Developing Alternatives to Antibiotics Use: Reduction of *Campylobacter* counts on chicken wingettes by a chitosan based coating or use of probiotic (*Lactobacillus* spp.) isolates

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ABSTRACT

The presence of *Campylobacter* on poultry products remains one of the leading causes for foodborne illness. The reduction in the use of antibiotics in animal agriculture has increased the need for alternative forms of improving food safety. The use of lactic acid bacteria (LAB) as a bio-preservative/protective culture in food commodities is an ancient technology that is safe and natural. In this study, 13 *Lactobacillus* spp. isolates were screened by a chicken skin dipping model to evaluate the potential to reduce *C. jejuni* counts. From this screening assay, 4 isolates (isolates 1-4) which produced >1 log reduction in *Campylobacter* counts were chosen for further evaluation in a chicken wingette model. In replicate trials, chicken wingettes were inoculated with *C. jejuni* (~7 Log CFU/mL) and treated with either a *Lactobacillus* broth culture or a BPD control (n=5 samples/treatment). *Campylobacter* counts were determined at 0, 1, 3, 5 or 7 days post treatment. *Campylobacter* counts were $\log_{10}$ transformed and data were analyzed using ANOVA with the PROC MIXED procedure of SAS. Isolates 2 or 4 were the most effective and consistently reduced *Campylobacter* counts from day 1 through day 7 ($P<0.05$). In follow-up studies, isolates 2 and 4 were subjected to additional testing aimed at assessing potential synergistic activity between the *Lactobacillus* isolates and their combination with a 2% chitosan (CH) solution. Each isolate by themselves, CH or their combination significantly reduced *Campylobacter* counts (~1-2.5 log reduction) from day 1 through 7. The combination of isolates+CH reduced *Campylobacter* counts on wingettes, but this treatment did not demonstrate any additional reduction compared to each individual treatment alone. Our studies demonstrate the potential use of CH or *Lactobacillus* isolates as a protective culture to reduce *Campylobacter* counts on raw poultry.

INTRODUCTION

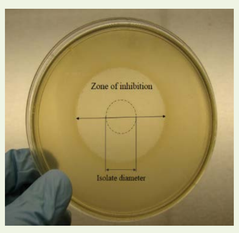
Campylobacter is one of the most commonly reported bacterial causes of human food-borne illness worldwide. A strong correlation exists between *Campylobacter* infections and consumption of contaminated or mishandled raw/cooked poultry products. *Campylobacter* contamination of poultry meat is a significant problem because it is consumed as one of the major sources of protein in the United States and indeed globally. The challenge to poultry producers arises from increased consumer demand for minimally processed foods while maintaining a microbiologically safe product.

An emerging strategy for the improvement of food safety without the use of chemicals is by the addition of lactic acid bacteria (LAB) as a protective culture or biopreservative. This method works by introduction of live organisms onto the food matrix and the subsequent inhibition of growth of microbial pathogens or spoilage organisms, thereby increasing the safety of the food product and extending its shelf-life. The use of coating treatments on raw poultry products can be considered another intervention to add to the multiple hurdle approach for enhancing the food safety of poultry products. Prior research performed in our laboratory has demonstrated that a 2% concentration of 190-310 kDa chitosan, when applied as a surface coating, reduces *C. jejuni* counts on chicken wingettes. This study investigated the potential of previously characterized LAB isolates in reducing *C. jejuni* on chicken wingettes either alone or in combination with chitosan.

