

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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Summary

The aim of this project is to generate experimental data on the performance of environmental monitoring (EM) programs in fresh produce processing facilities, including cut lettuce, cut fruits and prepared salad bowls. Special emphasis is given to *Listeria monocytogenes* (Lm) prevalence and concentration in Zone 1 and between clean and unclean processing plants. This valuable information will be used to improve management of the processing environment and the intervention strategies. Achieving this goal is being made possible thanks to the collaboration of CEBAS-CSIC with three major fresh product companies. The project will provide valuable information about the establishment of common contamination patterns within Zones, processing plants and industrial practices, as well as the effectiveness of disinfection operations to eliminate Lm from the processing environment.

Objectives

1. Assessment of the environmental contamination by *Listeria spp.* in fresh-cut processing plants for the detection of harbor places and potential transfer vectors.
2. Establishment of the genetic correlations of the Lm isolates. 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

Three processing lines (cut lettuce, cut fruits and salad bowls) were sampled at three times every 3–4 months (Table 1). A total of 702 samples were analyzed, including 607 samples from environmental surfaces corresponding to Zone 1 (n=203), Zone 2 (n=137) and Zone 3 (n=267), plus 95 samples of raw materials and end products. The EM sampling of the processing lines was performed at the end of the working day, before the cleaning and disinfection operations. Samples were processed following standardized protocols. All the presumptive Lm colonies isolated from the enrichment step in ALOA/OCLA (n=178) were confirmed by PCR. A selection of 100 isolates from the three processing plants is being analyzed by whole genome sequencing (WGS) for source tracking to determine if Lm strains are persistent or transient.

Results to Date

When the EM was conducted in the unclean plants, the highest Lm prevalence was found in the prepared salad plant, followed by the cut fruit plant and cut lettuce plant (Table 2). Regarding Zones, in the cut fruit and prepared salad plants, the prevalence of Lm was higher in Zone 3, followed by Zone 1 and Zone 2; whereas in the cut lettuce plant, the highest prevalence was found in Zone 3, followed by Zone 2 and Zone 1 (Table 3). Some of the sampling points that were positive for Lm presence in Zone 1 included the slicers, washing stations, conveyor belts and the vibratory filling (Figure 1), while in Zone 3 these sampling points were linked to drains, floors, ceilings and wheels. The positive Lm samples were associated with variable *Listeria spp.* counts (0.5 to 2 logs cfu/unit) (Figure 2).

Benefits to the Industry

Environmental sampling for Lm testing in fresh-cut processing facilities is giving relevant information regarding the most common contamination scenarios and patterns. Information obtained in Zone 1 and Zone 2 is relevant, as results are not always correlated among different Zones (Zone 3). The results obtained highlight the relevance of cleaning and disinfection practices. After a working day, the high prevalence of Lm in Zone 1 observed in the three processing plants emphasizes the need for testing the frequency of the cleaning and disinfection practices and the need for the process to stop for implementing corrective actions. The WGS results will allow evaluation of the critical points of transmission to prevent the transfer of this pathogen from the environment to products.

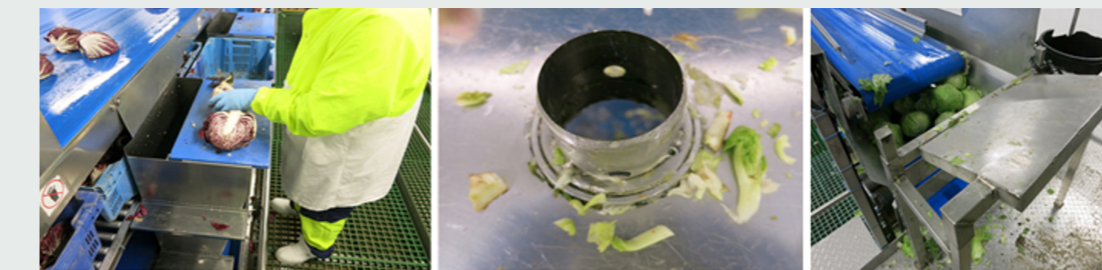


Figure 1. Details of Zone 1 sampling points from the shredded iceberg lettuce processing plant.

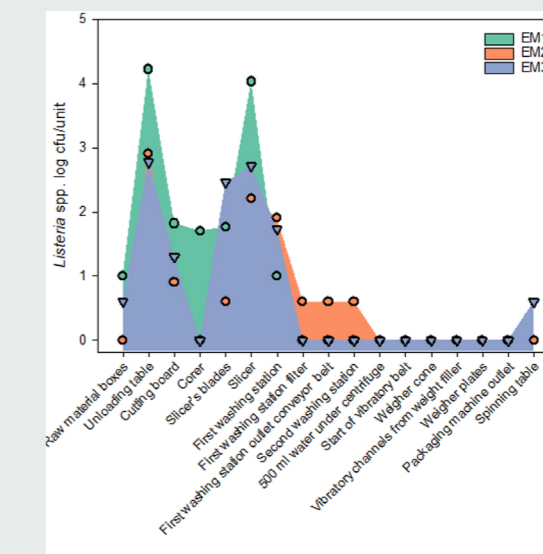


Figure 2. Levels of *Listeria spp.* (log cfu/unit) in sampling points from Zone 1, Zone 2, and Zone 3 at three EM samplings in the shredded iceberg lettuce processing plant.

Processing plant	#EM	Sampling dates 2021	# Surfaces
Shredded iceberg lettuce	1	15 th April	68
	2	1 st July	68
	3	25 th October	68
Cut Fruit	1	25 th May	62
	2	1 st September	63
	3	1 st December	72
Salad bowl	1	20 th June	70
	2	20 th September	71
	3	15 th December	71

Table 1. The number of samples collected in each processing plant.

Processing line	Positive samples	Prevalence (%)
Cut lettuce	56/204	27.5
Cut fruit	53/190	27.9
Prepared salads	77/213	36.2
Total	186/607	30.6

Table 2. Number and prevalence of Lm-positive samples at three EM samplings in the produce processing plants.

Processing line	Zones	Positive samples	Prevalence (%)
Cut lettuce	1	7/51	13.7
	2	12/63	19.0
	3	37/90	41.1
Cut fruit	1	11/68	16.2
	2	4/44	9.1
	3	38/78	48.7
Prepared salads	1	11/84	13.1
	2	3/30	10.0
	3	63/99	63.6

Table 3. Number and prevalence of Lm-positive samples from sampling at different Zones in the produce processing plants.