CHANGES IN THE LAMB MEAT MICROBIOME DURING COLD STORAGE

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I. INTRODUCTION

Raw meat provides an ideal environment for the growth of microorganisms due to its high-water activity and nutrient content. Fresh meat is particularly susceptible to spoilage and has a relatively short shelf life. Recent advances in high throughput sequencing (HTS) technology have revolutionised analysis of the species composition of microbial communities (the microbiome), in contrast to traditional microbiological culturing methodology which is inherently selective. Microbiomes can be determined by extraction and sequencing of total genomic DNA (gDNA) (metagenomics) or by PCR-sequencing of discrete regions amplified from gDNA such as the 16S or 18S rRNA genes (microbial community profiling). These non-culture based approaches have been applied to determine the microbiomes of beef and pork [1-3], but fewer studies have been done on lamb meat with most published research focusing on vacuum-packed lamb [4]. Yet, lamb is a significant percentage of the total meat diet consumed in many countries [5]. The objective of this research was to explore variability in the microbiome of fresh lamb meat purchased from three different sources and changes over time in community composition following cold storage.

II. MATERIALS AND METHODS

Fresh lamb meat cuts (leg, shoulder and loin) were obtained from three different butchers in Melbourne, Australia. The cuts were sealed in plastic bags and placed on ice for transfer to the laboratory. Six replicate samples (10g each) from each cut were used for analysis: three samples to be analysed on day 0, and three after storage at 5ºC for 6 days (54 samples total). Each sample (10g) was homogenized in 20 ml of ice-cold phosphate buffered saline, then each homogenate (1 ml) was centrifuged at 8000×*g* for 5 minutes. The supernatant was discarded and pellets were stored on ice. A MoBio PowerLyzer® PowerSoil® kit was used to extract DNA from the pellets. The 16S rRNA gene V4 region from DNAs was PCR amplified using barcoded universal bacterial primers 515F and 806R. A Nextera® XT DNA library preparation kit was used to prepare the DNA library sequencing. DNA sequencing was done using an Illumina MiSeq machine and Nextera® XT chemistry. A total of 3464605 reads were obtained, with numbers of reads used in analyses ranging from 10^4 (minimum) to $>10^6$ per sample. Sequencing reads were filtered and analysed by Qiime version 1.0.0 [6].

III. RESULTS AND DISCUSSION

In this study microbial community profiling based on HTS of bacterial 16S rRNA gene amplicons was used to profile the core and spoilage-related bacterial taxa present on different cuts of fresh lamb meat stored aerobically at 5ºC for a 6-day period. A total of 205 genera from 21 different phyla of bacteria were identified. The predominant genera were: *Photobacterium*, *Acinetobacter*, *Pseudomonas, Brochothrix* and *Psychrobacter*, with *Carnobacterium* and *Lactococcus* (lactic acid bacteria) present in lesser amounts (data not shown).

During cold storage at 5ºC the relative abundance of *Photobacterium*, *Acinetobacter*, *Brochothrix Pseudomonas* and *Psychrobacter* increased and overall diversity decreased. There was also some variability between replicate samples (Figure 1). This change has occurred presumably because some of the bacterial species present on the meat surface have efficient adaptation mechanisms for growth under cold conditions (psychrotrophic growth), allowing them to outcompete other bacterial species.

An interesting observation in our study was the dominance of *Photobacterium* (a gram-negative facultatively aerobic microorganism) after cold storage of some lamb meat samples (Figure 1). This species is more commonly associated with spoilage of fish, although in one study involving beef it was suggested it could have a significant role in meat spoilage under cold conditions [7].

