understanding of the factors influencing basic meat characteristics (such as tenderness, colour, and water-holding capacity), the molecular mechanisms underlying these characteristics, and the biochemical pathways involved. The term "omics" came into being when it was coined by Dr. Thomas H. Roderick in the year 1986. It basically refers to the new molecular-based technologies that attempt to research genomes, metabolites, proteins, and cellular transcription in order to understand the dynamics, structure, and function of biological systems in a holistic manner(5) and such an approach may deepen our understanding of how microorganisms that causes foodborne illnesses infect, endure, and persist across food systems. Future use of this knowledge can forecast the likelihood that a pathogen will be consumed and cause disease, which in turn will have a substantial impact on risk assessment, and consequently, risk management strategies. The practice of dividing microorganisms into discrete subgroups according to different traits, such as genetic makeup, biochemical characteristics, antigenic profiles, or other pertinent features, is known as subtyping. With the use of this classification, researchers can more fully comprehend the diversity seen within microbial populations and pinpoint particular strains or variants that might possess special qualities like virulence, resistance to antibiotics, or epidemiological significance. The use of molecular tools additionally gives rise to some concerns, such as the possibility that fast detection techniques could result in a decrease in pathogen isolation, which would then make it more difficult to perform subtyping (because there would be fewer pure isolates from food samples or human specimens). This problem could, at the very least, be partially resolved by using omics' techniques that enable fast detection and subtyping without the need for bacterial isolation.

2 Application of Omics Tools for Assessing Meat Quality and Safety

Numerous omics tools have the potential to significantly increase our capacity to stop foodborne illness cases and outbreaks, as will be discussed in this review. It is hence vital to thoroughly evaluate the advantages and drawbacks of utilizing these tools in order to ensure that their full potential is realized.

2.1 Genomic Approaches

The number of bacterial genomes sequenced has increased dramatically in recent years due to the comparatively modest size of bacterial genomes and advancements in largescale sequencing techniques. In fact, genome sequencing has become quite simple and is frequently contracted out to specialized service providers. Most sequenced genomes are publicly accessible through databases like the National Center for Biotechnology Information Genome Web site (http://www.ncbi.nlm.nih.gov/sites/entrez), which makes most genomes freely available. The most common foodborne pathogens have had one or more of their strains sequenced as one cannot stress the significance of genetic and genomic information in comprehending the ecological and evolutionary adaptations that fuel the persistence of food-borne diseases. Over the past ten years, the use of whole-genome sequencing (WGS) to characterize foodborne pathogens has yielded remarkable insights into their epidemiology, biology, evolution, and population structure. This has allowed for the precise arrangement of these pathogens into a phylogenetic hierarchy that essentially recapitulates the natural population structure of each species. The WGS of pathogenic strains now consistently offers a very dependable and predictive way to assign different phenotypic and diagnostic features to a particular isolate(6). Important phenotypic tests like serotyping, antimicrobial resistance (AMR) testing, and phage typing (PT) were previously tedious and costly during surveillance and diagnostics. However, genetic and genomic alternatives have since been developed that can produce results that are comparable and extremely reliable just by examining the genomic sequence of a specific isolate. Many large-scale genome sequencing projects have helped to sequence thousands of foodborne pathogen genomes globally. Examples of these projects include the FDA's GenomeTrakr project and the Centers for Disease Control and Prevention's PulseNet network(7). Furthermore, foodborne pathogen genome sequencing and analysis is still being conducted by university research institutes, public health organizations, and private businesses in an effort to improve our knowledge of foodborne illnesses and guide public health initiatives.

Many computer-aided techniques can be used to evaluate a whole genome sequence once it has been identified. Genomes may now be mined to produce an enormous amount of valuable information thanks to significant advancements in bioinformatics(8). For instance, in silico analyses are able to discover genes important in growth and survival in various environmental niches and assemble comprehensive metabolic pathways. To find shared and distinct genes, the genome sequences of foodborne pathogens

