Produce surface treatments based on bacteriophages and bacteriocin-producing cultures to consistently reduce 2-log of *Listeria monocytogenes* on leafy greens and pre-cut fruit and vegetables

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Summary

This project will test commercially available post-process treatments able to provide a consistent 2-log reduction of *Listeria monocytogenes* (Lm) on the surface of leafy greens and pre-cut fruit and vegetables (**Figure 1**). A risk categorization will be performed to provide the produce industry with information about which commodities are more susceptible and therefore require the application of a post-process treatment. Promising results against *Listeria* spp. and Lm have already been reported for several commercial treatments tested under lab-scale experiments. However, experience shows that results obtained in the laboratory are difficult to extrapolate to the industry. This project focuses on the validation and verification of control strategies in commercial processing plants. The impact of the selected post-process treatments on the quality aspects will be evaluated during shelf-life.

Benefits to the Industry

The main objective of this project is the elaboration of a user-friendly guideline including specific operational standards that indicate step-by-step the conditions required to apply these treatments to particular commodities successfully. This guideline will include the most appropriate treatments for different scenarios under industrial conditions. The specific outcomes are: i) Identification of the most susceptible commodities where application of produce surface treatments is necessary as an additional control point; ii) Selection of effective treatments and operational standards that assure the efficacy of the selected treatments; iii) Verification of operational standards in commercial processing lines to guarantee the feasibility of the treatments via experimentation; and iv) A user-friendly guideline for producers through the CPS website including evidenced-based standards for the application of the selected treatments.

Objectives

1. Risk prioritization analysis of critical fresh produce commodities based on the ability of Lm to grow at different temperatures in different fresh commodities, and search for commercially available produce surface treatments that meet regulatory requirements for use on food.
2. Establishment of the efficacy of commercially available post-process treatments against Lm and *Listeria* spp. by lab-scale trials mimicking commercial conditions.
3. Evaluation of the impact of the selected post-process treatments on the organoleptic quality and shelf-life of selected fresh produce.
4. Validation of selected post-process treatments in commercial fresh produce facilities and establishment of operational standards. Collaboration with two industry partners – Flensted (http://www.flensted.dk/) from Denmark and Florette (http://www.florette.es/) from Spain – will allow validation of the most efficient treatment/s under industrial conditions.

Methods

For the risk prioritization, quantitative and qualitative methods are being performed, including expert knowledge elicitation. Data on Lm growth in different commodities have been extracted from the literature and analyzed using ComBase predictive models. Model outputs will be combined with expert knowledge. Several meetings were performed to define the commercially available post-process treatments that will be tested in the 3–5 top commodities identified in the risk prioritization by lab-scale trials mimicking commercial conditions (**Figure 2**). Protocols have been developed for the cocktail preparation of three Lm and three *Listeria* spp., and inoculation procedures of fresh produce for commercial post-process treatments based on bacteriophages and bacteriocin-producing cultures. Establishment of operational standards in lab-scale trials will then be validated in commercial fresh produce facilities of the industry collaborators.

Results to Date

An extensive literature review was performed using the software CADIMA®, resulting in the selection of 154 papers for data extraction. Data retrieved from the comprehensive literature review was used to calculate the exponential growth rate (EGR) of one specific strain or a pool of different Lm strains by using the primary growth model fit with the DMFit tool from ComBase. Results are currently being analyzed. Additionally, ISI-FOOD performed challenge tests to evaluate the potential growth of Lm in 28 fresh vegetable and fruit products (**Figure 3**). Products have been classified based on risk segmentation into three groups, including produce allowing >2.0 log of Lm growth during storage (e.g., Edamame beans, Iceberg lettuce, red and white cabbage, Lollo bionda, cauliflower, mushrooms, and parsley) (**Figure 4**).

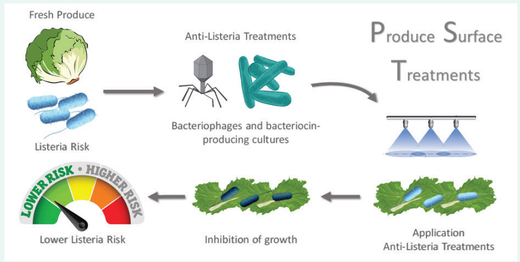

Figure 1. Overall schematic of the project objectives.

Figure 2. Kick-off meeting of the project celebrated on the 4th of February, 2020.

Figure 3. Some products selected for the challenge tests – supplied by the industry collaborator Flensted – the test products were washed and cut as commercial ready-to-eat formats.

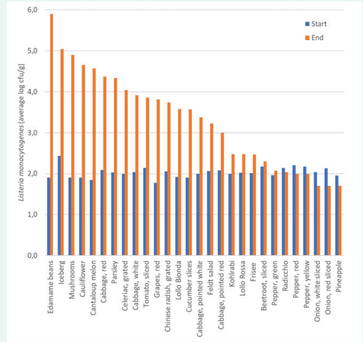

Figure 4. Summary of the results obtained in the challenge tests to evaluate the potential growth of Lm in ready-to-eat cut vegetables and fruit products. Analyses were performed at **start** (day 0: day of inoculation) and **end** of shelf-life (typically 6–9 days, depending on the commodity). Products were stored at 5°C for 2 days and then at 10°C.