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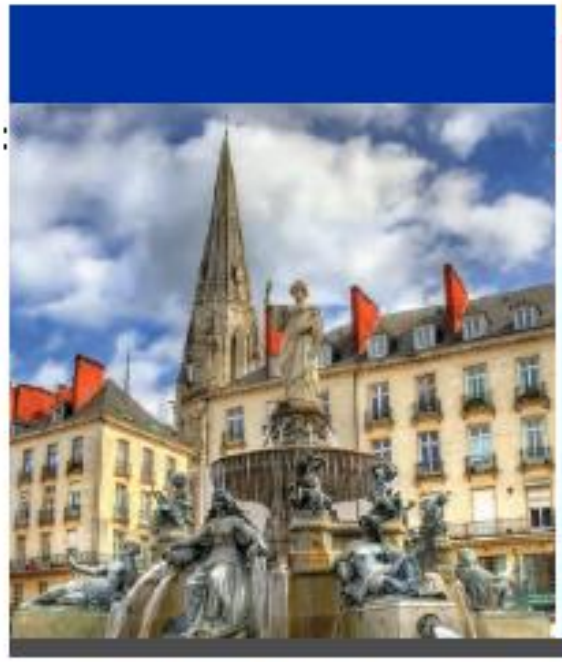

IAFP'S EUROPEAN SYMPOSIUM ON FOOD SAFETY

PROGRAMME

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* Student Award Competitor

P2-34 Exploring Yeast Biodiversity from Dry-Salted Naturally Black Olives with Culture Dependent and Independent Molecular Methods

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Introduction: Dry-salted olives are cultivated mainly on the island of Thassos and they are traditionally prepared by placing the fully mature olives in layers with coarse salt. Despite the low water activity of the final product due to high NaCl concentration, the olives are susceptible to spoilage microorganisms such as yeasts and fungi.
Purpose: To explore the yeast biodiversity of dry-salted table olives from Greek retail outlets with culture-dependent and independent molecular methods.
Methods: Nine samples of dry-salted olives were analyzed. One hundred eighty isolates were clustered after PCR amplification of repetitive DNA with the oligo-nucleotide primer $(GTG)_5$ and profiles with identity percentage higher than 90% were considered to belong to the same group. The typical isolates of each group were further clustered after the amplification of ITS region using ITS$_1$ and ITS$_4$ as primers and the implementation of RFLP analysis with the restriction endonucleases Hinf I, Hae III and Cfo I. A total of 20 isolates with discriminant differences were subjected to sequencing of the ITS region of rRNA gene and the results obtained were aligned with BLAST to determine the closest known relatives. Next generation sequencing was performed after the amplification of 26S rRNA gene using LS2F and NL4bttS as primers.
Results: *Pichia membranifaciens*, *Candida sorboxylosorans*, *Citeromyces cyanensis*, *Candida etchellsii*, *Wickerhamomyces subpelliculosus*, *Candida apicola*, *Wickerhamomyces anomalus*, *Torulaspora delbrueckii*, and *Wickerhamiella versatilis* were the dominant species among the samples according to ITS sequencing (culture-dependent method), while the dominant species showed by NGS (culture-independent method) were *Candida etchellsii*, *Pichia triangularis*, *Pichia membranifaciens* and *Candida versatilis*.
Significance: Dry-salted olives are an economically important product for the Greek table olive industry and the biodiversity of yeasts in the final product is revealed for the first time by means of molecular techniques.

P2-35 Control of Undesirable Microbial Growth in Table Olive Fermentation Using Selected Yeast Strains with Multifunctional Potential

