Microbial and genetic determinants of *Listeria monocytogenes* colonization of *A. thaliana* roots

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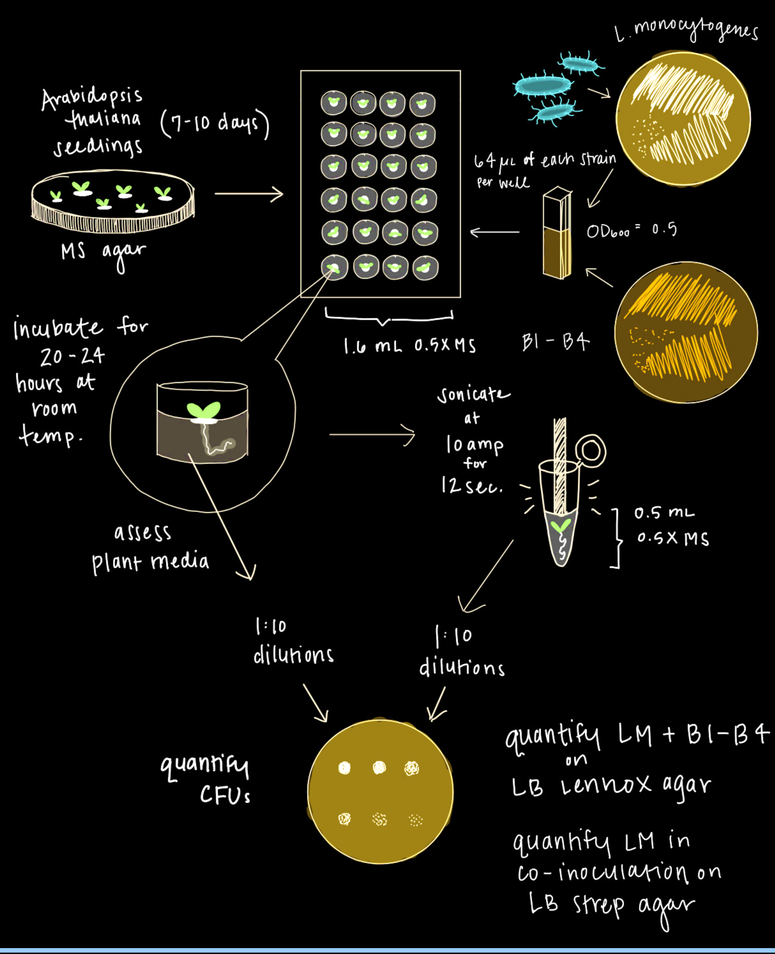
Abstract

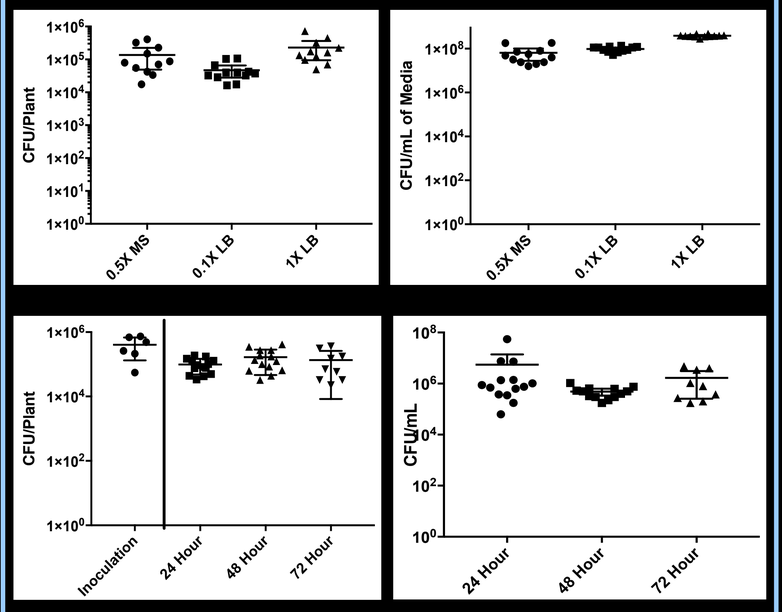
Listeria monocytogenes (LM) is a human pathogen and agent of listeriosis, a foodborne illness that can cause severe complications and death in immunocompromised individuals. LM-related illnesses have one of the highest mortality rates of foodborne illnesses, with 30% of patients dying due to infection. LM occurs ubiquitously in the environment, where it can attach to crops that humans consume. LM contamination of food products poses a major threat to human health, and to the food industry, costing between $2.3 and $22 billion per year.

Current research on LM largely investigates the clinical aspects of LM infection, with very little understanding of LM in an environmental context. Thus, little is known about LM in the environment and how it interacts with the flora and other soil microbes. To investigate the factors that influence LM plant root colonization, we utilized a hydroponic assay in which *Arabidopsis thaliana* seedlings were suspended in media and inoculated with LM. We first tested several media and found that LM readily colonizes and persists on *A. thaliana* roots in minimal-nutrient media. We then investigated the effects of LM pre-assay growth temperature on colonization and found that it was significantly enhanced at 30˚C and 37˚C, however, this enhancement is not due to the virulence gene regulator PrfA. Finally, we investigated the impact of coculturing LM with other soil microbes and found that *Pseudomonas fluorescens* increased LM colonization, while other bacteria predominantly decreased LM colonization. These data demonstrate LM readily colonizes *A. thaliana* in this hydroponic assay, and that genetic and microbial factors may influence LM colonization of plant roots.

