final report

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Executive Summary

This multi-faceted project commenced in September 2013 and was completed in April 2016. The project focused on the microbial quality/shelf life and safety of vacuum-packed beef and lamb meat. The primary goal was to innovate in the areas of carcass safety, and shelf-life prediction and extension, and to develop new personnel with scientific expertise in these areas to support the Australian red meat industry.

On the basis of its expertise and international reputation for food microbiology research and training, the Food Safety Centre at the University of Tasmania was appointed as a Meat and Livestock Australia *Principle Research Organisation* on “Microbial Ecology and Physiology” (‘PROMEP’). The objective was to further develop the UTas group’s expertise in the microbiology of red meat such that PROMEP could constructively respond to current meat industry needs and opportunities and so that it could be ready to contribute to future industry needs and opportunities.

The PROMEP project involved five sub-projects involving scientific research, scientific advice and science/technology dissemination to the industry and that was intended to promote:

- technological innovation,
- dissemination of industry-relevant science news,
- provision of high level and contemporary science-based advice, and
- development of people with good understanding of the Australian meat industry to fill future roles as scientists/technologists and advisors.

A consultative committee composed of industry experts ensured that the research provided value to the Australian meat industry and that the work progressed at a satisfactory rate.

Outcomes of the work undertaken to date include:

- **new technologies** for carcase decontamination with respect to enteric pathogenic bacteria are close to being trialled at pilot scale in a commercial abattoir: these technologies exploit earlier findings from MLA-funded research;
- **new predictive models and software tools** to enable the Australian export meat industry to better manage quality/shelf life through transport and distribution and to meet international market requirements have also been developed and are now being used by industry;
- **new human capital** - two doctoral scientists have received industrially-relevant research training, through: direct involvement with processors and industry experts who provided industry-relevant advice; having undertaken specific meat-science training courses; having sourced and information and news relevant to the industry through regular newsletters, and shared research results and potential applications industry through meat industry conference presentations. Several PhD candidates and Honours students have also received meat science training through the PROMEP.

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1 Background

This multi-faceted project commenced in September 2013 and was completed in April 2016.

The PROMEP project focused on the microbial quality/shelf life and safety of vacuum-packed beef and lamb meat. The primary goal was to innovate in the areas of carcass safety and shelf-life prediction and extension, and to develop new personnel with scientific expertise in these areas. PROMEP involved five sub-projects, which are briefly described below.

The work was needed because exports markets for Australian red meats were increasing their demands for demonstration that pathogenic *E. coli* are not present on red meat products. Associated with those expectations was the likely requirement for the implementation of "interventions", *i.e*., additional processes during meat processing that could reduce levels of pathogenic *E. coli* that may be present on carcases. From previous MLA-funded research there was evidence that air chilling can kill *E. coli*, *i.e.*, such that chilling could be considered as an intervention. The benefits were expected to be enhanced public health, industry reputation and industry productivity by minimally expensive approaches to meet international standards.

Similarly, Australian vacuum-packaged meat primals are internationally recognised for an extended shelf-life. However, there was little science-based information to explain this advantage. Previous MLA-funded projects had measured changes in sensory and microbiological properties of vacuum-packaged beef primals from six Australian export abattoirs, showing significant differences in microbiological profiles. These findings led to a second phase of research, indicating that differences in bacterial growth profiles and communities were more likely due to properties of specific bacterial strains. Subsequent research showed that bacterial strains from abattoirs with low growth were more sensitive to pH, lactic acid and to low concentrations of glucose. These abattoirs also had a higher proportion of strains that produced inhibitory compounds against other bacteria, with greatest effects against bacteria of the same species. It was proposed that such interactions might limit the overall growth of the bacterial community, resulting in longer shelf-life and a higher quality. The aim was to better understand the factors that control these strain interactions to explain how the structure of spoilage communities is formed and potentially exploit that understanding to further extend red meat shelf life.