Bacterium	Relevant characteristics	Number of genome
		sequences available
		on NCBI genome
		database
Listeria monocytogenes	Gram positive	24
Yersinia enterocolitica	Gram negative	
Aeromonas hydrophila	Gram negative, toxin producer	$\overline{2}$
Clostridium botulinum	Gram positive, toxin producer, spore former	15
Bacillus subtilis	Gram positive, toxin producer, spore former	5
Bacillus licheniformis	Gram positive, toxin producer, spore former	$\overline{2}$
Bacillus cereus	Gram positive, toxin producer	20
Salmonella	Gram negative	20
$\overline{Vibrio\ parahaemolyticus}$	Gram negative, toxin producer	
Escherichia coli	Gram negative	20
Staphylococcus aureus	Gram positive	20
Clostridium perfringens	Gram positive	9
$\overline{Campylobacter}$ jejuni	Gram negative	13
Source: Begley and Hill, 2010		

Table 1: Food Borne Pathogens and Their Genome Sequence Availability

can be compared with those of non-pathogenic species and with each other. Moreover, the ability to generate testable hypotheses from genome sequence analyses is perhaps its most significant use in the design of functional genomics research. Additionally, information can be used to plan the experimental setup and evaluate the outcomes of transcriptomic, proteomic, and metabolomic research.

2.2 Metagenomics Approaches

The concept of a metagenome encompasses a theoretical collection of every genome from individuals in a microbiological community from a certain environment. Such a holistic point of view makes it possible to untangle the intricate microbial communities that are present in meat habitats in significantly greater detail than ever before when used in conjunction with conventional techniques. The genus Companilacto bacillus, Dellaglioa, Lacticasei bacillus, Lactiplanti bacillus, Latilacto bacillus, and Paucilacto bacillus have recently been added to the taxonomy, replacing Lactobacillus, which was formerly the most common genus in meat and meat-associated matrices(9; 10; 11; 12). The nextgeneration sequencing techniques can be used in a variety of sequencing strategies, depending on the research topics to be addressed and the resources (budgets, manpower, etc.) at hand. Metagenomics techniques were employed by(13) to determine that Campylobacter jejuni was the cause of a foodborne illness case that could not be diagnosed using traditional microbiological culture. In summary, metagenomic analyses revealed that C. jejuni DNA was detected in a faecal sample taken from a patient who had symptoms similar to campylobacteriosis, but was absent from a faecal sample taken from the same patient three months after the infection had cleared up, indicating C . jejuni as the causal agent. In amplicon-based HTS, a specific area of a phylogenetic marker gene is amplified by a polymerase chain reaction (PCR), most frequently the 16S rRNA gene for bacteria, and then sequenced. All bacterial species possess

the 16S rRNA gene, making it the ideal target for investigations of bacterial diversity, particularly in complex food matrices where an overabundance of eukaryotic hosts may restrict the depth of sequencing. For other categories of microbes, amplicon targets can be employed, such as ITS for fungi, 18S rRNA for eukaryotes, or RdRP for RNA viruses. In case of samples expected to be containing a significant amount of eukaryotic host DNA, such as milk, species- or genus-specific metabarcoding techniques (e.g., gnd gene for Escherichia coli), multiplexed marker metabarcoding, host DNA depletion, and/or deep shotgun sequencing may be n ecessary $(9; 14)$ This is however a frequently used, quick, and affordable technique for high-level profiling of microbial community. However, the majority of sample preparation and processing-related sources of bias exists which include choice of amplification region, amplification reaction (template concentration, template GC content and secondary structure, primer mismatches, and polymerase errors), number of target gene copies per cell, chimeric reads, and metabarcoding sequencing mistakes, and such biases may cause some bacteria to be overrepresented (15) . Also, since metabarcoding often confines taxonomic classification to the genus level, it may be unable to differentiate between pathogenic and non-pathogenic species (e.g., L. monocytogenes versus L. innocua or Shiga toxin–producing E. coli [STEC] versus commensal $E.$ $\text{coli}(9)$. In a more recent study, (16) conducted an intriguing investigation in which they collaborated with 15 laboratories (an inter-laboratory ring trial) to standardize an analytical technique based on DNA metabarcoding assay for the detection of adulteration from poultry and mammalian species. In this European investigation, 16 anonymously labelled samples (8 samples, 2 subsamples each) comprising six combinations of DNA extract, one from maize, which served as the trial's negative control, and another from a model sausage were sent to each research team for analysis. The method's evaluation criteria enabled the researchers to verify the DNA metabarcoding approach's dependability for meat species

