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TECHNOLOGICAL EVOLUTION AND REVOLUTION IN FOOD MICROBIOLOGY

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ABSTRACT BOOK

Keynote 8.2

JOINT FOOD AND AGRICULTURE ORGANIZATION (FAO)/WORLD HEALTH ORGANIZATION (WHO) EXPERT MEETING ON VIRUSES IN FOODS: FOOD ATTRIBUTION, ANALYTICAL METHODS, AND INDICATORS

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The Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on microbiological risk assessment of viruses in foods was convened in September 2023 in response to the request by the Codex Committee on Food Hygiene (CCFH). The Expert Committee: 1) reviewed the literature and available surveillance databases, which ranked foodborne viruses according to frequency and severity; 2) ranked the relevant food commodities of highest public health concern; 3) discussed methods for virus testing performed for outbreak investigation and product testing as part of surveillance and monitoring strategies; and 4) reviewed current and potential indicators for viral contamination.

Human norovirus was identified as the leading cause of viral foodborne illness, followed by hepatitis A and hepatitis E viruses. Hepatitis A virus and hepatitis E virus were ranked equally but higher compared to norovirus in terms of clinical severity. When considering both frequency and severity, the ranking for these viruses are: 1) Norovirus; 2) hepatitis A virus and hepatitis E virus ranked in order; 3) rotavirus, sapovirus, enterovirus, astrovirus, and enteric adenovirus ranked in order.

Despite the methodological advancements, there remain challenges in their use, most notably ensuring accurate interpretation; application to other viruses and/or matrices; integration of sequencing technologies; and implementation in low resource countries. A variety of indicators for viral contamination have been investigated, including bacteria, bacteriophages, and plant and animal viruses. Additional research is needed to determine if there is an appropriate viral indicator for use in other commodities associated with foodborne virus contamination.

Oral 6.1

COMPARATIVE GENOMICS OF *ARCOBACTERACEAE* SPECIES AND INSIGHT ON *ARCOBACTER BUTZLERI* TRANSCRIPTOME DURING INFECTION OF A GUT MUCUS PRODUCER HUMAN CELL LINE

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The *Arcobacteraceae* bacterial family includes species isolated from several matrices and hosts. The division of *Arcobacteraceae* in several genera was proposed recently. *Arcobacter butzleri* represents the species most associated with food and waterborne outbreaks.

Aims of this work were the obtainment of genomics and transcriptomics information related to *A. butzleri* strains and linked to virulence potential. The study was consequently centered on *Arcobacteraceae* type strains to conduct pangenome assessments.

The *A. butzleri* virulence potential was evaluated on gut mucus producer (MP; Caco-2/HT29-MTX-E12) and not mucus producers cell models (NMP; Caco-2; Caco/HT29) infected with 32 strains isolated from humans and animals. Colonization and invasion capacities were evaluated jointly to whole genome sequencing (Illumina) and data analysis. Subsequent RNA-seq analysis on MP models after 30 and 90 minutes of infection were performed, including chemical analysis (HPLC) aimed at validating the gene expression of genes of particular interest. Finally, the study focused on the pangenome of 20 *Arcobacteraceae* was aimed to link their genomic traits with phenotypical characteristics and isolation sources.

A. butzleri has shown the ability to colonize host cells but demonstrated higher colonization in MP. Among the virulence genes, the *tonB* operon (iron transport and virulence) was detected in all *A. butzleri* strains. *A. butzleri* has demonstrated similarities between strains isolated from the same intestinal tract of pig. Transcriptome analysis demonstrated the overexpression of *tonB* operon in strain LMG11119 (higher colonization). Furthermore, an overexpression of genes involved in acetate metabolism was observed, among them *actP-yjcH*, whose functionality was confirmed by a decrease in acetate levels during host-bacterium contact. The *Arcobacteraceae* comparative genomics demonstrated a genome size reduction in animal-related species suggesting an evolutionary adaptation to the host with variation in gene classes percentages and with correlation to specific orthogroups (e.g. TonB-related genes). The pangenome partitions do not support the division of *Arcobacteraceae* family into different genera revealing partitions with numbers comparable to other Gram-negative bacterial genera.

The obtained results provided insights into the virulence genes of *A. butzleri*, enhancing understanding of its virulence mechanisms and associated pathways. Pangenome analysis of *Arcobacteraceae* suggested adaptive capabilities in animal-related species that showed smaller genomes.

Oral 6.2

NOVEL APPROACH FOR THE CONTROL OF CAMPYLOBACTERIOSIS USING *CAMPYLOBACTER*-SPECIFIC PHAGES

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Campylobacter, in particular *Campylobacter jejuni* and *Campylobacter coli* species, are the main causative agent of one of the most prevalent foodborne diseases worldwide, campylobacteriosis. This zoonotic pathogen has been included by the World Health Organisation in its list of priority bacteria resistant and multi-resistant to available antibiotics, in order to urgently seek solutions for its control. *Campylobacter*-specific bacteriophages, campylophages, are a promising solution to address human health, animal health and food safety issues.

With the aim of developing a new biocontrol tool against this pathogen, more than 300 campylophages were isolated from different environmental and food samples, especially from chicken skin. After a host range study, the most promising campylophages were selected and their main stability, specificity and safety properties characterised. Finally, the efficacy of single phages and phage-cocktails was evaluated both *in vivo,* in the animal model *Galleria mellonella,* and in chicken skin.

Morphological analysis by transmission electron microscopy revealed that the phages belong to the family *Myoviridae*, with an icosahedral head and a long contractile tail. They are stable under different pH and temperature conditions of application, and designed cocktails infect more than 70% of the tested *Campylobacter* strains.

Studies in *G. mellonella* suggested that the combined use of antibiotic and bacteriophages enhances treatment efficacy compared to their individual use, resulting in lower mortality (16.2%), than the application of antibiotic alone (41.7%) after *C. jejuni* infection.

Regarding bacteriophages efficacy on food, more than 1.5 Log (CFU/g) reduction in *C. jejuni* load was found when artificially contaminated chicken skin samples were treated and stored under refrigerated conditions.

These results suggest that the application of specific bacteriophages targeting *Campylobacter* have great potential as a food safety strategy for the prevention and control of this pathogen throughout the entire agri-food change, from the farm-to-table.

Oral 6.3

MOLECULAR MECHANISMS MEDIATING THE SURVIVAL AND GROWTH OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* ON DICED ONIONS

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Salmonella enterica subsp. *enterica* is one of the most important foodborne pathogens worldwide. In recent years, many salmonellosis outbreaks were caused by the consumption of contaminated fresh produce. Recent outbreaks in the years 2023, 2021 and 2020 were linked to the consumption of raw onions. An in-depth understanding of the key mechanisms that mediate pathogen fitness on

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