1.1 Sub-Project 1 – Antimicrobial interventions

The aim of this program was to understand and exploit the effects of carcase chilling (including both air and spray chilling) on *E. coli* stress physiology and survival, with a view to enabling chilling processes to be recognised as a pathogen "intervention", or to develop modifications of Australian chilling methods to enable them to be accepted by international markets as "interventions". Sub-project 1 involved 5 sub-programs:

- **Subprogram 1** – Determine behaviour of pathogenic *E. coli* cells after abrupt cold and osmotic shift
- **Subprogram 2** – Develop beef carcass treatments that exploit weaknesses of *E. coli*

exposed to cold and/or water activity (a_w) stress that occurs during Australian carcase chilling

- **Subprogram 3** – Determine if colanic acid produced by *E. coli* under chill-stress conditions protects them against those combined cold and water activity stresses
- **Subprogram 4 –** Further 'mine' the detailed proteomic and transcriptomic data developed in MLA-funded project A.MFS.0127 ("Effects of Chilling on the Survival of *Escherichia coli* on Carcasses") to aid development of chemical-based interventions for carcase decontamination
- **Subprogram 5** – Evaluate, at pilot scale, any novel intervention process developed from laboratory studies

1.2 Sub-Project 2 – Shelf-life extension

The aims of this Sub-project were to:

- Identify and model the influence of factors that promote the shelf-life of Australian lamb and beef through broth-based model studies:
    - as a function of extrinsic and intrinsic factors influencing the microbiology of packaged meat
    - as a function of interactions between specific microorganisms within the microbial community on Australian vacuum-packed red meats
- Validate model predictions through meat-based studies for industry-relevant products, packaging and storage environments, including evaluation in commercial supply chains into Middle Eastern and Asian markets
- Produce model interfaces that describe how changes in the meat environment affect:
    - intrinsic properties of meat
    - structure of the spoilage community

Sub-project 2 involved 4 sub-programs, and sets of objectives:

- **Subprogram 1** – to model factors that determine shelf-life of VP beef and lamb including incorporating inter-abattoir variations
- **Subprogram 2** – to model bacterial community influences on shelf-life including *in situ* interactions, spoilage community development, and inter-abattoir variations,
- **Subprogram 3** – to develop and validate predictive mathematical models for the shelf life of Australia vacuum-packed beef and lamb
- **Subprogram 4** – Develop user interfaces for those models

1.3 Sub-Project 3 – Expert advice

This program provided expert knowledge and opinion, and literature-based research, to Meat and Livestock Australia and its stakeholders. This was accomplished through participation of PROMEP staff in MLA-convened expert panels etc. including the Scientific Risk Management panel, and provision of *ad hoc* scientific advice on microbiological quality and safety or meat both to MLA and its industry stakeholders.

1.4 Sub-Project 4 – Knowledge Management

The objectives of sub-project 4 were to:

- produce (and disseminate to the industry) current and relevant food safety and quality information relevant to the Australian meat industry.
- produce a “Shelf-life Manual” (with annual updates), in collaboration with industry experts

These objectives were to be delivered to the Australian red meat industry *via*:

- regular provision, *via* email bulletins, of current information on food safety and spoilage
- preparation and periodic dissemination of the “MLA Meat Safety Digest”

1.5 Sub-Project 5 – Capability Development

The objectives of Program 5 were to:

- To increase scientific capabilities in the Australian meat industry in the areas of shelf-life science and through training of:
    - a new Post-Doctoral shelf-life/product preservation expert and
    - a new Post-Doctoral red meat microbial safety expert, with specific expertise in physiology and ecology of bacterial pathogens on red meat, to identify and respond to market challenges of the Australian red meat industry

in addition to training of other research students at Honours, Masters and PhD levels with expertise in microbiology relevant to Australian red meat products and processing.

2 Project Objectives

2.1 Overarching Objectives

The PROMEP was required to

- perform a significant body of research relevant to the microbial ecology and physiology of bacteria on red meats, and more specifically pertaining to:
    - the behaviour of *E. coli* (and similar pathogens) on meat and their control, and
    - the growth of bacteria that influence spoilage processes on meat during meat storage and distribution,
- develop personnel to work on the Project and related issues relevant to the Australian red meat sector, and
- develop a high level of expertise and consultancy capability to contribute to decisions regarding the direction of research and research investments, responding to industry issues, and providing advice to individual businesses in the Australian red meat industry.