verification in routine analysis. Shotgun metagenomics is the process of sequencing every bit of DNA found in a sample. The assembly of the metagenomic sequence reads into entire genome sequences, known as Metagenome-Assembled Genomes (MAGs), followed by genome annotation, enables taxonomic profiling at a higher resolution, i.e., species-level identification. This method is advantageous due to the absence of amplification bias, increased specificity of identification and representation of variety, and capacity to identify organisms from many kingdoms, outweigh the fact that it is more expensive than metabarcoding sequencing(17). Following sequencing, data is evaluated in accordance with the study's aims, which may include assessing the community's taxonomic diversity, gene prediction and functional annotation, or correlating community data with a specific condition. This also makes it possible to examine the functional potential of the present microorganisms and may identify species that are still undiscovered(18). When only a few species within a genus are pathogenic, shotgun metagenomics can also be employed for identification at the species and subspecies levels, which is obviously advantageous. Of all the techniques, long-read metagenomics is the newest and least developed approach, but it has the potential to combine the best properties of both shotgun and metabarcoding approaches. The long reads produced mean that amplification is not needed because whole target regions (e.g., 16S rRNA genes) are frequently recovered intact. It is also often possible to recover intact genomes of microorganisms and plasmids, potentially gathering valuable phylogenetic and virulence data. Sample preparation is also simpler than that for the shotgun approaches. However, cost of analysis per sample is higher, and as discussed in the sequencing technology section, the sequencing error rate is higher but is improving all the time In general, NGS techniques are potent tools for learning more about the microbial populations of meat and meat-derived products. There is still a lot of promise in this field because the use of NGS technologies in studies on meat and products generated from meat is still in its infancy. Such technologies provide a new way of hazard identification for use in Microbial Risk Assessment (MRA) based on the use of specific markers (genes, transcripts, proteins or other molecular signatures) rather than whole microorganisms (3). Knowing the genetic composition and expression profile of a bacterial isolate may help determine its host range, how it might persist and survive through the food chain and whether or not it has the potential to cause disease in humans. This can be achieved if particular genes or combinations of genes may infer greater potential to cause disease in humans or a greater ability to survive through the food chain. The impact of processing on refrigerated pork sausages has also been investigated(19). In this instance the researchers described the dynamic microbiota of pork sausage throughout its storage, with its initial microbiota being first replaced by Pseudomonas spp., and then subsequently by the lactic acid bacteria Lactobacillus graminis and Carnobacterium divergens. Apart from microorganisms, a study by(20) shows how novel parasitic infections causing foodborne illness can be identified using metagenomic techniques. In this particular investigation, the myxosporean parasite Kudoaseptem punctata was found to be the most likely causative agent behind several foodborne illness outbreaks linked to the consumption of a particular fish species (olive flounder, Paralichthys olivaceus) through a combination of epidemiological investigations, metagenomic studies, and animal studies. The use of metagenomics as a technique for the identification of foodborne pathogens in food-associated environments and foods still confronts a number of challenges, despite the fact that a number of papers highlight the promise for metagenomics applications in food safety. Metagenome sequencing, for starters, can identify DNA from living and dead things. Although samples may test positive for a foodborne pathogen because of dead cells, even if the DNA from dead cells may deteriorate with time (e.g., for food samples tested after pasteurization or environmental samples tested after sanitation). Moreover, the generation of large sequence data sets linked to specific food or food-related facilities (such as farms or processing facilities) by metagenomics approaches presents another difficulty. These sets are likely to contain some sequence data that could be mistakenly interpreted as indicating a risk to food safety (such as the presence of virulence or antimicrobial resistance genes). Nevertheless, in the recent years, metagenomics has benefited from numerous forward-thinking financial and intellectual initiatives. The scientific community should strive to share, compare, and critically evaluate the findings of metagenomic studies in order to guarantee that those investments are used as effectively as possible. New methods for analysis, storage, and visualisation will be needed as datasets become more intricate and extensive. They will ensure that metagenomics is used as effectively as possible to answer fundamental questions about the ecology, evolution, and diversity of microorganisms as well as to generate and test novel hypotheses.

