Development of an immunoassay for quantitative detection of *Erwinia carotovora* subsp. *carotovora* in hydroponic solutions

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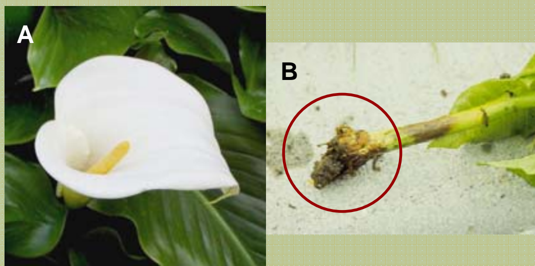
Background and Introduction

- Gram-negative bacterium ***Erwinia carotovora* subsp. *carotovora*** (*E.c.c.*) causes **soft rot** disease in calla lily, poinsettia, tomato and pepper (Senchenkova *et al.*, 2003)



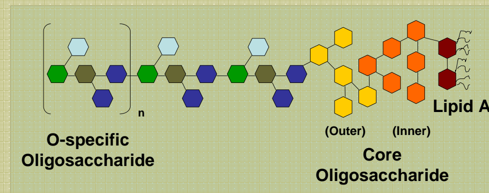
Source: HortResearch and Kansas State University

A: Calla lily; B: Bacterial soft rot

- Ontario greenhouse yield – 480 ha worth $725 million (year 2005)
- Yield losses – 12% to 20% ($87 to $145 million)
- Hydroponic solutions are routinely circulated, increasing the likelihood of spreading disease; bacterial species in solution cause disease (Blom, 2003)

Source: EagleSprings Farm

- The outer membrane of *E.c.c.* contains lipopolysaccharide (LPS) that initiates specific pathogenesis in plants (Senchenkova *et al.*, 2003)



- LPS is composed of three distinct regions: O-specific oligosaccharide (consisting of long chains of repeating units), core oligosaccharide and lipid A (responsible for endotoxicity) (Yi and Hackett, 2000)
- ***Growers of Ontario require a user-friendly test for quantitative detection of E.c.c. to limit its effects in hydroponic solutions***

Objective

- To develop a sensitive enzyme-linked immunosorbent assay (ELISA) for detection and quantification of *E.c.c.* LPS in hydroponically grown flowers and vegetables.

Method

- *E.c.c.* LPS was isolated from whole E.c.c. cells; electrophoresis of LPS to confirm purity and stability of *E.c.c.* LPS.
- Immunization of New Zealand white rabbits with *E.c.c.* LPS to raise polyclonal serum against *E.c.c.*
- Blood was collected every two weeks followed by injection with LPS (three month immunization period). Plasma (sera) was separated from red blood cells and used for ELISAs.
- Indirect polyclonal ELISAs were performed throughout the immunization period to monitor titer.
- Once high antigen-specific binding (~1.0) values were observed, cross-reactivity and inhibition ELISAs were conducted using sera from the fourth bleed. The fourth immune bleed took place approximately 56 days after start of the immunization period.

Results

- The inhibition ELISA data presented in Figure 1 indicates that non-immobilized (free) *E.c.c.* LPS molecules compete with immobilized *E.c.c.* LPS for binding to antibodies present in rabbit sera (blue line).
- Conversely, free *E. coli* LPS molecules do not compete with immobilized *E.c.c.* LPS for binding to *E.c.c.* LPS specific antibodies (red line).

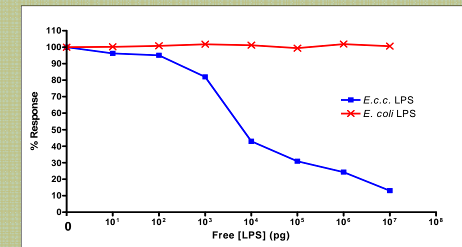


Figure 1: *E.c.c.* and *E. coli* LPS inhibition ELISA examining free LPS concentration versus %response. No inhibition equals 100% response; inhibition equals less than 100% response. As the concentrationn of free LPS increases, the % response decreases and vice versa, indicating that free LPS inhibits immobilized LPS binding to specific antibodies. Values indicated have been corrected for background absorbance.

- Gram-negative bacteria such as *E.coli* does not cross-react with *E.c.c.* Preliminary cross-reactivity results using an Erwinia spp. such as *E. chrysanthemi* showed no cross-reactivity.
- Gram-positive bacteria such as *Listeria monocytogenes*, the oomycetes *Pythium apanidermatum* and the fungi *Fusarium graminearum* do not cross-react with *E.c.c.*

Conclusion

- Results indicate that a specific ELISA for detection and quantification of E.c.c. has been developed.
- Polyclonal antibodies will be used to create a user-friendly dipstick assay.

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