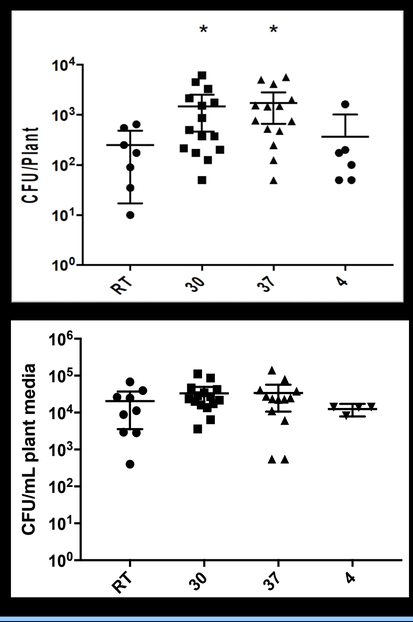
Listeria monocytogenes (LM) plant colonization workflow



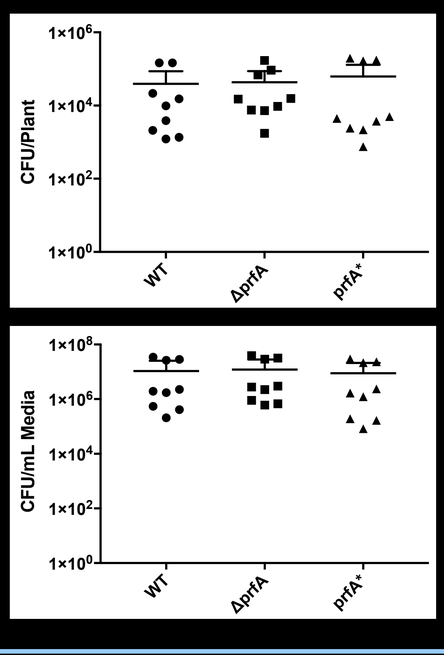
Listeria monocytogenes (LM) readily colonizes and persists on *A. thaliana* roots



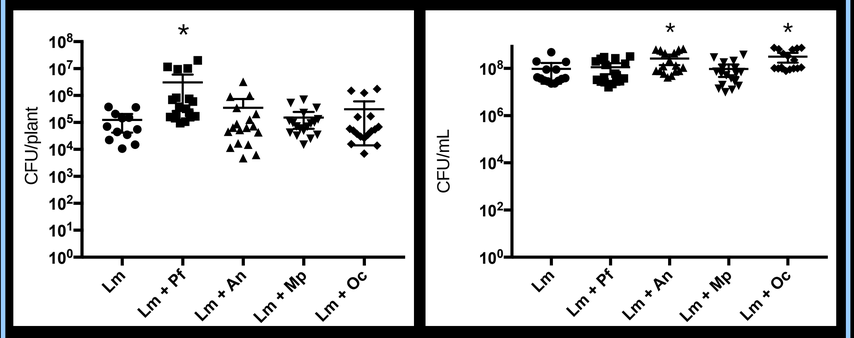
Pre-assay growth of LM at 30 and 37℃ enhances root colonization



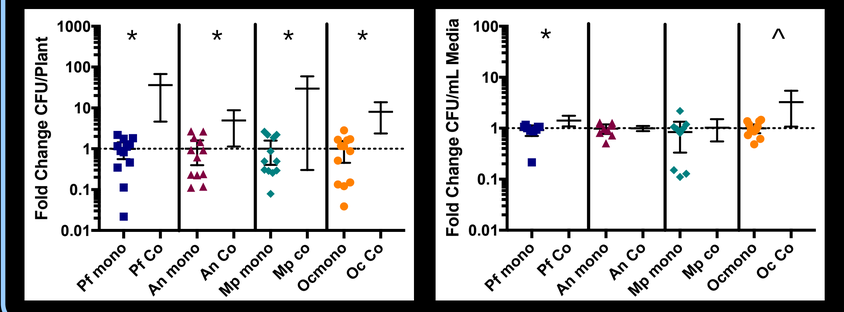
Enhanced attachment at 30 and 37 is not due to PrfA (Virulence gene regulator)



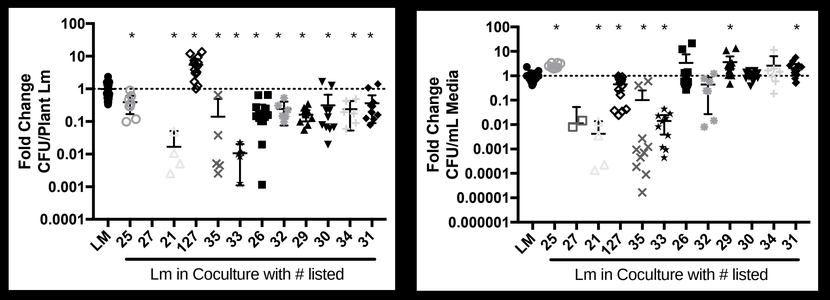
Coculture with *Pseudomonas fluorescens* increases LM root colonization without enhancing overall growth



Coculture with LM enhances plant colonization of plant associated isolates



Coculture with plant associated *Pseudomonas* species, predominantly elicits negative impacts on LM plant colonization



Statistics

- All statistics were performed using a Kruskal-wallis test
- Plots are represented as the mean with standard error of the mean
- Each dot represents a biological replicate

Summary

- *L. monocytogenes* readily attaches to *Arabidopsis thaliana* in minimal-nutrient media (0.5x MS)
- LM colonization was enhanced at 30˚C and 37˚C
- PrfA regulated genes do not impact LM plant colonization
- *P. fluorescens* enhances LM plant colonization

Future Directions

- Determine if there is a difference between clinical and environmental LM isolates plant colonization and persistence.
- Perform invasion experiments to investigate the effects of colonization of inhibitive microbes on plant roots pre-colonized with LM
- Conduct assays using media conditioned by inhibitive microbes to observe their effects on LM pre-colonized roots