RESEARCH IN PROCESS

Identifying competitive exclusion microorganisms against *Listeria monocytogenes* from biological soil amendments by metagenomic, metatranscriptomic, and culturing approaches

SUMMARY

Listeria monocytogenes is a leading foodborne pathogen that can contaminate fresh produce at both farm and processing environments. Fully understanding the ecology of *L. monocytogenes* in biological soil amendments is essential to reduce produce contamination with this pathogen. Next generation sequencing approaches are powerful tools for understanding microbial composition and functions at the metagenomic level in complex samples. In considering compost as a rich source of microorganisms with a diversity of species, this project will identify compost-adapted competitive exclusion microorganisms against *L. monocytogenes* using 16S/18S rRNA and metatranscriptomic sequencing approaches along with culturing methods. Preliminary sequencing analysis indicated incubation time and moisture level as the main factors driving variation in compost microbial community composition. Analysis by denaturing gradient gel electrophoresis revealed that *L. monocytogenes* should be inoculated at 7 log CFU/g or higher for 72 h in compost before sequencing analysis. Also, multiple competitive exclusion strains with anti–*L. monocytogenes* activities have been isolated.

OBJECTIVES

1. Use 16S rRNA and 18S rRNA sequencing to profile the microbial communities of biological soil amendments.
2. Analyze functional metatranscriptomics of *L. monocytogenes* interactions with indigenous microorganisms in composts.
3. Optimize culturing conditions to isolate and validate competitive exclusion (CE) microorganisms with antagonistic activities against *L. monocytogenes*.

METHODS

For a preliminary study, turkey litter compost was adjusted to moisture levels of 40 and 60%, and inoculated with *L. monocytogenes* strain LCDC 81-861 (~5 log CFU/g) or not. DNA was extracted from compost samples after propidium monoazide (PMA) treatment with different incubation conditions. High-quality sequenced reads were analyzed using a custom modified QIIME analysis pipeline. Alpha diversity, principal component analysis, and canonical correspondence analysis were performed with PAST3 software. The significance of different environmental factors was tested by analysis of similarity and permutational multivariate analysis of variance, and the top genera driving variation in the microbial community were identified by similarity percentage (SIMPER) and Random Forest analysis.

Additionally, the inoculation level and incubation conditions of *L. monocytogenes* in the biological soil amendments were optimized by denaturing gradient gel electrophoresis (DGGE).

Potential CE strains against *L. monocytogenes* from three biological soil amendments were isolated and characterized.

RESULTS TO DATE

- The top ten taxonomically assigned families, namely Flavobacteriaceae, Trueperacea, Halomonadaceae, Balneolaceae, Pseudomonadaceae, Alteromonadaceae, Alcaligenaceae, Xanthomonadaceae, Bacillaceae, and Sphingobacteriaceae, were observed in all turkey litter compost samples (**Figure 1**).
- The top five genera in turkey litter compost that were found to be most impacted by *L. monocytogenes* inoculation included KSAI, B-42, Halomonas, Marinimicrobium, and Gillisia (belonging to the families Balneolaceae, Trueperaceae, Halomonadaceae, Alteromonadaceae, and Flavobacteriaceae, respectively), as screened by similarity percentage (SIMPER) and Random Forest analysis (**Figure 2**).
- The results from DGGE indicated that *L. monocytogenes* should be inoculated at 7 log CFU/g or higher and incubated for 72 h in compost prior to metagenomic analysis (**Figure 3**).
- A total of 58 isolates were confirmed with various levels of anti–*L. monocytogenes* activities (**Figure 4**).

BENEFITS TO THE INDUSTRY

This project will directly impact the fresh produce industry and the compost industry in California and nationwide as well as impact consumers of these products. Water, soil, and compost are three of the major inputs in the production of fresh produce. As California produces nearly half of U.S.-grown fruits, nuts and vegetables, it is imperative that California's produce industry can verify the absence of human pathogens including *L. monocytogenes* in these inputs that are used to produce their crops. This research on understanding compost microbial communities with the goal to identify competitive exclusion microorganisms as a biological control tool against *L. monocytogenes* will have direct impacts on the practices of biological soil amendments, and contribute to the safe production of fresh produce. Also, our research findings will help the compost industry to understand their products better at the microbial species and gene levels, and lead to an increase in the value of their products.

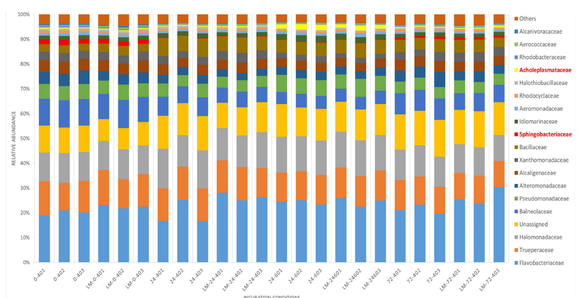


Figure 1. Relative abundances of family in each sample.