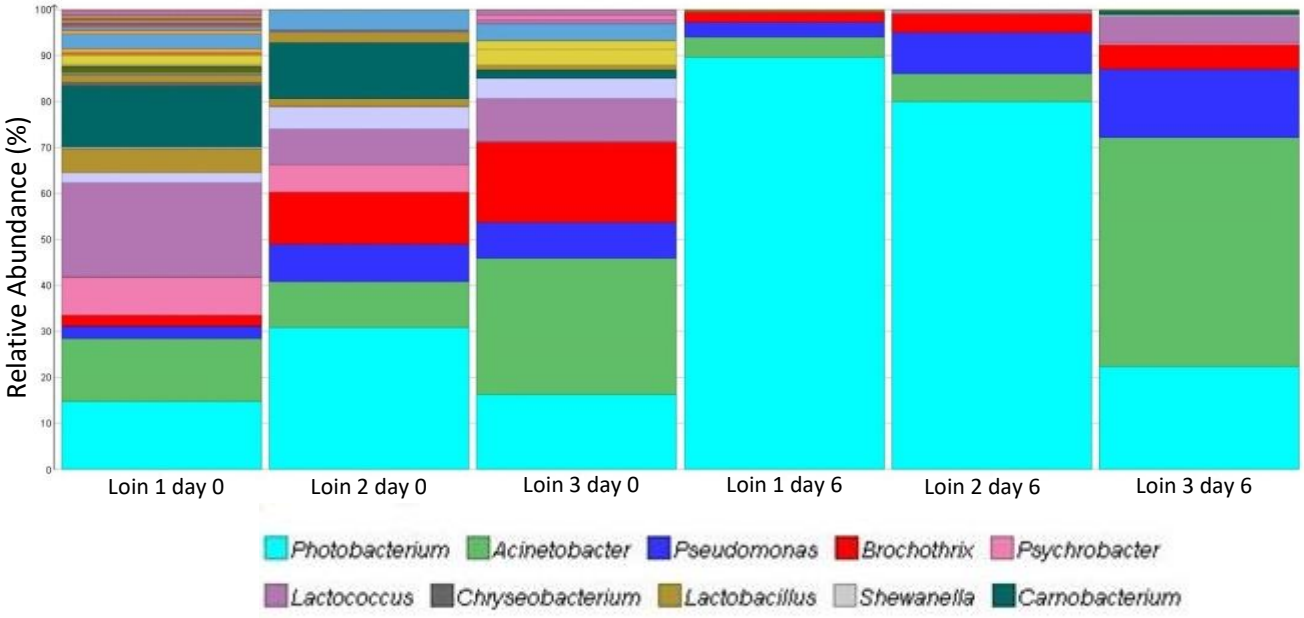

Figure1: Relative abundance (%) of different bacterial genera determined by sequencing of DNA from microbial communities on lamb loin meat samples obtained from a Melbourne butcher at time of purchase (day 0) and after storage at 5ºC for six days.

IV. CONCLUSION

The results of this study show that psychrotrophic Gram-negative aerobic and facultative anaerobic bacteria have outcompeted the other microbiota present on lamb meat during cold storage, leading to a reduction in species diversity. Many of these Gram-negative bacterial genera have been previously implicated in meat spoilage. Future studies will assess levels of bacterial variability between different lamb cuts and butchers.

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REFERENCES

1. Ercolini, D., I. Ferrocino, A. Nasi, M. Ndagijimana, P. Vernocchi, A. La Storia *et a*l. (2011). Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. Applied and Environmental Microbiology 77(20): 7372-7381.
2. De Filippis, F., A. La Storia, F. Villani and D. Ercolini (2013). Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. PLoS One 8(7):e70222.
3. Zhao, F., G. H. Zhou, K. Ye, S. Wang, X.-L. Xu and C. Li. (2015). Microbial changes in vacuum-packed chilled pork during storage. Meat Science 100: 145-149.
4. Mills, J., A. Donnison, and G. Brightwell, (2014). Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. Meat Science 98(1): 71-80.
5. Locke, R. and J. O'Connor. Sheepmeat's unique global position. Meat & Livestock Australia. Report, April, 2017
6. Caporaso, J. G., et al. (2010). "QIIME allows analysis of high-throughput community sequencing data." Nature Methods **7**(5): 335.
7. Pennachia, C., D. Ercolini and F. Villani (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. Food Microbiology 28(1): 84-93.