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

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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* serovar 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
This project will generate practical knowledge on potential risk sources and transmission routes of pathogen contamination in CEA, as well as suitable corrective actions for implementing disinfection strategies. To accomplish this main goal, four objectives have been identified:

1. Risk-assessment of *Salmonella* serovars 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 associate 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
This project is specifically designed to develop a keen understanding of potential sources and routes of contamination as well as the identification of effective sanitation procedures and sampling plans. Two technology-advanced CEA systems will be characterized in three environmental monitoring (EM) samplings. About 300 samples will be tested for the detection of *Salmonella* serovars and *L. monocytogenes* and enumeration of *Listeria* spp. based on standard methods. Characterization of the isolates using WGS will allow the identification of transient or persistent *L. monocytogenes* isolates. Special attention will be given to enhance the detection of potential pathogenic isolates using different enrichment steps and selective culture media. The use of abiotic surrogates will allow the evaluation of potential distribution of contamination patters within a CEA operation. (**Figure 1**)

Methods (continued)
Listeria spp. based on standard methods. Characterization of the isolates using WGS will allow the identification of transient or persistent *L. monocytogenes* isolates. Special attention will be given to enhance the detection of potential pathogenic isolates using different enrichment steps and selective culture media. The use of abiotic surrogates will allow the evaluation of potential distribution of contamination patters within a CEA operation. (**Figure 1**)

Results to Date
The first EM sampling has been performed in one of the technology-advanced CEA systems after harvest in the first week of May (**Figures 2** and **3**). **Table 1** lists the samples taken during the EM. The samples are being analyzed for the detection of *Salmonella* serovars and *L. monocytogenes* and enumeration of *Listeria* spp. Considering that *L. monocytogenes* and *Salmonella* are poor competitors against routine background microflora, two different enrichment steps and two selective media have been selected for *Salmonella* serovars, and in the case of *L. monocytogenes* the confirmation of potential isolates will be performed in two selective culture media. The analyses are in progress.

Benefits to the Industry
The project aims to acquire science-based knowledge to help growers in developing risk-based preventive measurements. The output of the project will help to identify:

- Contamination niches with Salmonella serovars and L. monocytogenes and transference pathways from contact or adjacent surfaces to the product
- Persistent and transient isolates through genetic correlations
- Traffic patterns associated with cross-contamination
- Efficacy of sanitation strategies

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 of 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 eliminate foodborne pathogens from CEA facilities.

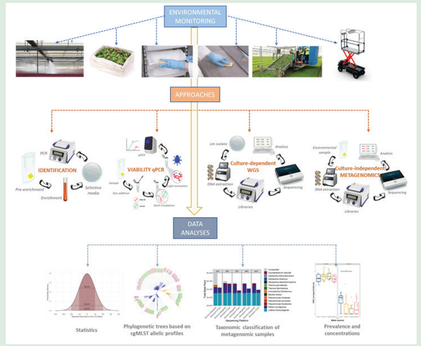

Figure 1. Schematic of methodologies and expected outcomes of the project

Figure 2. Sampling points considered in the environmental monitoring

Figure 3. Sampling in CEA facility A

Table 1. Selected sampling points in CEA facility A

SAMPLING POINTS	SAMPLE
Soil amendments	
Soil	
Water reservoir	
Irrigation water	
Crop	
Boxes harvest	
Boxes cooling	
Leaf vacuum cleaner machine	
Harvesting machine	
Wheels vacuum cleaner machine	
Wheels harvesting machine	
Walls	
Booties	