UMSAEP ACTIVITIES 2021

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Proposal: Food spoilage associated with *Chryseobacterium* species

With Dr. Andrew Clarke, Associate Professor, Division of Food Systems & Bioengineering, 246 Stringer Wing of Eckles Hall, University of Missouri- Columbia

clarkea@missouri.edu

The visit to the University of Missouri in 2021 was my first visit to the university. My application was approved in 2020 and it seemed unlikely that I would be able to visit the university due to the ongoing Covid 19 pandemic. I was very lucky to visit the University during the time I did. I arrived in Columbia, Missouri on Sunday, October 3, 2021, on a 4-week visit to start a collaboration with Dr. Andrew Clarke. The aim of the project was to identify *Chryseobacterium* strains present on chicken carcasses in the USA. *Chryseobacterium* is known to spoil food and this study would shed light on the wide distribution of this bacterial strain in the food industries of South Africa and the United States of America. Dr. Clarke and I had regular meetings during 2021 to keep up to date and discuss my potential trip. Prof. Uphoff was instrumental in me traveling to the USA and made various arrangements, including changing flights when travel to certain countries was restricted to South African citizens. Prof. Uphoff's determination to allow me to travel to the USA gave me an opportunity to learn and grow in my career.

Food spoilage is a multifactorial process, of which microbial spoilage plays an important role. There are significant gaps in knowledge regarding microbial spoilage, the organisms that contribute to it, and ways to manage spoilage. The spoilage of microbial food affects the presentation and taste of the products and is a complex interaction of various microbial species. In this project, we are interested in looking at the presence of microbial spoilage organisms on chicken carcasses after slaughter and testing various disinfection protocols to prevent them. Poultry and chickens are the most abundant source of protein in South Africa (SAPA, 2018). Currently, the most widely used antimicrobial intervention in the poultry industry, including the USA industry, is the use of chlorine-based antimicrobial washes (Killenger et al. 2010).

Chryseobacterium sp. are known for their ability to spoil food due to its production of proteolytic enzymes and its psychrophilic nature (Bernadet et al., 1996). It has been isolated from various environments and is frequently isolated from meat- and meat-containing food products. However, its contribution to food spoilage is diminished or neglected due to the presence of significant food spoiling bacteria such as Pseudomonas, Bacillus, Moraxella, and Clostridium. In recent studies, this organism was isolated from freshly slaughtered chicken (Oosthuizen, 2019). *Chryseobacterium* is an

important opportunistic pathogen and has been known to cause severe infections in humans. There is no evidence that species of *Chryseobacterium* associated with food spoilage cause infections in humans, but its role in the spoilage of meat products is worth investigating. It is a psychrophilic microorganism that can cause spoilage at refrigeration temperatures and can survive chlorine wash treatments. Therefore, the objective of the project is to investigate various methods to control bacterial spoilage in poultry.

Objectives

• Investigate the ability of *Chryseobacterium* strains to cause chicken spoilage. Various strains of *Chryseobacterium* have been isolated from various areas, including portions of chicken. These strains will be investigated for their potential to cause food spoilage.

• Compare various antimicrobial rinses of poultry carcasses at harvest to determine the effect on various spoilage organisms. The effect of antimicrobial rinses on meat quality will also be evaluated.

Details of the collaborative study

Dr. Clarke was able to secure freshly slaughtered chicken carcasses from a small abattoir in Versailles, Missouri (Figure 1) that will supply us with 54 chicken carcasses. We collected 18 chicken carcasses per week and repeated the shelf life study three times.

