Identification of quantitative and qualitative patterns of environmental contamination by *Listeria* spp. and *L. monocytogenes* in fresh produce processing facilities, and evaluation of practical control measures able to eliminate transient and persistent contamination

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Project funding dates
January 1, 2021 – December 31, 2022

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Summary

Prevalence studies on *Listeria* spp. and *L. monocytogenes* (Lm) in the fresh produce processing industry are being performed. Collaborative research between the industry and academia has been established to (i) understand *L. monocytogenes*/*Listeria* spp. ecology, (ii) identify primary contamination sources for preventing cross-contamination, and (iii) select the most suitable mitigation options to reduce or eliminate the primary contamination sources. This project aims to help the fresh produce processing industry in several ways: reducing *L. monocytogenes* contamination in fresh produce processing facilities, establishing common contamination patterns within a processing plant and among different plants, and selecting the most efficient sanitizing treatments to eliminate *L. monocytogenes* in the processing environment.

Objectives

1. Assessment of environmental contamination by Listeria spp. in fresh produce processing plants:

   a. Categorization of the processing lines based on zoning (**Figure 1**), sanitary design, location connectivity, and the level of contamination

   b. Quantification of the weight of each specific factor in the probability of contamination

   c. Development of a set of guidance for the industry

2. Establishment of the genetic correlations of the *Listeria* spp. isolates to understand the distribution patterns:
   a. Baseline data across environmental samples

   b. Distribution of identical or similar isolate WGS types in different locations and times

   c. Identification of persistent and transient *Listeria* spp. strains

3. Evaluation of the efficacy of control measures currently implemented in commercial fresh produce processing plants against transient and persistent *Listeria* spp. contamination.

Methods

During the first trimester of the project, the research team focused on assessment of environmental contamination by *Listeria* spp./*L. monocytogenes* in fresh produce processing plants (Objective 1a). The first environmental monitoring (EM) was performed in a fresh-cut processing plant for leafy vegetables: a total of 80 potential sampling points were identified (**Figure 2**). Sampling was performed 12 h after the work finished, before the cleaning and sanitation process. HydraSponge sterile swabs were used for swabbing. For detection of *Listeria*/Lm, two analyses were performed in parallel (**Figure 3**). In each sample, levels of *Listeria* spp. were enumerated by filtration through sterile cellulose-nitrate filters using a vacuum filtration system. The prevalence of *L. monocytogenes* was estimated following the specific ISO standard method for detection (EN ISO 11290-1).

Results to Date

The presence of *Listeria* spp. was detected in about half of the samples (51.4%), with highly variable counts (**Table 1**). Among samples positive for *Listeria* spp., 42.8%, 14.3%, and 78.5% were found in Zone 1, Zone 2 and Zone 3, respectively (**Table 2**). The distribution of environmental samples positive for *Listeria* spp. varied across the different areas of the processing plant (processing, clean and packaging areas) (**Figure 1** and **Table 1**). Colonies positive for *Listeria* spp. were isolated onto ALOA/OCLA, and those positive for Lm were confirmed by PCR. Four *L. monocytogenes* were confirmed from Zone 3. The prevalence of Lm in Zone 3 was 28.6%.

Benefits to the Industry

The current project aims to impact the fresh produce processing industry in several ways, including:

- Development of an improved set of guidance based on empirical data for the industry

- A reduction in *L. monocytogenes* contamination in fresh produce processing facilities

- Establishment of common contamination patterns within one processing plant and among different processing plants

- Recommendations on the most efficient sanitizing treatments to eliminate *L. monocytogenes* in processing environments
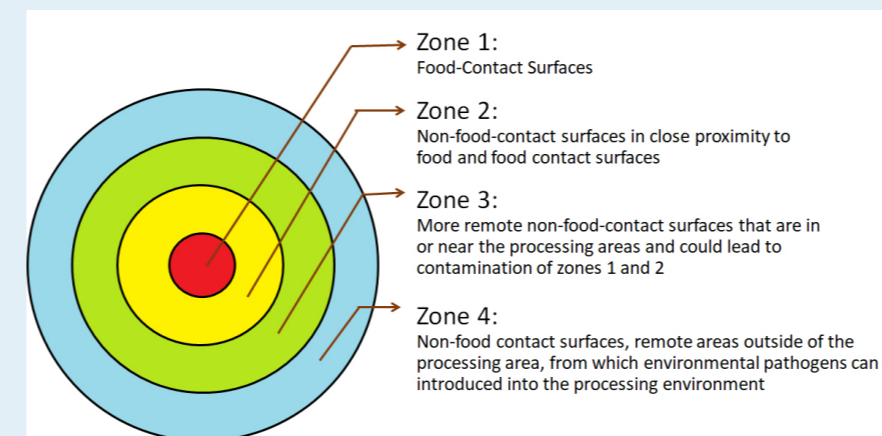

Figure 3. Diagram of detection and quantification of *Listeria* spp. and *L. monocytogenes*.

