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EVALUATING THE SPOILAGE POTENTIAL OF *VAGOCOCCUS* SPP. IN MODIEFIED ATMOSPHERE PACKAGED BROILER

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INTRODUCTION

Microbial spoilage is a primary cause of economic losses in the meat industry, significantly reducing shelf life and consumer appeal. While a diverse community of bacteria contributes to this process, recently *Vagococcus* species have been identified as potential key spoilage organisms in broiler meat products . Vagococci have been detected in humans [1] as well as in chicken and fish [2] . While several specific *Vagococcus* species has been definitively established as primary spoilers in the existing literature, their increasing prevalence in spoilage microbiota suggests a potential role. Understanding their specific spoilage mechanisms within the complex meat microbiota is crucial. To address this, our study investigates the spoilage potential of novel strains of *Vagococcus fessus* and *Vagococcus proximus* in commercially produced broiler chicken packages. The introduction of streptomycin resistance allows for targeted tracking and quantification of these strains within the natural microbial background. In the present study, we hypothesize that these *Vagococcus* strains will exhibit distinct spoilage characteristics, which will be evaluated through comprehensive sensory analysis and volatile organic compound (VOC) profiling during refrigerated storage (6°C) over a two-week period. This targeted approach will provide valuable insights into the specific contribution of *Vagococcus* spp. to meat spoilage

MATERIALS AND METHODS

This research investigated the role of streptomycin-resistant *Vagococcus fessus* and *Vagococcus proximus* strains in chicken spoilage. The materials employed included: six strains from two distinct *Vagococcus* species, streptomycin (Sigma-Aldrich, purity ≥ 98%), various culture media (Tryptic Soy Broth - TSB, de Man, Rogosa and Sharpe agar - MRS, Plate Count Agar - PCA, and Elliker agar - Eliker), commercial broiler chicken packages (sourced from a local super-market), Gas Chromatography (GC) equipment (Agilent 7890B GC system coupled with a 5977A MSD).

The methodology involved first generating streptomycin-resistant cocktail strains of *V. fessus* and *V. proximus*. This was achieved through a stepwise selection process where the initial wild-type strains were exposed to increasing concentrations of streptomycin in growth media, starting at 200 µg/mL and escalating to 1000 µg/mL. This procedure ensured the selection of stable streptomycin-resistant mutants. The resistant strains were then cultured in TSB medium under aerobic conditions and subsequently combined in equal proportions to create a bacterial cocktail.

For the spoilage study, commercial chicken packages were inoculated with the prepared *Vagococcus* cocktail. Control samples consisted of uninoculated chicken packages. The experimental design involved three treatment groups (chicken inoculated with *V. fessus* and *V. proximus* cocktail and uninoculated control) with triplicate samples for each group at each time point. All inoculated and control packages were stored at 6°C for a duration of 14 days. Enumeration of streptomycin-resistant *Vagococcus* spp. was performed on selective media at predetermined intervals to quantify bacterial growth associated with spoilage. Spoilage was assessed through sensory analysis (odor, color, texture) and volatile organic compound (VOC) profiling using Gas Chromatography (GC) to characterize spoilage metabolites produced by the *Vagococcus* strains.
This research did not involve human subjects, animals, or sensitive personal data; therefore, ethical approval was not required. All experiments were conducted in a controlled laboratory environment following standard microbiological practices to ensure the integrity and reproducibility of the results.

RESULTS AND DISCUSSION

Preliminary investigations, conducted with the wild-type *Vagococcus proximus* strain (PNs007T) using a similar experimental setup involving modified atmosphere packaged broiler meat (0.045% O2, 36.5% CO2, 63% N2), revealed differential growth patterns across various culture media, suggesting media-specific metabolic activity and highlighting the importance of the growth medium on the spoilage potential of *Vagococcus*. Strain PNs007^T was

detected during a independent study related to the identification of spoilers of commercially manufactured modified atomosphere packaging broiler meat [3].

As shown in Figure 1, the growth curves demonstrate that *V. proximus* (PNs007^T) exhibited distinct growth patterns in each medium, with TSB and PCA supporting higher final cell densities (reaching approximately 10^7 - 10^8 CFU/g) compared to MRS and Elliker media (reaching approximately 10^6 - 10^7 CFU/g). This observation, derived from a meat-based experimental setup, underscores the influence of nutrient availability within a food matrix on *Vagococcus* growth dynamics and justifies the need to investigate these dynamics further in the current study using streptomycin-resistant strains.

The current study is designed to utilize these data to elucidate specific role of *Vagococcus* spp. in meat spoilage using streptomycin resistance as a tracking mechanism. We hypothesize that the streptomycin-resistant strains will exhibit measurable spoilage characteristics, detectable through sensory and VOC analysis, and that their growth dynamics will correlate with these spoilage indicators. We anticipate that the selective enumeration of the inoculated resistant strains will enable a precise determination of their contribution to the overall spoilage process, distinguishing them from the background microbiota and providing insights into their competitive interactions within the spoilage community. Specifically, we expect to quantify the streptomycin-resistant *Vagococcus* strains over time in chicken packages and correlate their growth curves with changes in sensory attributes and the production of specific volatile compounds (VOCs) identified by GC analysis. These results will be discussed in the context of current knowledge on meat spoilage mechanisms and the role of specific bacterial groups in these processes.

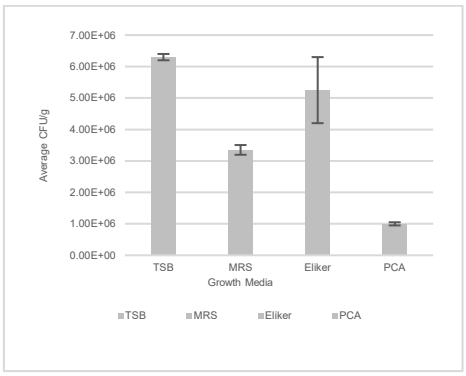


Figure 1. Growth of wild-type *V. proximus* (PNs007^T) in TSB, MRS, Elliker, and PCA media over time.

CONCLUSION

This research will provide valuable insights into the spoilage potential of *Vagococcus* in commercial meat products. By combining microbiological, sensory, and volatile compound analyses, the study will contribute to a more detailed understanding of meat spoilage mechanisms and inform strategies to enhance meat quality and safety.

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