2.2 Sub-Project Objectives

In addition to the overarching objectives identified above, objectives for each of the sub-projects were established and are detailed below.

2.2.1 Sub-Project 1 - Antimicrobial Interventions

This program of work aimed to develop underpinning science and observations to demonstrate that air chilling, or modifications of it, can be proven to be “interventions”, *i.e.*, methods that can reduce the levels and frequencies of pathogenic *E. coli* on carcases. More explicitly, Project 1 proposed to extensively study the effects of air chilling (and variations including spray chilling) on *E. coli* physiology and survival, to enable the process to be recognised as an “intervention”, or to develop modifications of Australian processing methods that would enable them to be accepted by international markets, and their regulatory authorities, as “interventions”.

2.2.2 Sub-Project 2 – Shelf-life Extension

This program was intended to consolidate and extend findings from previous MLA-funded projects that aimed to produce science-based information to explain the long shelf-life of Australian export primals compared to analogous products from other exporting nations. The objectives of the proposed research were to:

- provide science-based information to Australian red meat exporters that would enable them to manage and extend their market advantage, by
    - determining specific physicochemical factors that control the growth of spoilage bacteria
    - defining properties of bacterial strains in Australian beef that might provide an explanation of the observed extended shelf-life of Australian product

- provide industry with tools (specifically mathematical models) to monitor and manage processing and distribution conditions, and
- provide foundation-knowledge for innovation.

2.2.3 Sub-Project 3 – Expert Advice

The aims of this sub-project were to provide expertise, opinion and literature-based research to Meat and Livestock Australia and its stakeholders through participation in expert panels *etc*., including the Scientific Risk Management panel and provision of *ad hoc* scientific advice on microbiological quality and safety or meat, to assist in decision-making and problem solving on behalf of the industry, related to microbial quality and safety of meat.

2.2.4 Sub-Project 4 – Knowledge Management

The aims of this sub-project were to:

- produce (and disseminate) on a regular basis current and relevant food safety and quality information relevant to the Australian meat industry, and to
- produce a “Shelf-life Manual” (with annual updates), in collaboration with industry experts.

2.2.5 Sub-Project 5 – Capability Development

This sub-project was intended to increase scientific capabilities in the Australian meat industry through training of a new Post-Doctoral shelf-life expert, who would add capacity to improvements in shelf-life and another Post-Doctoral expert in red meat microbial safety. The development of these experts was intended to support and respond to market challenges and add capacity to maintain and improve the microbiological safety and shelf life of Australian red meat through innovations relevant to the Australian industry.

3 Approaches, Results and Discussion

The following section presents and briefly discusses the approaches used and the key Project results, outputs and outcomes according to each of the Sub-Projects. Full details are available in the relevant Project Milestone reports cited, as appropriate, in the following text. The Appendices to this report also provide details of publications and reports that further describe the work undertaken and its potential impact for the Australian red meat industry.

3.1 Sub-Project 1: Antimicrobial Interventions

3.1.1 Sub-Project 1: Where we were in 2013

Escherichia coli are bacteria that are endemic in the gastrointestinal tracts of cattle and may contaminate carcases at slaughter. Some groups of *E. coli*, called Shiga toxin-producing *E. coli* (STECs such as serotype O157:H7, O26, O111 etc.), can cause serious (*i.e*., potentially fatal) food-borne illness. Regulatory authorities in export markets were imposing increasingly stringent criteria concerning the frequency of their presence on meat products. The presence and detection of these pathogenic *E. coli* on Australian-produced meat, both for domestic and export trade, presents a threat to the reputation and profitability of the Australian meat industry.

Improved methods to control carcase contamination or to eliminate contaminating *E. coli* from carcases are continually being sought to reduce potential food safety risks and to satisfy export market requirements. In 2013, authorities in export markets - particularly in north America – seemed ready to demand that Australian processors implement "interventions" (*i.e*., microbial decontamination processes) to reduce levels and frequencies of a range of pathogenic *E. coli* (i.e., O157:H7 and the "big six" non-O157 STEC serotypes[1]) on red meats. Intervention technologies approved in USA, for example, were potentially costly to implement and administer in Australian abattoirs.

