Isolation, Characterization, and Application of Antilisterial Isolates in a Raw Milk Cheese Model to Inhibit *Listeria monocytogenes*

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Background

The bacterium *Listeria monocytogenes* has been responsible for many foodborne outbreaks. In artisanal raw milk cheeses, *L. monocytogenes* can survive and cause severe illness and/or death in consumers, especially older adults, the immunocompromised, and pregnant women and their newborns[1]. *L. monocytogenes* may be found in biofilms in dairy processing environments, which can provide the organisms with a physical barrier protective against sanitizers. Some lactic acid bacteria (LAB) have been shown to produce proteins called bacteriocins that can inhibit *L. monocytogenes*[2]. However, bacteriocin production depends on the organism's species and biotic and abiotic environmental conditions. LAB are commonly used as starter cultures or probiotics in fermented foods and beverages due to their ability to convert carbohydrates into lactic acid, reducing the overall pH of the product, making for a more shelf-stable and favorable product[3].

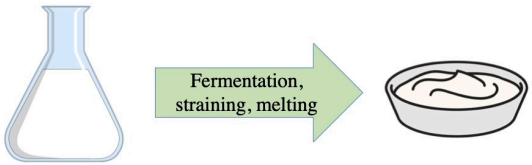

Figure 1: Simplified process of cup cheese production.

This study aimed to obtain LAB isolates from dairy food processing environments and evaluate their inhibitory potential against *L. monocytogenes*. Isolates that inhibit *L. monocytogenes* were further characterized to understand the mechanism of their antilisterial activity. Finally, the cultures were evaluated for the ability to inhibit *L. monocytogenes* in a cup cheese, which is a raw milk cheese, primarily produced by the Amish in Pennsylvania[4].

References

(1) Loessner, M., Guenther, S., Steffan, S., & Scherer, S. (2003, March). *A Pediocin-producing lactobacillus plantarum strain inhibits listeria monocytogenes in a multispecies cheese surface microbial ripening consortium*. Applied and environmental microbiology. Retrieved March 4, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC150062/

(2) Coelho, M. C., Silva, C. C., Ribeiro, S., Dapkevicius, M., & Rosa, H. (1970, January 1). *Figure 1 from control of listeria monocytogenes in fresh cheese using protective lactic acid bacteria.: Semantic scholar*. undefined. Retrieved March 4, 2022, from https://www.semanticscholar.org/paper/Control-of-Listeria-monocytogenes-in-fresh-cheese-Coelho-Silva/3e70f63240ca2a74a33c8bfbd660ea0426ac0618/figure/1

(3) Ruiz Rodríguez, L. G., Mohamed, F., Bleckwedel, J., Medina, R., De Vuyst, L., Hebert, E. M., & Mozzi, F. (1AD, January 1). *Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in northern Argentina*. Frontiers. Retrieved February 27, 2022, from https://www.frontiersin.org/articles/10.3389/fmicb.2019.01091/full

(4) *Cup cheese*. Cheese.com - World's Greatest Cheese Resource. (n.d.). Retrieved March 4, 2022, from https://www.cheese.com/cup-cheese/

Methods

Isolation of Cultures

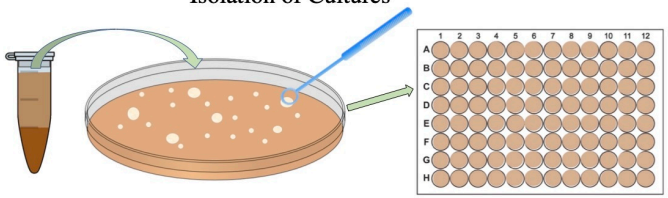

Figure 2: Direct plating method was used to isolate bacteria from environmental samples. Environmental samples were plated on Brain Heart Infusion (BHI) agar and grown at 35ºC for 24 hours to obtain isolates for further testing.

Characterization of Antilisterial Activity

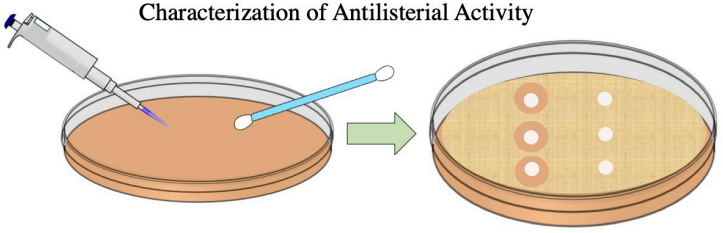

Figure 3: Spot inoculation test was used to test antilisterial activity of isolates. Isolates were spot-inoculated onto a lawn of *L. monocytogenes* (~10^7 CFU/ml) and incubated at 20ºC for 72 hours. Antilisterial activity was observed as a zone of inhibition around the inoculated spot.

