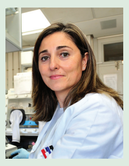
Occurrence and transfer of pathogens from the production environment to leafy greens grown in controlled environment agriculture



Contact
Ana Allende, PhD
CEBAS-CSIC, Spain
aallende@cebas.csic.es

Authors
Maria I. Gil (Co-PI),
Pilar Truchado (Co-PI)

Project funding dates
January 1, 2023 – December 31, 2024

Acknowledgements
Support provided by Juan A. Tudela, the technicians involved in the project (Silvia Andújar, Natalia Hernández, Diego Sabater and Virginia Serrano), and the PhD student, María Ayala-San Nicolás, is highly appreciated. The collaboration of the industrial partners is also recognized.

Summary
Production of leafy greens in controlled environment agriculture (CEA) has significantly increased over the last few years. These protected systems promote the efficient production of crops in an environmentally friendly way but they are not inherently safer than open systems, as contamination can occur through different production practices and procedures that introduce hazards into the environment. Little is known about the likelihood of *Listeria monocytogenes* and *Salmonella* persistence in CEA systems. The main benefit of this project will be the acquisition of science-based knowledge to help develop risk-based preventive measurements that fulfill current FDA requirements and recommendations for CEA growers to reduce potential hazards such as *Salmonella* and *L. monocytogenes*.

Objectives
1. Risk-assessment of *Salmonella* and *L. monocytogenes* contamination in CEA facilities: detection of potential sources and routes of contamination.
2. Establishment of genetic correlations of isolates to identify the distribution patterns of *Salmonella* and *L. monocytogenes* across different sources and routes of contamination.
3. Evaluation of foodborne associated traffic patterns using abiotic surrogates in indoor production environment to leafy greens.
4. Assessment of the efficacy of practical and feasible sanitation strategies implemented in CEA facilities against *Salmonella* and *L. monocytogenes* contamination.

Methods
Three CEA systems—soil, hydroponic, and substrate in trays—producing leafy green crops (Batavia baby lettuce, Pak choi, and Lamb's lettuce, respectively) were selected. Each facility was sampled three times during a 1-year period. Identification of potential sources of contamination was done in selected sampling points (**Figures 1–3**). About 300 samples were tested for detection of *Salmonella* and *L. monocytogenes* based on standard methods. Presumptive isolates were identified using MALDI-TOF MS analysis (Shimadzu). Positive isolates will be characterized using WGS. Isolated *L. innocua* will be used as a surrogate to evaluate the potential transfer within a research CEA, mimicking commercial operations. Efficacy of current sanitation strategies will be assessed on some of the contaminated surfaces.

Results to Date
In CEA system 1 (soil), three *L. innocua* isolates were identified on the first visit (May 2023) in plastic bins (2 positive out of 3 samples) and on the columns of the facility. Only one *L. monocytogenes* isolate was found on the third visit (April 2024) in the booties (**Figure 4**). In CEA system 2 (hydroponic), the booties were also positive for *L. fleischmannii* on the second visit (September 2023).

In CEA system 3 (substrate in trays), one water sample taken from the water reservoir and one sample of the nutritional solution were positive for *Salmonella* (**Figure 5**).

In summary, booties and plastic bins were the hot spots for *Listeria* spp. contamination, while irrigation water sources were shown as an important vector for *Salmonella* contamination.

Benefits to the Industry
The project aims to acquire science-based knowledge to help growers in developing risk-based preventive measurements. The impact of this new knowledge will be translated into:
- Development of an improved set of guidance based on evidence-based practice for the industry,
- Achievement the food safety standards of excellence in CEA facilities for growing leafy greens,
- Establishment of contamination patterns within a CEA facility, and
- Selection of the most efficient sanitizing treatments to prevent foodborne pathogen contamination in CEA facilities.

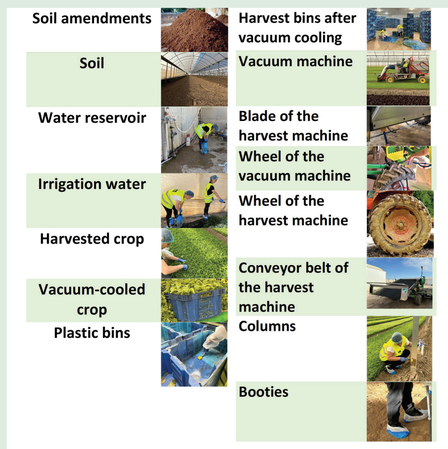


Figure 1. Sampling points of CEA system 1 where soil was use as substrate and several re-growths of Batavia baby lettuce were performed.

Figure 2. Sampling points of CEA system 2. A hydroponic system for Pak choi cultivation.

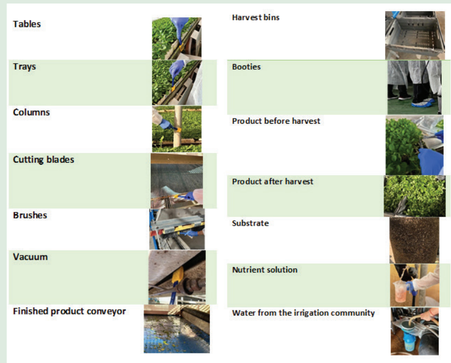


Figure 3. Sampling points of CEA system 3 where the substrate in trays was placed in beds and several re-growths of Lamb's lettuce were performed.

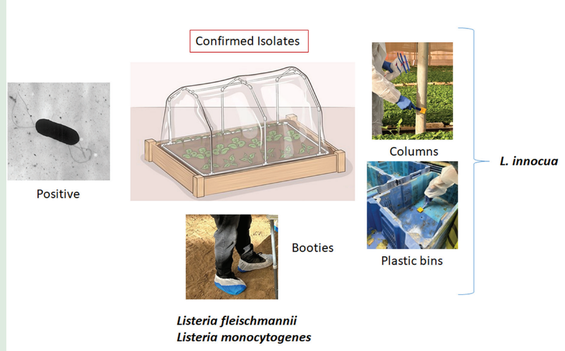


Figure 4. Sampling points positive for *L. innocua* and *L. monocytogenes* (CEA System 1) and for *L. fleischmannii* (CEA System 2).

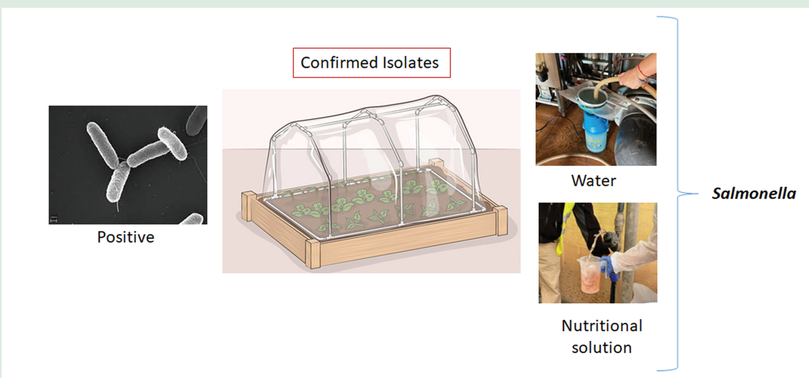


Figure 5. Sampling points positive for *Salmonella* (CEA System 3).