Although not specifically recognised as an intervention or 'Critical Control Point', there was evidence that air chilling of carcasses as employed in many Australian abattoirs could reduce the levels of microbial contamination on carcasses. Through an earlier MLA-funded project (A.MFS.127), *E. coli* had been shown to exhibit a complex pattern of responses including 'injury and recovery' phenomena when exposed to simultaneous changes in temperature and water activity, as occurs during air chilling of beef carcases. These 'injury and recovery' phenomena were hypothesised to provide a 'window of opportunity' of cell susceptibility during which a small, additional, environmental stress might induce cell death, rather than recovery. This suggested the potential for a mild, easily implemented, low cost *E. coli* 'intervention' for Australian meat processors. Further investigation using 'omics' technologies (*i.e*., proteomics and transcriptomics) to elucidate the mechanisms of the injury and recovery phenomena exhibited by *E. coli* suggested that *E. coli* on carcases may be

[1] In June 2012 the United States of America's Food Safety and Inspection Service (FSIS), a branch of USA's Department of Agriculture, classified six non-O157 STEC strains were as food adulterants effectively requiring that they not be detectable in raw meas. The serogroups nominated, in addition to serogroup O157, are O26, O45, O103, O111, O121, and O145.

more susceptible specifically to oxidative stress during particular stages of the chilling process. These observations suggested the prospect of a novel intervention involving use of low levels of an oxidant chemical at certain stages in the chilling process.

The aims of Sub-Project 1, then, were to:

- study the effects of carcase chilling on the physiology of *E. coli*, particularly during injury and recovery' phenomena, with a view to determining potential for its manipulation to permanently eliminate this organism from carcases during chilling, (including spray chilling processes); and

- to determine the efficacy of a range of oxidants during carcase chilling as an intervention against *E. coli* and related enteric pathogens (*e.g*., *Salmonella*).

3.1.2 Sub-Project 1: Where we are in 2016

Sub-Project 1: Outputs

A series of studies were conducted to address the aims of Sub-Project 1, including both basic and applied aspects. The results showed that addition of chlorine dioxide (ClO_2), an oxidant compound approved for food use, can achieve reductions in *E. coli* and *Salmonella* loads of from 2 to 4 $\log_{10}$cfu.g^{-1} (*i.e.*, 100 to 10,000-fold) during either air- or spray-chilling even when used at relatively low level (*e.g.*, ≥ 150 ppm). Detailed descriptions of the relevant studies have been presented in project Milestone reports and publications that are identified in the summary below and detailed in Section 5. Specific observations from the Sub-Project 1 are summarised below:

- *E. coli* exposed to combined chilling and desiccation stresses as occur during air chilling of carcases show a complex pattern of changes in population density including apparent inactivation (*i.e.*, cell death). This pattern of behaviour was shown to be due to a loss of culturability, rather than a loss of viability (Milestone Reports 1 and 4), and cannot be relied upon as an 'intervention'.

- At least two distinct subpopulations of *E. coli* are identifiable after exposure to rapid chilling and desiccation. These subpopulations are in different physiological states, and could have different levels of sensitivity to the stresses imposed. The apparent differences in the physiological states of cells may offer an explanation for the complex pattern of population behaviour observed (Milestone Report 5).

- Supporting the observations described above, changes in *E. coli* morphology were consistent with the observed (culturable) population changes upon imposition of combined chilling and desiccation stresses. This suggests that the morphology of cells could be linked to their ability to produce colonies on an enumeration medium, and that changes the underlying physiological changes causing the changes in cell morphology related to may provide insights into the complex patterns of population change observed (Milestone Report 4) that can be exploited to optimise the processes of inactivation.

- Comparison of the changes in *E. coli* populations subjected to the stress conditions relevant to air chilling and spray chilling revealed that only air chilling caused a loss of culturability, *i.e.*, air chilling imposes a greater physiological stress on *E. coli* than the spray chilling process (Milestone Reports 2 and 3).