Application of Antilisterial Cultures in a Raw Milk Cup Cheese Model

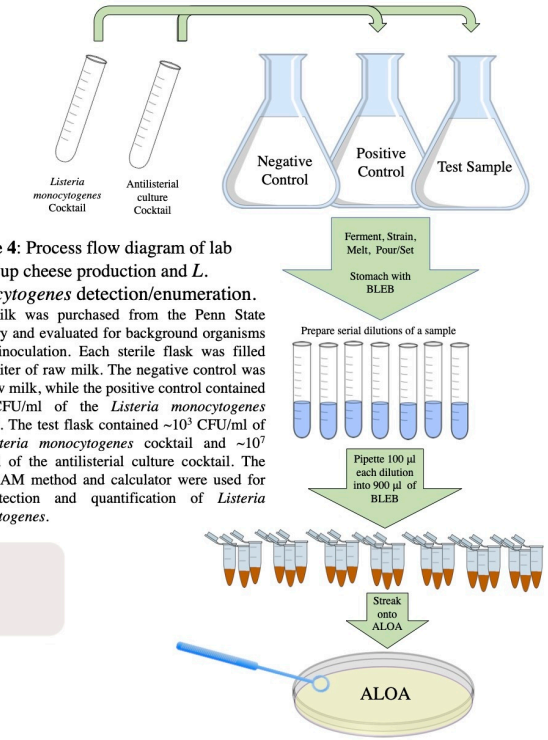

Figure 4: Process flow diagram of lab scale cup cheese production and *L. monocytogenes* detection/enumeration. Raw milk was purchased from the Penn State creamery and evaluated for background organisms before inoculation. Each sterile flask was filled with 1 liter of raw milk. The negative control was only raw milk, while the positive control contained ~10^3 CFU/ml of the *Listeria monocytogenes* cocktail. The test flask contained ~10^3 CFU/ml of the *Listeria monocytogenes* cocktail and ~10^7 CFU/ml of the antilisterial culture cocktail. The MPN BAM method and calculator were used for the detection and quantification of *Listeria monocytogenes*.

Results & Conclusions

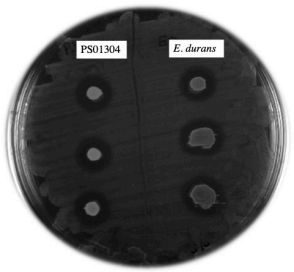

Figure 5: Characterization of Antilisterial Activity. Isolate PS01304 was shown to have antilisterial activity and was identified as *Enterococcus* spp. using 16S rRNA sequencing. *E. durans,* ATCC strain 152, is known to possess antilisterial activity and was included as a positive control.

Isolate PS01304 produced ~3mm zones of inhibitions comparable to other known bacteriocin producers.

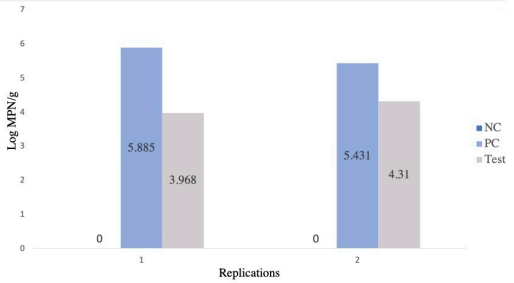

Figure 6: Results from Cup Cheese experiment Two independent replicates of cheesemaking were completed. A 1.917 and 1.121 Log CFU/g reduction of *L. monocytogenes* was detected in the cup cheese made with milk inoculated with isolate PS01304 and *E. durans* for both replicates, respectively. The negative control had no detectable *L. monocytogenes* , while the positive control had 5.885 and 5.431 Log CFU/ml respectively.

The addition of the antilisterial culture decreased the Log CFU/ml of *L. monocytogenes* in the cup cheese when compared to the positive control

Future Directions

In the future, the the mechanism of antilisterial activity of the isolate PS01304 can be characterized using whole-genome sequences and phenotypic assays. Additionally, more experimental work is needed to assess whether the isolate PS01304 is to be used as an adjunct culture in the cheese making process to reduce *Listeria monocytogenes*. Whole-genome sequences can reveal the taxonomic species of this antilisteral isolate and more information about possible bacteriocin production .