Comparative Efficacy of Novel Endolysins in Queso Fresco

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Problem

Listeria monocytogenes is problematic for the manufacturing, storage, and consumption of ready-to-eat foods. This bacterium may cause listeriosis upon consumption often with deadly complications. Queso Fresco (QF), a pasteurized Hispanic-style fresh cheese (HSFC), has been shown to support the growth of *L. monocytogenes*. QF is the most widely produced and implicated HSFC in the U.S., representing a significant health hazard to at risk populations such as infants, pregnant women, elderly, and the immunocompromised. Approximately 16 to 27% of all *L. monocytogenes* infections occur in pregnant women, making them 18 times more likely to contract listeriosis, often resulting in lethal complications for the mother and/or child.[1]

Introduction

Listeria monocytogenes is a gram-positive foodborne pathogen that may form biofilms within food processing facilities and in domestic refrigerators. *L. monocytogenes* can survive for years under refrigerated storage and high salt contents regardless of frequent sanitation. The FDA has declared *L. monocytogenes* a zero-tolerance organism—completely unfit for human consumption, that cannot be present, at any level, in ready-to-eat foods.[2]

Queso Fresco is a soft HSFC most commonly associated with foodborne disease outbreaks. QF is particularly susceptible to *L. monocytogenes* contamination due to a relatively low acid content, high water activity, and near neutral pH. Typically, pasteurization is an effective process for eliminating *L. monocytogenes* from the raw ingredients prior to cheesemaking. However, despite advances in food processing technology no suitable solution for post processing contamination has been discovered.[3]

Endolysins are enzymes derived from bacteriophages that hydrolyze bacterial cell walls leading to bacterial cell death.[3] Endolysins maintain their lytic activity when applied exogenously and have a specific lytic spectrum that only target bacteria within a certain genus. Unlike traditional antimicrobials, bacteria are incapable of developing antimicrobial resistance to endolysins.[4] In addition to the aforementioned properties, endolysins are desirable antimicrobials since they will not inadvertently affect the food or consumer's microbiome.

Objective

To evaluate the efficacy of ten novel antimicrobial endolysins in combatting *L. monocytogenes* contamination in a miniaturized lab-scale queso fresco model over a 28 day shelf life.

Methods

1. Production of Queso Fresco

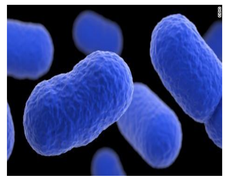
Rennet and $CaCl_2$ were added to warm milk (35°C).

The milk was divided into 1 mL batches.

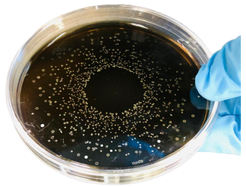
After incubation, the curds were cut with sterile needle and cooked again.

The whey was poured off of each curd, NaCl was added, and the batches were cooked a final time.

2. Application of experimental treatments



The curds were inoculated with L. monocytogenes and 2.5 U/mg of each endolysin was added.



Each cheese was enumerated by spiral plating.

Results

Highest Percent Reduction

- ❑ PlyP100 showed the greatest percent reduction on day 3 at 81.90%±5.87
- ❑ P118 demonstrated the highest percentage reduction at 14 days, with a 97.28%±9.52 reduction on day 7 and a 95.34%±11.41 reduction on day 14.

Lowest Percent Reduction

- ❑ P35 demonstrated the lowest percent reduction on day 3 at 35.15%±5.87.
- ❑ PlyP PSA showed the lowest percent reduction on both day 3 at 8.42%±5.87, and day 14 at -14.78%±11.41.

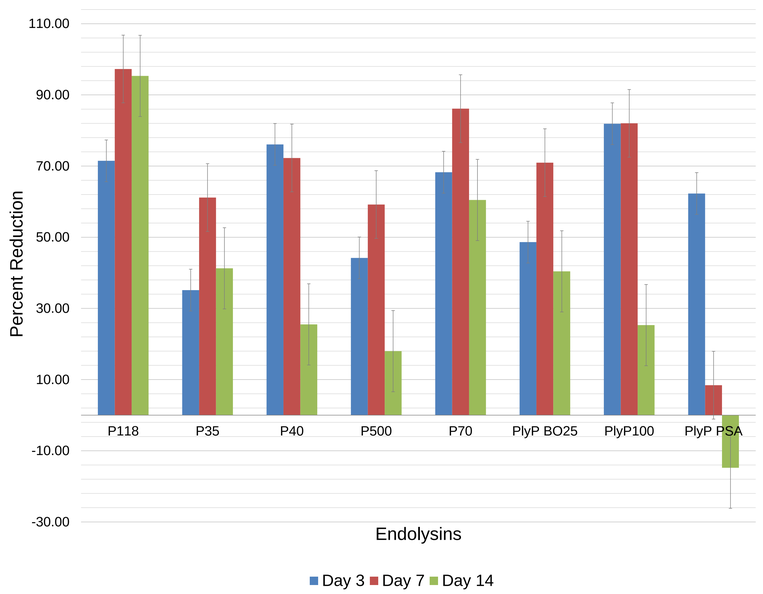


Figure 1. L.monocytogenes cell counts were determined in Queso Fresco treated with 10 different endolysins, stored at 4° C, after days 3, 7, and 14 . The percent reduction was calculated by comparing the resulting colonies/mL to the control cheese. Days 3 & 7 were done in duplicate while day 14 results were measured in duplicate across two biological replicates. Results means with ± SE

Conclusion

This research suggests that P118 was the most successful at inhibiting the growth of *L. monocytogenes* in QF. Although all of the cheeses were plated in duplicate, the accuracy for days 3 and 7 is limited due to there being only a single biological replicate as apposed to day 14 which contains two. In addition, PlyP PSA is shown to have inhibited growth on day 3, followed by a lack of inhibition at days 7 and promotion of bacterial growth on day 14. This result may be caused by an outlier in one of the biological replicates on day 14 possibly indicating an excessive inoculation of *L. monocytogenes*, deficient amount of PlyP PSA, or user error in spiral plating. The differences observed among the endolysins could be explained by differences in their respective lytic spectrums.

Future Work

Further research needs to be performed to determine the efficacy of the proposed endolysins in QF. Additionally, optimization of the endolysin concentrations should be determined for the most economical and industrial application. This may include combining endolysins to be used in tandem to evaluate synergy. These endolysins may also further lend themselves as antimicrobials to combat *L. monocytogenes* in other foods while maintaining quality structure and flavor.

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Acknowledgments

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