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Omic applications to understand food system microbiomes

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ABSTRACT

Understanding the microbial communities associated with food systems has traditionally used culture-based techniques that can provide a snapshot of the microorganisms present. However, this approach requires multiple media types and only allows for the identification of a limited number of culturable species. Culture-independent methods such as sequencing and omic techniques provide a deeper understanding of the microbial community, how they interact and function together across the entire food system. This review provides a brief introduction to omic techniques used in microbiome studies and touches on microbiome research that has been undertaken across the farm to fork continuum focusing on leafy vegetables where possible.

Keywords: food systems, metagenomics, metaproteomics, metatranscriptomics, microbiomes, omic techniques.

Leafy vegetables are a part of a healthy and balanced diet providing many nutritional qualities including vitamins, minerals, fibre and phytochemical compounds.[1,2] Production and sale of leafy vegetables has increased due to humans consuming leafy vegetables as part of a healthier diet. Between 2019 and 2020, the Australian retail supply of leafy salad vegetables increased by 9%.[3] As production has increased, so too has foodborne illnesses associated with leafy vegetables, with some major outbreaks linked to lettuce, spinach or ready to eat salads due to their raw and ready to eat nature.[4–6] As a result, important strategies, guidelines and policies have been developed both within Australia and overseas.[7,8]

Traditionally, food safety regulatory measures have focused on the identification of foodborne pathogens at various locations within processing facilities and on the final product prior to sale. While it is important to track foodborne pathogens, they occur at low prevalence and low numbers within a broader microbial community that are often present in complex environments such as food production and processing environments as well as food products. This leads to challenges in detecting pathogens in food systems, determining how they enter and survive throughout the food chain and how best to control them.

Understanding the microbial composition and potential impact these communities have on pathogens and spoilage organisms could provide new ways of improving the safety and shelf life of foods. A microbiome is a microbial community and includes the environmental components (chemical, physical and biological) of the ecological niche in which the community exist.[9] The microbiome can be composed of bacteria, fungi, viruses, algae, or small protists, which is known as the microbiota, in a mutualistic or competitive manner. Within food systems, microbiome research can occur within the farm arena to understand the microbial interactions with the crop or animal system and the production of a healthy commodity; within the processing environment to ultimately prevent cross-contamination of products by pathogenic species and reduce spoilage; and within the human gut to understand the dietary effects and impact on the native microbiota of the commodity on human health.

Food microbiome research has historically been conducted using culture-based methods through the identification of pathogens of interest as well as determining total viable counts of bacterial and fungal species. With the development of molecular and sequencing methods and the reduction of costs associated with sequencing, microbiome research nowadays is typically performed using omic techniques like *16S* rDNA amplicon sequencing and whole genome sequencing, metagenomics, metabarcoding, metatranscriptomics, metaproteomics and metabolomics and provides a greater depth of information. These techniques can be used singularly or in combination to help overcome any limitations individual techniques may have as well as providing an understanding of community

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structure, networks and interactions.[10] Utilising omic techniques allows the analysis of the taxonomic composition of all microbial members (understanding who is there), analysis of the metabolic potential (what they can do) and the microbial functioning (what are they doing).[9] The majority of food system microbiome research has focused on understanding the soil microbiome for improving plant/crop growth, with a small amount of research concentrating on microbial communities within the red meat and poultry industry, with limited microbiome research in the horticultural farm to fork space.

A summary of some of the microbiome research and potential applications that have occurred within food systems are highlighted below. Jackson *et al.*[11] utilised culture-dependent and culture-independent methods to assess the bacterial composition of leafy salad vegetables. Culture-dependent methods identified bacterial colonies from six phyla in comparison to culture-independent methods which identified 11 different phylas. While most of the dominant taxa identified in the leafy salad vegetable samples were characterised by both dependent and independent methods, pyrosequencing was able to identify two additional bacterial taxa, *Ralstonia* (endophytic) and *Actinobacter* (associated with the leaf surface). Although *Ralstonia* is capable of growth on trypticase soy agar, the colonies are typically small and may be missed in isolate sampling, therefore the identification of *Ralstonia* by pyrosequencing ensured its presence was detected. Culture-independent methods are able to identify low abundant taxa otherwise missed using culture-dependent methods. Determining the entire microbial community from leafy vegetable samples also allows for the identification of microbial species which may influence the survival of pathogenic or spoilage organisms.