Family	Genus	Contrib. %	Cumulative %
Halomonadaceae	CandidatusPortiera	1.775	85.43
Alteromonadaceae	BD2-13	0.09425	98.33
Trueperaceae	B-42	10.52	50.17
Bacillaceae	Gracilibacillus	0.2777	96.87
Bacillaceae	Lentibacillus	0.1112	98.03
Dietziaceae	Dietzia	0.4634	93.7
Halothiobacillaceae	Halothiobacillus	1.343	88.13
Flavobacteriaceae	Aequorivita	0.1582	97.65
Flavobacteriaceae	Gillisia	5.147	76.84
Alteromonadaceae	Marinimicrobium	6.262	65.49
Acholeplasmataceae	Acholeplasma	4.917	81.76
Pseudomonadaceae	Pseudomonas	1.896	83.66
Alteromonadaceae	Spongiibacter	0.1486	97.8
HTCC2188	HTCC	1.358	86.79
Nocardiopsaceae	Thermobifida	0.3729	94.88
Bacillaceae	Bacillus	0.5477	92.77
Halomonadaceae	Halomonas	9.06	59.23
Balneolaceae	KSA1	20.89	20.89
Nitriliruptoraceae	Nitriliruptor	0.07599	98.66
Sphingobacteriaceae	Sphingobacterium	0.06121	98.86
Vibrionaceae	Photobacterium	0.00461	99.85
Glycomycetaceae	Glycomyces	0.07343	98.73
Corynebacteriaceae	Corynebacterium	0.03497	99.25
Moraxellaceae	Psychrobacter	0.009871	99.71

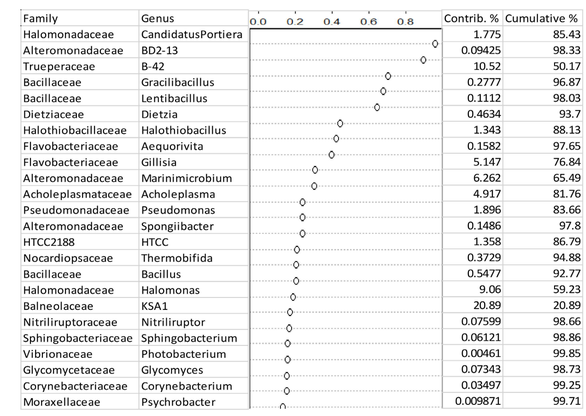


Figure 2. Random Forest and SIMPER analysis.

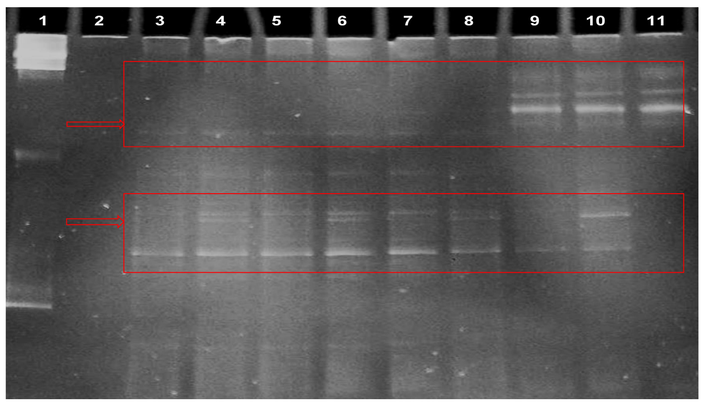


Figure 3. DGGE profiles of PCR-amplified 16S rDNA fragments from finished chicken litter compost with 80% MC. Lane 1, 1Kb Ladder; Lane 2, space lane without sample; Lanes 3-4, compost samples w/o *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 5-6, compost samples with ca. 5 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 7-8, compost samples with ca. 7 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 9-10, compost samples with ca. 9 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lane 11 *L. monocytogenes* strain only.

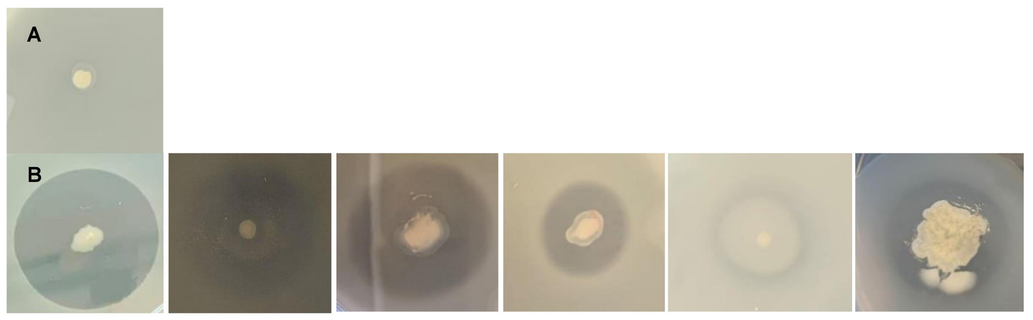
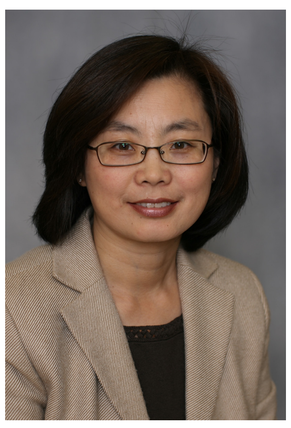


Figure 4. Selection of competitive exclusion microorganisms against *L. monocytogenes* from compost samples. The isolates showed no inhibition zone (A) and with various sizes of inhibition zones (B) on *L. monocytogenes* lawn.



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