Characterisation of bacterial diversity on vacuum packed beef after 9 weeks chilled storage with and without prior-rinsing with peroxyacetic acid

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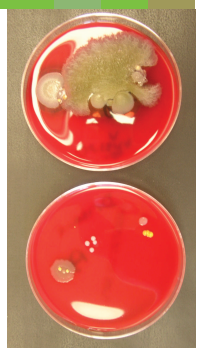
Introduction

- The high standard of New Zealand meat processing hygiene generally results in meat products with low microbial counts at the time of manufacture.
- In some instances, meat producers may use various chemical or physical carcass decontamination procedures to reduce levels of bacteria present on carcasses during the manufacturing process.
- Studies have reported the efficacy of peroxyacetic acid (POAA) as an antimicrobial intervention on carcasses and this agent is approved for use in New Zealand.

Why use molecular methods?



- Classical culture methods underestimate bacterial diversity.
- Culturing *in vitro* may fail to provide the necessary growth conditions for all types of bacteria present in the same sample, e.g. temperature, nutrient requirements and atmosphere.
- May not be able to culture stressed/crippled bacteria.
- Molecular methods
  - provide a way to look at the impact changes in a facility on microbe populations
  - gain a better understanding of the nature and distribution of microorganisms within a particular environment or product.

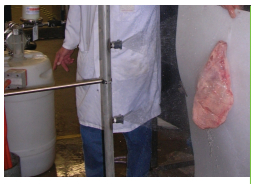
Important to ensure that changes in meat processing e.g. interventions do not create new hazards by alteration of microbial balance.

"Cause and effect – every action has a consequence"

Aim

To identify and compare bacterial community composition of POAA treated and untreated chilled vacuum-packed beef

Materials & methods

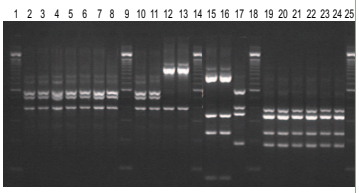


- Beef flaps were either treated with a POAA based carcass wash (INSPEXX™, 180ppm) or untreated, prior to vacuum packaging and chilled storage.

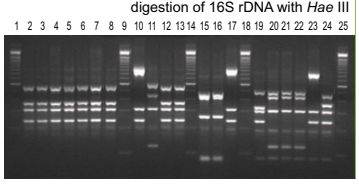
Culture independent approach

- 16S rRNA gene clone libraries were screened to investigate bacterial diversity associated with vacuum-packaged meat following 9 weeks storage at -1.5ºC.
- Clone libraries were generated following DNA extraction of genomic DNA from replicate meat samples.
- Total community 16S rRNA genes were amplified by PCR and cloned in *E. coli* using the Zero Blunt TOPO PCR cloning kit. Inserts were amplified from plasmids by PCR with vector specific M13 primers.
- Unique clones were determined by Restriction Fragment Length Polymorphism (RFLP) analysis, and selected for sequencing.
- GenBank database was searched using the BLAST tool to identify bacterial sequence identities.

Representatives of RFLP groups generated by digestion of 16S rDNA with *Cfo* I



Representatives of RFLP groups generated by digestion of 16S rDNA with *Hae* III



Culture dependent approach

- Total aerobic plate counts were performed on treated and untreated meat samples following chilled storage.
- Identified representative isolates by differential plate count (MIMM Manual).

Results

Culture independent

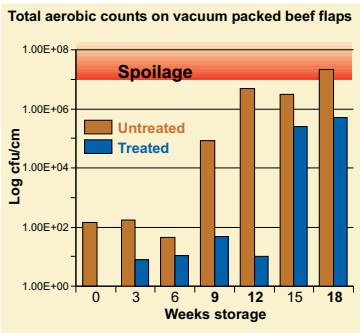
- Untreated beef and POAA treated beef samples were predominantly populated by facultatively anaerobic *Carnobacteria* following chilled storage.

Sequence identity	% of clones detected in each 16S rDNA library: Beef untreated	Beef POAA treated
Carnobacterium maltaromaticum	100 %	98 %
Clostridium algidicarnis / Cl. putrefaciens	0 %	2 %

- Psychrotolerant anaerobes, *Clostridium algidicarnis / Cl. putrefaciens* were exclusively detected in the POAA treated beef clone libraries.

Culture dependent

- Dramatic reduction in total aerobic bacteria counts were observed with POAA treatment up to **12** weeks of storage.
- No "off odours" were observed with POAA treated samples at **18** weeks storage.
- Untreated samples at week **9**
  - 96% Lactic Acid Bacteria
- Treated samples at week **9**
  - 87% Lactic Acid Bacteria
  - 9% Enterobacteriaceae



Conclusions & considerations

- **Culture method may not distinguish *Carnobacteria* from other Lactic Acid Bacteria** Lactic Acid Bacteria may be involved in spoilage, also produce bacteriocins (+ve and –ve effects).
- **Detection of spore forming anaerobes in POAA treated beef clone library but not untreated beef** (*Clostridium algidicarnis / Cl. putrefaciens*). Psychrotolerant anaerobes have been implicated in spoilage of chilled vacuum packaged meats. However, the % prevalence of these organisms is low based on clone library data and the results obtained from the 18 week storage gave no indication of clostridial spoilage having occurred.
- **Dramatic reduction in total aerobic bacteria counts** were observed with POAA treatment up to 12 weeks of storage.
- **No observed spoilage odours associated with treated product after 18 weeks storage.**

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Te Ahuwhenua, Te Kai me te Whai Ora. Tuatahi

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