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Introduction: The implementation of microbial starters related to table olives with multifunctional features during the fermentation process can reduce the risk of spoilage and the growth of undesirable microorganisms.
Purpose: To assess the survival of five autochthonous yeast strains belonging to *Candida boidinii* and *Saccharomyces cerevisiae* inoculated in the brines of Kalamata natural black table olives during fermentation and monitor the changes in the microbiological and physicochemical profile.
Methods: Olives were fermented in seven percent w/v NaCl and inoculated in monoculture with four yeast strains belonging to *C. boidinii* (Y27, Y28, Y30, and Y31) species and one strain of *S. cerevisiae* (Y34) during the first day of the fermentation to a final concentration of 2×10^6 CFU/mL. Changes in microbiological counts and pH were analyzed for a period of 150 days. The survival of the inoculated strains has been estimated by gel electrophoresis from PCR amplification of repetitive DNA elements with the oligonucleotide primer $(GTG)_5$ at different fermentation points.
Results: At the end of the fermentation, yeasts' populations ranged from 3.8 to 5.3 log CFU/g in olives, while they reached a population between 3.6 and 6.4 log CFU/mL in brines. Moreover, pH values ranged from 3.7 to 4.9 among the treatments for both the olives and the brines. The results obtained from rep-PCR for the first day of fermentation showed 100% survival of the starters. At the end of the process three out of five starters namely, Y27, Y30, and Y31 (seven isolates, one isolate and two isolates, respectively) showed survival on the olive flesh.
Significance: The survival of the strains used as starters during fermentation is very important as table olives are considered to be a good matrix for several microorganisms with probiotic potential.

P2-36 Prevalence and Relevance of *Salmonella* spp. Testing in *Camellia Sinensis* Tea

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Introduction: Tea is the second most commonly consumed beverage in the world. Its forecasted growing consumption is driven by consumer demand for new flavours and recognition of the health benefits, which results in the emergence of innovative herbal tea and tea blends with the addition of herbs and spices. Currently, no legislation specifies microbiological criteria for tea in Europe. However, available guidelines require the absence of pathogenic *Salmonella* spp. in finished products.
Purpose: This study evaluates the prevalence of *Salmonella* spp. in leaf and herbal teas to mitigate the risk associated with this pathogen. Findings are used to assess the relevance of finished product *Salmonella* testing for these commodities.
Methods: Information relating to the prevalence of *Salmonella* spp. in *Camellia sinensis* was collected following searches of electronic databases, scientific journals, books, technical reports and company internal data. The search strategy included keywords "microbiology", "tea", "prevalence, "quality", "*Salmonella*".
Results: An exhaustive search of the literature (over 2,100 publications screened) and internal data yielded 17 publications. The overall analysis indicated low prevalence (48 positive samples of total 2,338 identified) of *Salmonella* at the level of 2%, with no study reporting pathogens in black and green tea. All positive samples were in herbal teas (e.g., jasmine, lemongrass, rooibos, peppermint or lemon verbena tea).
Significance: Findings of this review align with a history of safe use of tea over thousands of years and equally confirms that control of *Salmonella* spp. within factory facilities manufacturing black and green tea should rely on preventative approaches, e.g., Ingredient quality programmes, Good Manufacturing Practices, Hazard Analysis Critical Control Point and environmental monitoring programmes, rather than finished product testing. Considering that the pathogen has been sporadically isolated from herbal teas, robust sampling plans aligned to finished product testing based on statistical methods are suitable to control the risk of *Salmonella* spp. associated with tea blends containing herbs, dried flowers, fruit pieces, and spices.

P2-37 Exploring the Virulence Gene Expression of *Arcobacter butzleri* during Simulated Infection of Human Gut Models

DAVIDE BUZZANCA, Valentina Alessandria, Cristian Botta and Kalliopi Rantsiou
University of Torino-DISAFA, Grugliasco, Italy

