*** Student Award Competitor**

Significance: Based on good manufacturing practices as a prerequisite, PEA application to RTE products might be an additional hurdle to limit *Lm* growth in foods whereas its biofilm inhibitory effects suggest a potential role for PEA as a surface disinfectant in food processing environments.

T8-02* Fluorescence-Activated Cell Sorting Enables the Characterization of Sublethal Injury and VBNC State in *Listeria monocytogenes*

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Introduction: Exposure of *Listeria monocytogenes* to sub-lethal stressors related with food processing may induce sub-lethal injury and the viable-but-non-culturable (VBNC) state that is stochastically expressed at single-cell level, with varying resuscitation capacity.

Purpose: i) To outline the proportion of metabolically active, injured, VBNC and dead cells using flow cytometry and CFDA/PI staining; ii) to evaluate the physiological state and resuscitation capacity of sorted cells on agar *versus* Broth; and iii) to determine single-cell lag times.

Methods: Acetic (AA) and hydrochloric acid (HCl) (adjusted to pH 2.5-3.0; 20ºC for 5h) and peracetic acid (PAA) (20, 30, 40 ppm; 20ºC for 3h) were used to evaluate the induction of injury and VBNC state of *L. monocytogenes* Scott-A. To define injured ($CFDA^+/PI^+$) and VBNC ($CFDA^+/PI^-$) cells, flow cytometry coupled with CFDA (metabolically active) and PI (dead) staining was used. Stressed $CFDA^+/PI^-$ or $CFDA^+/PI^+$ cells were sorted on Tryptic Soy Agar or Broth supplemented with 0.6% Yeast Extract (TSAYE or TSBYE), to evaluate culturability. Resuscitation capacity was monitored by visual inspection on TSAYE and by optical density measurement on TSBYE for 5 days at 37ºC. The time to detection (Td), time to reach OD 0.2, was calculated for each experiment and individual cell's lag time was determined by the formula lag = Td – (log ND- log No)/ µmax.

Results: AA pH 2.8 induced VBNC state after 240min and 300min of exposure. 30% of sub-lethally injured (CFDA+/PI+) / AA-treated cells (pH 2.8) were non-culturable on TSAYE and 39% on TSBYE after incubation at 37ºC for 120h. PAA 20, 30 and 40ppm induced the VBNC state. As stress intensity increased from 20 to 40ppm, the distribution of the lag times for different times of exposure presented a bimodal pattern.

Significance: Assessing the heterogeneity and dormancy in *L. monocytogenes* sheds light into risks of underestimation of a product's actual microbial status.

T8-03 Impact of Disinfectants Neutralizing Buffers Used for Sampling Methods on the Viability of *Listeria monocytogenes* Cells in Monospecies Biofilm

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Introduction: The ready-to-eat products can be contaminated during processing by pathogen and/ or spoilage bacteria, which persist in the industrial environment. To check the bacterial contamination present on the surfaces in the food processing plants, the professionals must regularly use surface sampling methods (sponge, swab, gauze pad...) to detect the pathogen such as *Listeria monocytogenes*. Due to the presence of disinfectant residues on the surface, many sampling methods are moistened in a nutrient broth combined with a neutralizing buffer to inactivate disinfectant residues that can have a slight deleterious impact on bacterial cells. This could be a source of false negatives.

Purpose: The objective of this study was to evaluate the impact of the neutralizing buffer on the viability of *L. monocytogenes* after sampling.

Methods: In this study, biofilms of *L. monocytogenes* were cultivated on stainless steel for 24 hours at 8°C or 20°C. The biofilms were treated with two different disinfectants or with sterilized water (control) and then were neutralized with 6 different commercially neutralizing buffers. The bacterial populations were detached by swab and analyzed directly after sampling and after 24 hours of incubation at 8°C to simulate the transport time before samples analysis (EN ISO 18593 standard, 2018). The analyses included agar enumeration to quantify the viable culturable (VC) population and qPCR and PMA-qPCR assays to quantify the p dead and viable populations of *L. monocytogenes*.

Results: *T*his study showed that in our conditions tested, neutralizers have a variable effect depending on the type of biocide used (quaternary ammonium or hydrogen peroxide), the culture temperature and the type of neutralizer. No neutralizer systematically allowed us to enumerate only the VC population of *L. monocytogenes*.

Significance: The Dey-Engley and sponge neutralizer were the most suitable neutralizers in the majority of the conditions tested to enumerate the maximum of the VC cells of *L. monocytogenes* in monoespecie biofilm.

T8-04* Targeted and Untargeted Monitoring of Pathogens Along Infant Food Processing Chain

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Introduction: Pathogens that persist after various stages of the process line is a crucial challenge for food industries and more specific those of infant food as the absence of them is indispensable. The focus is on infant food as it reaches a group of the population that has not yet thoroughly developed its immune system.

Purpose: The aim of this work was to shed light on how the untargeted metataxonomic analysis can decipher the prevalence of pathogens complementing the targeted detection of each pathogen by isolation through enrichment and Real-Time PCR approaches.

Methods: More than a hundred samples were collected during various infant food process runs in a commercial facility and more specifically environmental samples, raw material, intermediate and final products. On each sample, 16S rRNA amplicon-based sequencing has been performed and Amplicon Sequence Variants (ASVs) distribution was investigated. The presence of *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella enterica*, *Staphylococcus aureus* and *Clostridium perfringens*, was examined before and after twenty-four hours of enrichment, with pathogens isolation and Real-Time PCR.

Results: Different bacterial communities have been observed in between the samples in relation to the product's composition, while less clear segregation was seen in the environment. It is worth noting that detection through metataxonomic analysis is unlikely to be sufficient for the identification of low abundant ASVs, which is the case of the pathogen. However, this does not exclude the possibility to correlate specific metataxonomic profiles with the potential presence of a pathogen.

Significance: Undoubtedly, when we focus only in specific targeted method, we need to consider the risk of underestimating the presence of other emerging microorganisms. This gap could be filled by the implementation of an untargeted metataxonomic technique.

Technical Session 9 – Microbial Food Safety and Spoilage

T9-01 Impact of pH and CO_2 on the Thermal Resistance of *Aspergillus niger* Spores in a Carbonated Liquid Medium

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Introduction: Carbonated beverages include several types of drinks, such as carbonated soft drinks and waters, beers, and sparkling wines. They are characterized by the presence of carbon dioxide (CO_2), and a pH usually comprised between 2 and 6. While the CO_2 has firstly been added for organoleptic reasons, it might also have an impact on the resistance of spoilage microorganisms to thermal treatments. Indeed, carbonated beverages can be pasteurised (up to 70°C for 20 min). However, as far as the authors are aware, the impact of CO_2 on the thermal resistance of microorganisms has never been studied.