- After exposure to combined chilling and desiccation stresses as occur during air chilling, the application of an oxidant compound (*i.e.*, ClO_2 at ≥150 ppm) caused significant (~1000-fold) and permanent reduction of *E. coli* populations but the timing of application was also found to be important to obtain maximum efficacy (Milestone Reports 1-3) and to minimise the use of chemicals (both in terms of cost and potential presence of residues)

- In laboratory–based experiments, application of ≥150 ppm chlorine dioxide to meat during a simulated spray chilling process caused ≥ 3-log reduction in *E. coli* levels (on the fat surface only) within 24 after commencing the simulated-chilling process but the process efficacy requires adequate contact time of ClO_2 (Milestone Report 4). Curiously, *E. coli* reductions on fat surfaces were much greater than reductions on lean surfaces. Importantly, however, trials involving ClO_2 treatments of ≥150 ppm on primals in *a pilot-scale facility at a commercial abattoir* achieved significant reductions of *E. coli* populations (up to 3 $\log_{10}$cfu.g^{-1}) on carcases during spray chilling and subsequent storage (at -1°C) (Milestone Report 4) on both lean and fat surfaces. (This also supports the suggestion that the susceptibility of *E. coli* to oxidative stress is specifically related to the effect of temperature (Milestone Report 3)).

- Application of ClO_2 (at 150 ppm, for 60 s) on meat before subjecting it to the process of air chilling or spray chilling was less effective against *E. coli* when compared to ClO_2 application (at the same concentration) during the simulated spray chilling regime (Milestone Report 5).

- Application of ≥200 ppm ClO_2 for at least the last 20 cycles during the spray chilling process used is required to achieve the maximum antimicrobial effects on meat (Milestone Report 5).

- A ClO_2-based intervention under optimised conditions (*see* immediately above) caused reductions of *E. coli* populations of up to 4 $\log_{10}$cfu.g^{-1} on carcase surfaces during chilling and subsequent storage (Milestone Report 6).

- Growth of the spoilage microbiota of vacuum-packed meat (measured as total aerobic count and/or lactic acid bacteria) was suppressed by ClO_2 treatment. This suggests that ClO_2 application during carcase chilling might lead an extension of shelf life of red meat (Milestone Report 6).

- *Salmonella* spp. exhibited a similar response to *E. coli* when exposed to ClO_2 during spray chilling indicating that the ClO_2-based intervention (initially developed for *E. coli*) is equally effective against *Salmonella* spp. (Milestone Reports 3-5).

Sub-Project 1: Outcomes

More complete and specific knowledge of how *E. coli* (and other enteric organisms) behave on carcases during chilling has been developed and is being exploited in the development of new interventions, including pilot scale trials, that show great potential for reducing E. coli prevalence on Australian meats. Use of this technology, and presentation of its scientific underpinning, will greatly assist in meeting the demands of export markets for Australian meats.

The transient reduction of *E. coli* population observed on carcases during air chilling appeared to be due to damage followed by recovery of damaged cells rather than growth of surviving cells. The observations support previous suggestions (*see* PRMS.043c, 2005) that testing for *E. coli* and other enteric organisms on carcasses too soon after chilling may lead to a falsely low estimate of contamination levels compared to analyses done later in the supply chain, even if the products were correctly stored at temperatures that precluded *E. coli* growth. However, it should be noted that the apparent loss of culturability that occurs during air chilling of carcases might not occur during the process of spray chilling where changes in temperature only occur. Nonetheless, interventions relevant to spray chilling are being developed and showing similar antimicrobial effects.

Application of ClO_2 during spray chilling of carcases has been developed as an antimicrobial intervention that is suitable for Australian processing conditions and existing infrastructure. This intervention has been shown to be effective against pathogenic *E. coli* and other enteric bacterial pathogens (*i.e.*, *Salmonella*) on carcases at a pilot scale. The proposed intervention also appears to have the potential to extend the shelf life of meat.

3.1.3 Sub-Project 1: Where to from here?

Studies in Sub-Project 1 have provided science-based information and preliminary demonstration of the potential for commercial application of an oxidising agent at specific times during carcase chilling to be a very effective antimicrobial intervention against *E. coli* and related pathogens (*e.g.*, *Salmonella*) on carcases, as well as having the potential to increase shelf life. To further develop this novel intervention as a practical, industry-ready, technology several basic aspects remain to be addressed.