Bacterial *16S* and fungal ITS amplicon sequencing was employed to distinguish the bacterial and fungal communities on spinach and swiss chard with consideration of leaf damage.[12] The fungal community at all stages (baseline – manual harvest and no wash step, machine harvest, washing, packaging) remained consistent for both spinach and swiss chard with *Ascomycetes* followed by *Basidomycota* phyla the most dominant. Whereas the bacterial diversity varied with specific species abundant at different harvesting and processing stages. Spinach and swiss chard communities were both dominated by the phylum *Moraxellaceae*; however, a significant reduction in its abundance occurred following the washing stage. The family *Pseudomadaceae* increased and was the most abundant family in the washing and packaging microbiota.[12] A *16S* rRNA analysis before and after sanitisation in a fresh produce processing facility found greater reduction in bacterial populations and shifts in microbiomes following effective sanitation.[13] The microbial populations on production floors were also found to be consistently reduced by sanitation in comparison to peripheral surfaces like doors and walls. Several species were identified at multiple sites throughout the facility suggesting these species comprised part of the core microbiota of the processing facility. Understanding the microbiota at various stages of processing and how these communities are influenced by the various hurdles and processes associated with food production provides important information on the microbiota the consumer is exposed to and the potential influence it may have on consumer health.

A meta-transcriptomic study performed by Jung *et al.*[14] on the interactions of lactic acid bacteria during a kimchi fermentation identified *Leuconostoc mesenteroides* was most active during early phase fermentation and *Lactobacillus sakei* and *Weissella koreensis* dominated the later fermentation phase. They identified genes typical of heterolactic acid fermentation from pathways relating to carbohydrate transport, hydrolysis and lactate fermentation. The identification of active populations, gene expression and interaction of community members at important stages of the fermentation process would not have been possible with the use of culture-based methods. Proteomics was employed to assess the inhibition potential of modified atmospheric conditions, 30% carbon dioxide (CO_2) and 70% oxygen (O_2), of five typical meat spoilage microorganisms on a simulated meat medium.[15] Proteomic analysis identified the five species were able to co-exist as a result of alternative species-specific metabolic pathways in which synergistic spoilage occurred. Three of the meat spoilage species utilised a variety of mechanisms to reduce oxidative stress, maintain intracellular pH, osmotic balance and oxygen levels and alteration of the fatty acid composition. The use of proteomics provided an insight into nutrient utilisation and adaptation to industry adopted modified atmospheres designed to reduce the growth of spoilage microorganisms and therefore spoilage in general. Identification of members of the core microbiota, particularly if they are spoilage or pathogenic microorganisms, provides valuable awareness of the species which may support the survival of undesirable microorganisms. Metagenomic analyses also provide greater insight into the effectiveness of cleaning and disinfection treatments and offers the ability for facilities to tailor their sanitation methods to target species of interest.

There is substantial research in the human gut microbiome arena demonstrating the value in understanding microbial community interactions. However, microbiome research across food systems and in particular non-fermentative and or leafy vegetables is lacking. The availability of sequencing and omic technologies has the power to rapidly expand our understanding of microbial community members and interactions in this space. Understanding how community members interact and move from one area of the food chain to another area may allow for the development of rapid screening techniques or the development of healthy state (ideal) microbiomes that may increase crop production, reduce contamination by spoilage and pathogenic microorganisms, increase product shelf-life and improve health benefits for consumers.

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Biography

Dr Jessica Gray is a post-doctoral fellow within the Microbiome for One System Health Future Science Platform at CSIRO within the Food Microbiology group. Jess's research focuses on understanding the various microbiomes across the farm to fork continuum.

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