Figure 1. Identification of four sanitary zones based on their proximity to food.
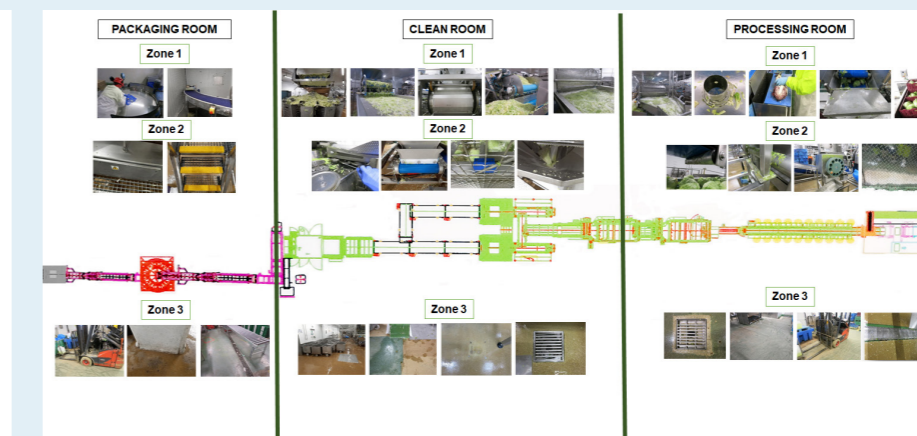
Figure 2. Examples of sampling points from Zone 1, Zone 2 and Zone 3 of a fresh-cut processing plant.
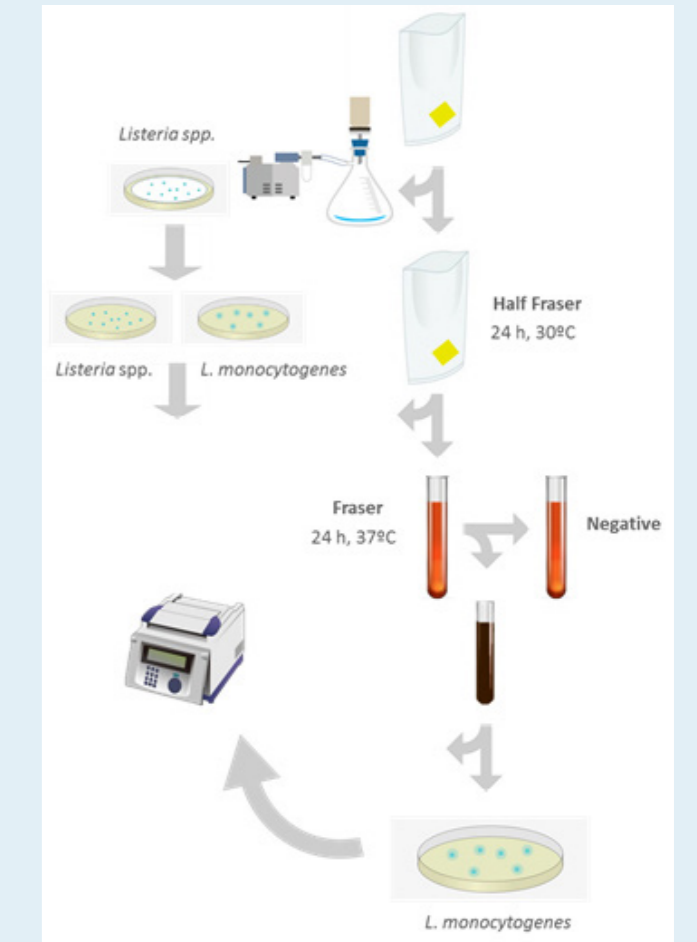
Zone	Sample	Sampling point	Sample type	Listeria spp. CFU/ 900 cm² or unit
1	1	3	Feedstock boxes	Non-detected (ND)
	2	4	Unloading table	216
	3	9	Cutting board	236
	4	10	Cover	56
	5	21	Slicer's blades	21
	6	26	First washing station filter	ND
	7	34	Second washing station filter	ND
	8	40	Conveyor belt to centrifuge	ND
	9	42	500 ml water under centrifuge	ND
	10	44	Centrifuge B	ND
	11	50	Beginning of vibratory belt	3
	12	59	Vibratory channels from weight filler	ND
	13	74	Blue conveyor belt to warehouse	ND
	14	78	Spinning table	1
2	15	6	Prepared product conveyor belt roller	224
	16	18	Waste shovel	ND
	17	23	Frame under slicer	ND
	18	43	Centrifuge cage	ND
	19	55	Vision system's waste conveyor belt	ND
	20	61	Electrical panel's rubber junction	ND
	21	83	Weight filler's rubber seals	ND
3	22	13	Drain near to the elevator	15
	23	15	Chromed wall	106
	24	30	Forklift's wheel	7
	25	32	Forklift's fork	133
	26	81	Floor near elevator	212
	27	46	Floor's holes near the centrifuge	ND
	28	52	Drain before the vibratory belt	ND
	29	54	Drain under vision system	ND
	30	62	Stairs to weight filler	11
	31	66	Cart wheels	13
	32	62	Floor's holes near a pillar	2
	33	75	Pillar junction	4
	34	79	Drain	5
	35	80	Forklift wheel	1

Table 1. Description of surfaces sampled in the different zones of the lettuce processing plant.

Sample type	Positive Listeria spp. samples	Positive Lm samples	Listeria spp. Prevalence (%)	Lm Prevalence (%)
Zone 1	6/14	0/14	42.8	0
Zone 2	1/7	0/7	14.3	0
Zone 3	11/14	4/14	78.5	28.6

Table 2. Prevalence of positive samples of *Listeria* spp. and *L. monocytogenes* (Lm) in the different zones of the lettuce processing plant.