MATERIALS AND METHODS

Selection of *Lactobacillus* spp. isolates

Isolation and identification of GRAS bacterial isolates from healthy chicken ceca → Selection for *in vitro* anti-*Campylobacter* activity (Soft agar overlays) →

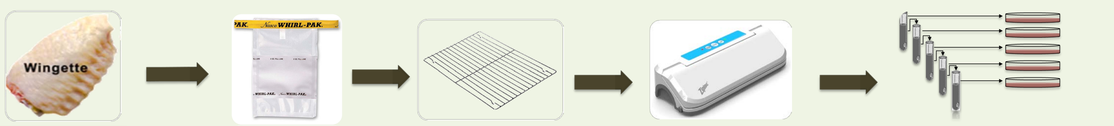


→ 13 *Lactobacillus* isolates with anti-*Campylobacter* activity were selected for *in vivo* studies in broiler chickens

Initial Screening of *Lactobacillus* Isolates: Skin Model

Inoculation of skin samples with *C. jejuni* (30 min)
↓
Treatment - 20ml broth culture (5 min)
↓
Drying for 2 min
↓
Direct plating on CLA for enumeration

Treatment protocol: Wing model



- **Inoculation**: • *C. jejuni* inoculum • 30 min
- **Treatment**: • 10 mL broth culture • 30 sec
- **Drying**: • 15 min per side • 30 min total
- **Vacuum sealing**: • Storage 1,3,5 or 7 days • 4^0C
- **Sample processing and plating for enumeration**

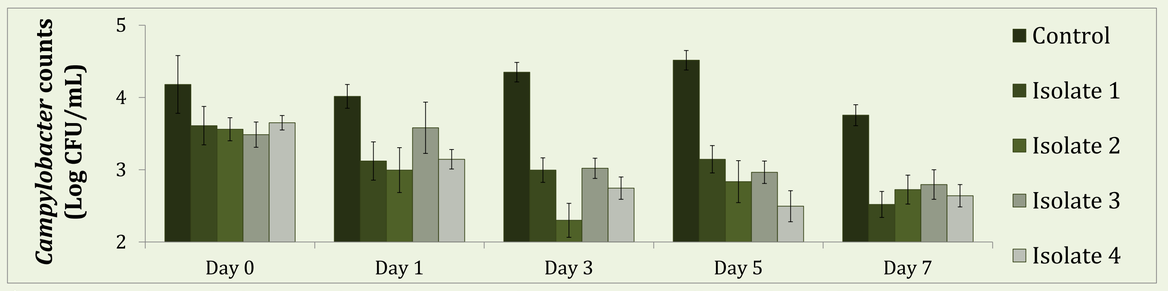
Experimental design: Isolates, chitosan or the combination on chicken wingettes

- **Isolates**: Control (BPD); Isolate 2; Isolate 4
- **Chitosan**: Control (50mM Acetic acid); 2.0% MMW
- **Chitosan+Isolates**: Control (BPD:Acetic acid); 2.0% MMW + Isolate 2; 2.0% MMW + Isolate 4

- n=5 wingettes per treatment
- The 2% medium molecular weight (190-310 kDa) Chitosan concentration was determined in a prior study
- Treatment protocol, sample processing & enumeration was done similar to the wing model

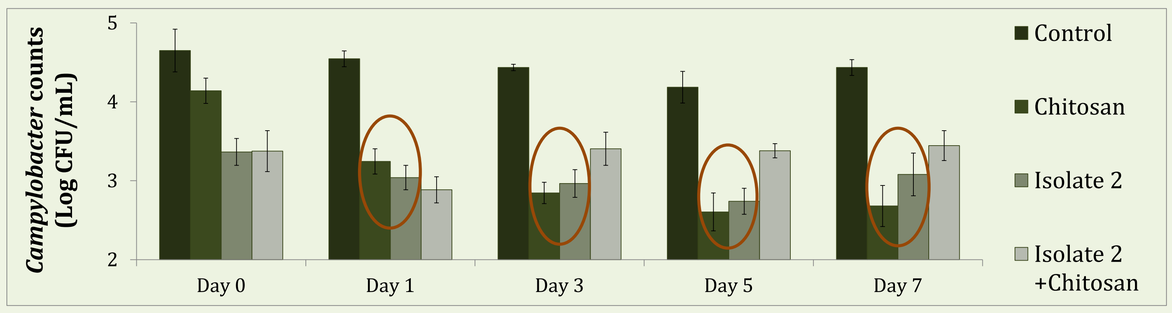
RESULTS

The ability of selected *Lactobacillus* spp. isolates to reduce *Campylobacter* counts on chicken wingettes (Trials 1 and 2)*