Introduction: *Arcobacter butzleri* is an emerging foodborne pathogen often isolated from pork, chicken and beef meat, which causes different gastrointestinal diseases in human due to its invasive behaviour. However, the pathogenicity of *A. butzleri* is still underestimated due to a substantial lack of information on its virulence mechanisms, metabolic and genomic features.
Purpose: This study aims to explore, in simulated host-pathogen interactions, the expression of nine genes that are currently correlated with virulence traits of *A. butzleri*.
Methods: After the ex novo design of primers for the nine genes (cadF, ciaB, cj1349, tlpA, tlecA, tlecB, mviN, pldA, flyA) on an *A. butzleri* reference genome (type strain LMG 10828[T]), their relative expression was quantified by quantitative RT-PCR under simulated host-pathogen interaction conditions. Briefly, in Vitro gut models of mucus-producing (HT29-MTX) and non-producing (HT29, Caco-2) human cells were co-incubated with the pathogen and total bacterial RNA was recovered at different time points. At the same time, bacterial counts were performed to describe the colonization and translocation capabilities of *A. butzleri*, by using two dimensional and three dimensional gut models, respectively.
Results: As the first outcome, an RT-qPCR protocol, suitable to quantify the relative expression of the nine virulence genes of *A. butzleri* in the presence of human cells, was optimized. Applying this protocol, an upregulation of part of those genes along the co-incubation time was observed. In addition, a favourable role of the mucus for the pathogen colonization was observed whereas the data from the three-dimensional models suggested an intracellular passage of *A. butzleri* through the epithelial barrier.
Significance: The results of this study represent a first step in the understanding of *A. butzleri* pathogenicity and are important to explain its role in gastrointestinal diseases.

P2-38 Survival of *Listeria* monocytogenes in Food Residues on Packaging Materials for Dairy Products

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Introduction: *Listeria monocytogenes* is known for causing foodborne infections often associated with a high mortality rate. Survival under adverse conditions of *L. monocytogenes* for extended periods of time has been reported. *L. monocytogenes* contamination

P2-37 Exploring the Virulence Gene Expression of *Arcobacter butzleri* during Simulated Infection of Human Gut Models

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Purpose: This study aims to explore, in simulated host-pathogen interactions, the expression of nine genes that are currently correlated with virulence traits of *A. butzleri*.
Methods: After the *ex novo* design of primers for the nine genes (*cadF, ciaB, cj1349, irgA, hecA, hecB, mviN, pldA, tlyA*) on an *A. butzleri* reference genome (type strain LMG 10828^T), their relative expression was quantified by quantitative RT-PCR under simulated host-pathogen interaction conditions. Briefly, In Vitro gut models of mucus-producing (HT29–MTX) and non-producing (HT29, Caco-2) human cells were co-incubated with the pathogen and total bacterial RNA was recovered at different time points. At the same time, bacterial counts were performed to describe the colonization and translocation capabilities of *A. butzleri*, by using two dimensional and three dimensional gut models, respectively.
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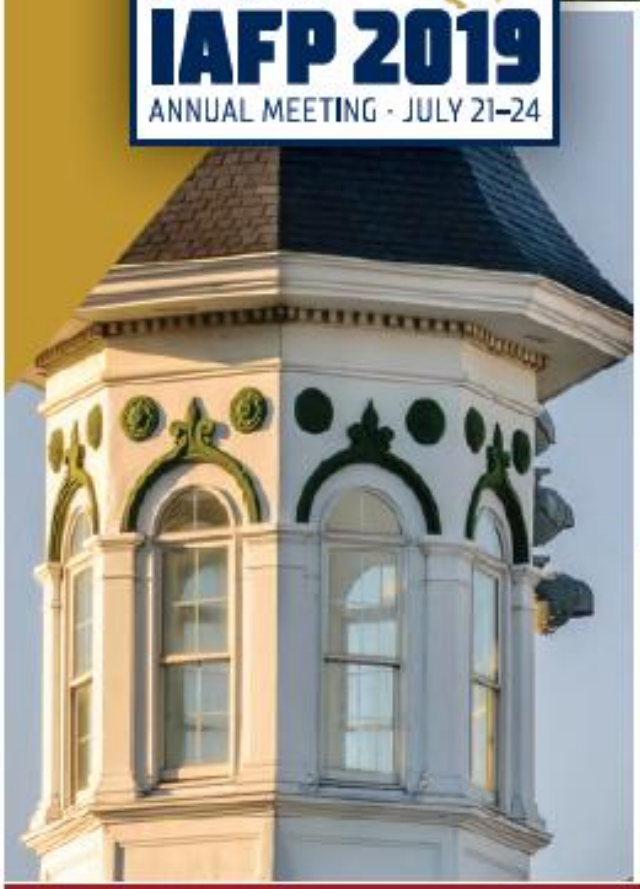

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