2.3 Transcriptomic and Proteomic Approaches

Determining the physiological condition of pathogens while they are present on meat is necessary in order to design logical control measures for foodborne pathogens in the supply chain. The physiological state of the pathogen under various circumstances can be ascertained by examining changes in gene and protein expression in response to stress, which can be used to signify the activation or repression of a particular physiological response. Over the past ten years, a number of studies have evaluated the transcriptomes and/or proteomes of bacteria under conditions that are similar to those that a pathogen may meet on food, such as the low temperature and low water activity that E. coli O157:H7 could experience during the chilling of beef carcasses(21). Transcriptomics can be utilized not only to comprehend the physiological status of foodborne pathogens but also to evaluate the response of bacteria to physical, chemical, or biological food preservation methods. Many times, the antimicrobial activity of a particular chemical is well-established, but nothing is known about the antimicrobial's molecular actions. Data from transcriptomics and proteomics have enormous potential for the logical creation of novel foodborne pathogen control methods. Using the data from such research to find novel chemicals that precisely obstruct pathways crucial to food survival is a potential strategy. For instance, a recent study found that the small chemical fluorophenyl-styrene-sulfonamide (FPSS) selectively prevents L. monocytogenes from activating the general stress response sigma factor, SigB(22). Transcriptomics can be utilized not only to comprehend the physiological status of foodborne pathogens but also to evaluate the response of bacteria to physical, chemical, or biological food preservation methods. Many times, the antimicrobial activity of a particular chemical is well-established, but nothing is known about the antimicrobial's molecular actions. An analysis of E. coli O157:H7's transcriptional response to the antimicrobial cinnamaldehyde revealed that the pathogen first triggered the oxidative stress response before quickly becoming resistant to the antimicrobial stress by turning cinnamaldehyde into cinnamic alcohol(23). Often, an ensemble of food preservation techniques is employed; this is referred to as hurdle technology. Hurdle technology stops the growth of microorganisms by combining various preservation techniques. The growth inhibitors in the combination should ideally achieve a higher level of inhibition than the total amount of inhibition attained by each inhibitor alone. This is known as synergy among the obstacle components. In contrast, if a bacterium becomes accustomed to one barrier, the effectiveness of subsequent or contemporaneous hurdles may be diminished, resulting in cross protection from a multitude of hostile barriers. Comprehending the mechanisms of action of these growth inhibitors allows us to comprehend the mechanistic functioning of effectiveness. Apart from studies involving food borne pathogens, a number of studies have been performed in order to assess and improve quality of meat using -omics approaches. (24) evaluated various extraction techniques of the sarcoplasmic and myofibrillar sub-proteomes of the Longissimus thoracis et lumborum (LTL) in the context of the discovery and evaluation of biomarkers for beef quality in order to determine the most dependable protocol for the identification of biomarkers of dark-cutting beef condition, also referred to as dark, firm, and dry (DFD) meat. The authors examined the protein fractions of each extraction procedure using onedimensional sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Higher protein extractability was achieved inside the sarcoplasmic sub-proteome using extraction buffers including Triton X-100, while the TES buffer containing Tris, EDTA, and sucrose was useful in differentiating between the protein patterns of normal and DFD meat. The non-denaturing buffer permitted higher intensity protein bands inside the myofibrillar sub-proteome, but the lysis buffer enhanced protein extractability with greater sensitivity to treatment changes. (25) used a shotgun proteomics technique to find indicators of beef tenderness on young bulls raised in an Irish production system that were evaluated using the Warner-Bratzler shear force. Thirtyfour potential protein biomarkers that differentiate between tough and tender meat categories were disclosed by the scientists. These proteins are involved in metabolic processes that affect heat shock proteins, muscle structure, oxidative stress response, and apoptosis.