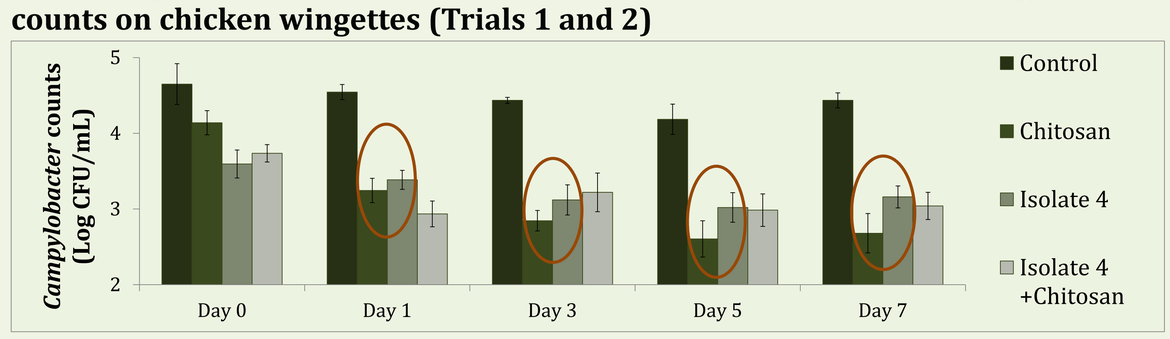


*Isolate 2 & 4 chosen for further evaluation for potential improvement of efficacy when added to a 2% MMW chitosan coating

The ability of isolate 2, chitosan or the combination to reduce *Campylobacter* counts on chicken wingettes (Trials 1 and 2)



The ability of isolate 4, chitosan or the combination to reduce *Campylobacter* counts on chicken wingettes (Trials 1 and 2)



DISCUSSION

Despite a plethora of postharvest interventions, *Campylobacter* remains a persistent contaminant on raw poultry products. A potential strategy for reducing this pathogen is by the treatment of raw poultry products with protective cultures. In the first two trials a chicken skin dipping model was utilized to screen 13 *Lactobacillus* isolates with previously demonstrated anti-*Campylobacter* activity. From this screening 4 isolates which produced greater than a 1 log reduction in *Campylobacter* counts were chosen for further evaluation in a chicken wingette model. The chicken wingette model was used to more closely resemble the heterogeneous nature of the skin on a chicken carcass and in addition, the treatment exposure time of 30 seconds more closely approximates a realistic exposure time in terms of integrating the coating treatment with intervention strategies already in place within the processing plant. The testing of 4 select *Lactobacillus* isolates has demonstrated the effectiveness of using specific lactic acid bacterial cultures as a protective culture to reduce *C. jejuni*. In an attempt to improve upon the efficacy of the cultures, selected *Lactobacillus* isolates were combined with a 2% chitosan solution and applied as a coating on the wingettes. From these trials we observed that both the isolates or chitosan treatments significantly reduced *Campylobacter* levels continuously through day 7 (1-2 log reduction). However, combination of isolates with chitosan did not demonstrate any additive or synergistic effect.

CONCLUSIONS

We have identified 2 LAB isolates, which consistently reduced the number of *Campylobacter* on wingettes and show potential to be used as a protective culture on raw poultry meat. Either *Lactobacillus* isolates or chitosan treatment can significantly reduce *Campylobacter* on chicken parts (1 to 2 log reduction). Use of these natural products could be part of a multifaceted approach to reduce *Campylobacter* counts in poultry and the incidence of this disease in humans.

ACKNOWLEDGEMENTS

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