A **B**

Figure 1A: *The small processing facility receives chickens from surrounding small chicken farms and slaughters and processes the chicken carcasses.* ***B:*** *The processing facility where the chicken carcasses were washed and sealed in plastic ready to be sold.*

We randomly divided 18 chicken carcasses and treated the carcasses with a 50 ppm chlorine wash, 2% lactic acid wash, and drinking water as a control. Each chicken carcass was swabbed before treatment to assess the bacterial load on the carcasses before treatment. The carcasses were treated for 30 seconds, the breast and wings were removed and packaged. The wings were stored at -20°C until needed. The breasts were halved, and one quater was processed on day 1 and the rest of the breasts were incubated at 4°C and sampled on days 3 and 6. Chicken breasts were subjected to microbial analysis to determine the efficacy of antibacterial treatments. The microbial analysis included the total aerobic bacterial count, the total coliform count, and the total lactic acid bacteria present in the samples. The microbial analysis was performed in Prof Mustapha's laboratory and her graduate students trained me to use some of the equipment that I was unfamiliar with.

The first set of breasts was processed on day 1. Breasts were placed in a sterile sampling bag containing 90 ml of neutralizing broth. The neutralizing broth was used to neutralize the antibacterial treatments to assess the bacteria present in the samples. The bag was messaged for 30 seconds to allow the bacteria present in the chicken breast to be washed off. The broth was collected and pooled in sampling bottles followed by plated on Tryptic soy agar and Pseudomonas broth to isolate *Chryseobacterium* and *Pseudomonas* species. The samples were plated on aerobic count plates, coliform count plates, lactic acid bacteria count plates, and Petri films to determine the bacterial counts.

The results were not conclusive, but it was estimated that the lactic acid antibacterial wash had generally lower bacterial counts than chlorine treatment and control. The coliform count was in the acceptable range (100-1000 CFU/ml), but it is recommended that it should be below 100 CFU/g for fresh meat. This is somewhat concerning, but it is possible that it is the result of laboratory error.

Table 1: *The effect of antibacterial treatment and storage time on bacterial counts*

Treatment	Total aerobic count (log cfu/ml)			Total coliform count (log cfu/ml)			Total Lactic acid bacteria (log cfu/ml)		
	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
Control	3.91 ±0.3	3.95 ±0.39	4.12 ±0.21	2.09 ±0.99	1.93 ±0.96	2.08 ±1.09	3.07 ±0.28	3.30 ±0.35	3.04 ±0.42
Lactic acid	3.47 ±0.45	3.21 ±0.41	3.21 ±0.48	1.92 ±0.96	1.15 ±0.61	1.28 ±0.64	2.74 ±0.37	2.81 ±0.58	2.31 ±0.27
Chlorine	3.95 ±0.32	4.02 ±0.21	4.32 ±0.52	2.23 ±1.06	1.84 ±0.91	1.81 ±0.99	3.05 ±0.47	3.05 ±0.27	3.19 ±0.6

Total bacterial counts before treatment: 3.55 log cfu/ml ±0.28

The coliform count should be tested before treatment to draw more conclusions from the results. Lactic acid bacteria are generally associated with food spoilage and this is an indicator that they generally survive chlorine treatments. It should also be remembered that total bacterial counts were generally very low. This should be repeated to test the efficacy of the methods. We were unable to identify the species *Chryseobacterium* or *Pseudomonas* from any of the carcasses of chickens. They may not be present in the US poultry setting, or sampling methods need to be amended to isolate bacterial strains.

Future plans

The rest of the chicken samples will be processed by a master's student that joined Dr. Clarke, Mr. Patrick Luo. Lactic acid has been shown to be a promising treatment method, and we will further investigate this method to determine the correct concentration that will not influence taste but retain the antimicrobial properties.

The visit to Columbia

During my first week in Columbia, I was orientated to the University and the laboratories. I prepared culture media to be used in the experiments and visited the poultry processing facility. On weekends, there was time to explore the area. Dr. Clarke was kind enough to take me on a tour of the surrounding towns. We visited Jefferson City, St Louis, and Fulton. I had a wonderful experience at the University of Missouri and had many discussions on how to strengthen the collaboration between our research groups. I had many cultural experiences and met some fantastic people at the University of Columbia. Some from the photos of my trip is included below.

References

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Figure 2: *Dr. Clarke gave me a tour of the surrounding areas during the days I was not busy in the laboratory. A: Jefferson City. B: St Louis, D: Lake of the Ozarks, E: Fulton.*