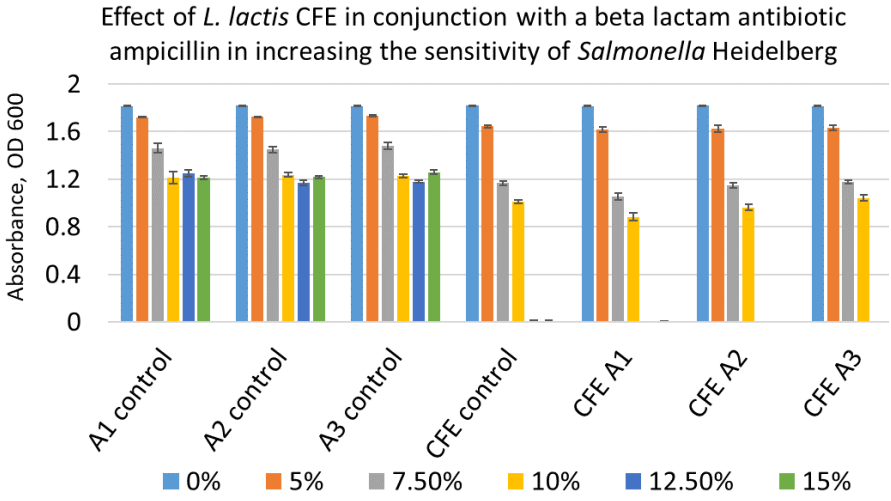
Effect of *Lactococcus lactis*-Derived Cell-Free Extract on Sensitizing Multidrug-Resistant *Salmonella* Heidelberg to a production antibiotic, Bacitracin

Introduction: The use of antibiotic growth promoters has led to the emergence of multiple drug resistant pathogens in animal production, including poultry, delaying the cure time for bacterial infections in humans.[1] Non-typhoidal *Salmonella* is the major cause of foodborne illness in the U.S., where approximately 1.2 million illnesses and 450 deaths occur every year.[2] Among the many serovars, MDR *S*. Heidelberg is one of the major foodborne Salmonellae with high potential for antimicrobial resistance.[3] *Salmonella* Heidelberg has been frequently isolated from retail poultry.[4]

Due to the introduction of Veterinary Feed Directive by the FDA, there are restrictions on the use of clinically important antibiotics in poultry. However, bacitracin, a polypeptide antibiotic, has been approved for improving feed efficiency, and to control necrotic enteritis and coccidiosis, in poultry. Bacitracin works by inhibiting the biosynthesis of peptidoglycan, a principal component of the bacterial cell wall, by suppressing lipid phosphorylase[5].

Use of probiotics would be an ideal approach to augment the activity of bacitracin against bacterial pathogens, since probiotics could work in multiple ways. *Lactococcus lactis* is a dairy – originated lactic acid bacterium (LAB) used for industrial microbiology purposes and has proven health benefits in humans. *L. lactis* produces several antimicrobial compounds, including lactic acid. It is previously reported that lactic acid can significantly damage the permeability of the outer membrane of Gram-negative bacteria, by affecting the lipopolysaccharide layer [6, 7]. The findings indicate the potential of LAB to work in conjunction with bacitracin to increase the bactericidal activity against *S*. Heidelberg.

Based on my completed URS project, it was found that *L. lactis* cell-free extracts (CFE) increased the sensitivity of MDR *S.* Heidelberg to a β-lactam antibiotic, ampicillin. Ampicillin, an anti-*Salmonella* antibiotic, inhibits bacterial division by inhibiting a membrane carrier molecule transporting peptidoglycan precursors across the inner cell membrane. The URS results presented on the right indicate significant reduction of *S.* Heidelberg populations obtained when 7.5% and 10% *L. lactis* CFE were used in conjunction with 16µg/ml of Ampicillin (CFE A1) when compared to the controls and other treatment groups.



A1 - 16µg/ml Ampicillin; A2 - 32µg/ml Ampicillin; A3 - 64µg/ml Ampicillin; CFE - Cell Free Extract; CFE A1, CFE A2, and CFE A3 - Combinations of CFE and Ampicillin

In this UROP proposal, I hypothesize that *L. lactis* CFE will increase the sensitivity of MDR *S.* Heidelberg to bacitracin. This hypothesis is based on the understanding that ampicillin and bacitracin act through independent mechanisms on bacterial cell wall and *L. lactis* could provide intermediates helping both processes. My research objective is to determine the effect of *L. lactis* CFE on sensitizing multidrug-resistant *Salmonella* Heidelberg to bacitracin.

Methods: The first step will be to create the stock culture for *L. lactis* and MDR *S.* Heidelberg (2011 turkey outbreak isolate) and grow them to 10^9 CFU for at least 3 generations in deMan Rogosa Sharpe (MRS) and Tryptic Soy Broth (TSB), respectively. The second step will be to extract CFE from *L. lactis* cultures grown in MRS by centrifugation and three-stage filtration of the supernatant. The last step will be to determine the effect of combination of minimum inhibitory

concentration (MIC – concentration of the antibiotic that will inhibit bacterial growth) of *L. lactis* CFE and bacitracin against MDR *S.* Heidelberg. The concentration of the *L. lactis* CFE that will be tested are 5%, 7.5%, 10%, 12.5%, and 15%, where the MIC could fall between 10% and 15% based on the URS results. The bacitracin dose that will be tested on *S.* Heidelberg will be 128, 256, and 384 mg/liter[8]. There will be 24 treatment (challenge) groups: a positive control (TSB adjusted to pH=4.1; My URS project results indicate that addition of up to 15% of *L. lactis* CFE would result in pH=4.1 and *S.* Heidelberg grew in this pH to an OD_{600}=1.8), 5 CFE control groups, 3 bacitracin controls; and combination of each CFE concentration with each bacitracin concentration (total 15 combinations). *S.* Heidelberg at 10^5 CFU/ml will be added to all treatments. Appropriate negative controls will be kept. The study will be repeated 5 times with at least 2 duplicate samples per experimental group. The growth of SH will be determined by using a microplate reader to obtain the optical density (OD) at 600nm. Once the optimal concentration is obtained, broth dilution assay will be performed to obtain accurate bacterial populations survived after the treatments. Data will be analyzed using SAS software with a significant difference detected between the groups at $P \leq 0.05$.

Significance and Intellectual Merit: My UROP research focuses on understanding probiotic interventions to tackle an emergent problem in animal industry – antimicrobial resistance. I will improve my independent research, critical thinking skills, and scientific writing skills obtained from my URS project with Dr. Johny to develop and complete this project. I will gain more knowledge on the ability of *L. lactis* to increase the sensitivity of *S.* Heidelberg to multiple antibiotics. This research will prepare me to become a successful food microbiologist focusing on antimicrobial resistance mitigation. The results will be reported and presented at the U of M Undergraduate Symposium and IFT conference to educate others on my research.

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