2.4 Metabolomics Approaches

Meat quality is a multifaceted attribute determined by various factors, including composition, texture, flavour and nutritional value. A cascade of reactions involving proteins, carbohydrates and lipids affects meat colour, tenderness and flavour, specifically, metabolites that are key biomolecules in biochemical reactions associated with attainment of acceptable meat quality. Metabolites which are small molecules <1000 Da, play an important role in attaining consumer-preferred meat quality traits such as visual appearance, tenderness and flavour of meat. Traditional methods for assessing meat quality often focus on physical and chemical parameters; however, metabolomics enables simultaneous quantification and identification of metabolites involved in various biochemical pathways, providing insights into the metabolic processes underlying meat quality traits(26). Metabolomics routinely utilizes sophisticated and high-throughput analytical platforms such as gas chromatography and liquid chromatography–mass spectrometry (GC-MS and LC-MS) and nuclear magnetic resonance (NMR) spectroscopy. With the advent in analytical techniques, metabolomics has greatly facilitated the study on metabolic fingerprinting and pathway networks. The flavour and aroma profile of meat are shaped by a diverse array of metabolites originating from various biochemical pathways. For instance, the Maillard reaction, a complex series of non-enzymatic browning reactions between amino acids and reducing sugars during cooking, generates excess of volatile compounds that contribute to the characteristic aroma of cooked meat. Previous studies have shown that the aromas in cooked meat are generated by not only the Maillard reaction of free amino acids and reducing sugars, such as glucose, ribose and mannose, but also heat degradation and auto-oxidation of lipids, sugars, amino acids and their reaction intermediates(27). In a different study, the metabolite analysis of the components that contribute to meat odour using a multispecies comparison of chicken, duck, pork and beef has revealed eight substances (E)-2-nonenal, (E,E)-2,4-decadienal, hexanal, heptanal, octanal, nonanal, 1-octen-3-ol, and dimethyl, tetrasulfide which were considered to be universal contributors to meat odour(28).Furthermore, metabolomics studies have elucidated the metabolic pathways underlying the production of bioactive compounds in meat, such as creatine, creatinine and carnosine, which have been implicated in various physiological functions, including muscle metabolism, antioxidant activity and flavour enhancement. Recently, metabolomics study also has revealed the spoilage characteristics in refrigerated ground beef inoculated with Pseudomonas lundensis and Brochothrix thermosphacta. This study has revealed 58 metabolic pathways, in which histidine metabolism was identified as an important pathway related to spoilage(29). Metabolomics has emerged as an effective means in determining authenticity and origin of meat, such as geographical origin and species origin, by characterizing its chemical composition and metabolite levels(14). In the recent past, there's a growing interest among customers regarding the geographical origins of meat. Employing advanced techniques like NMR-based and MS-based metabolomics, researchers have made sig-

Table 2: Recent Studies Conducted in Various Areas of Meat Safety

nificant changes in this area. For example, a study using NMR-based metabolomics distinguished beef from Australia, Korea, New Zealand and the United States based on primary metabolites like succinate and various amino acids. Similarly, another study by(30) utilized MS-based metabolomics to characterize beef samples from different countries, identifying twenty-four metabolites as biomarkers which included many amino acids and sugar metabolites. Metabolomics approaches have also proven successful in discriminating the origins of lamb meat, achieving remarkable classification and prediction abilities.

3 Conclusion

Although the application of omics techniques is still in its infancy in certain areas of microbiological food safety, it has the potential to have a significant impact in others. Some of the imminent challenges that researchers face while going on about an omics study are data integration and interpretation problems as omics generate vast amounts of data. Integrating and interpreting these diverse datasets to extract meaningful insights about meat safety can be complex and requires advanced computational and bioinformatic tools which in turn requires specialized bioinformatics expertise. Moreover, standardizing omics protocols and ensuring data quality and reproducibility are essential for reliable results. Variability in sample collection, processing, and analysis can introduce biases and affect the accuracy and comparability of findings. Implementing robust quality control measures is crucial to mitigate these challenges. Meat samples are already a complex and heterogeneous environment containing diverse microbial communities, proteins, RNA transcripts, and metabolites. Analysing such complex matrices using omics techniques can be challenging, and strategies for effectively capturing and representing the diversity within samples need to be developed that are better in terms of detection limit and robustness. Also, omics technologies can be expensive, and access to equipment, reagents, and expertise may be limited, particularly in resource-constrained settings. Lowering the cost of omics analyses and enhancing accessibility to technology and training are critical for widespread adoption and implementation in meat safety programs. Addressing these challenges requires collaborative efforts from multidisciplinary teams comprising scientists, policymakers, industry stakeholders, and regulatory agencies. By overcoming these obstacles, omics technologies have the potential to revolutionize meat safety assessment and ensure the delivery of safe and high-quality meat products to consumers. Therefore, in order to promote the continued application of these methods, scientific training programs that bridge the gaps in omics technologies, bioinformatics, and food and public health microbiology are required.

Conflict of Interest

The authors declare no conflict of interest in this reported communication.

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