Further studies should focus on optimisation of the proposed intervention. This includes evaluation of other oxidizing agents (*e.g.*, peroxyacetic acid, electrolysed water, ozone etc.) to augment chilling as an intervention and evaluation of their effects on different STECs, and other pathogens, found on red meat that have the potential to cause human illness.

Studies to assess the commercial feasibility of implementation of the intervention will also be required to facilitate commercial adoption of the intervention. This will require trials in a commercial facility, or pilot plant that closely simulates a commercial plant. Additionally, assessment of the intervention and evaluation of its cost of implementation, and ongoing costs, compared to the potential benefits of reduced detections of STECs in export products and reduced testing costs, and associated losses due to downgrading or rejection of product, and the potential benefit of increased shelf life is required. With regard to the latter,

rigorous studies will also be required to assess whether, or how, the intervention affects the quality and shelf-life of red meats.

It will also be necessary to compare the performance of the novel intervention on both beef meat and lamb meat because their microbiology is different. We suggest assessment of the efficacy of the intervention against pathogens on lamb, and also including its impact on lamb shelf-life. An intervention during lamb carcase processing may not only minimise the risks from pathogens, but may also reduce general microbial loads, or loads of 'specific spoilage organisms' that extend vacuum-packed shelf-life. Currently the shelf life of vacuum-packed lamb is considerably shorter than that of beef.

The results of the proposed subsequent studies will enable Australian meat processors to make decisions about implementation of the proposed intervention (including its variations) as a decontamination step for STECs and related pathogens during in their own plants and for their specific circumstances. The results of the proposed studies will enable processors to select treatments best suited to their operations and products (i.e., beef or lamb), and to evaluate the cost *vs.* benefits of implementation of that technology in their operations.

Finally, the relevant science/observations underpinning the novel intervention(s) and their efficacy against pathogens, or for extension of shelf-life, should be published in the peer-reviewed literature. This will facilitate acceptance of the technologies, and associated claims of increased product safety and shelf-life, by regulatory authorities and customers in export markets.

3.2 Sub-Project 2: Shelf-life Extension

3.2.1 Sub-Project 2: Where we were in 2013

The Australian red meat industry enjoys a unique position in the world marketplace. Its products are widely recognised for high-quality, safety, wholesomeness and long shelf-life. However, there was little science-based information to explain the longer than expected shelf-life. Previous MLA projects measured changes in sensory and microbiological properties of vacuum-packaged beef primals from six Australian export abattoirs, showing significant differences in microbiological profiles. However, comprehensive information regarding the effect of specific abattoirs on microbial community composition on meats and the response of these communities to varying storage temperature was lacking. As with beef, the impact of abattoir and storage temperature on microbial communities in vacuum packaged lamb primals was also largely unknown.

Subsequent research indicated that differences in bacterial growth profiles were more likely due to the properties of specific bacterial strains. Bacterial strains from abattoirs with observed lower levels of growth tended to be more sensitive to pH, lactic acid and to low concentrations of glucose. These abattoirs also had a higher proportion of strains that produced inhibitory compounds against other bacteria, with greatest effects against bacteria of the same species (MLA project A.MFS.0237). Consequently, it was proposed that such interactions might limit the overall growth of the bacterial communities, resulting in longer shelf-life.

Even though much had been reported about factors that influence shelf-life, it was not a complete understanding, particularly the complex interactions among intrinsic factors of meat, microbial communities and within the package environments. It was proposed if the factors that control these strain interactions could be characterised and understood it might be possible to explain how the structure of spoilage communities is formed and, from that, that a shelf-life predictive tool could be produced and validated for the industry to accurately analyse and design supply chains for specific markets. It was proposed that such a tool could reduce the uncertainty concerning quality and remaining shelf-life when meat products reach markets, especially where those markets apply microbiological and shelf-life limits exist at entry points, *e.g*., lamb into the Middle East and Asian markets.

Thus, the overarching objectives of Sub-Project 2 were to:

- provide science-based information to Australian red meat exporters that allow them to manage and extend their market advantage, by
    - determining specific physicochemical factors that control the growth of spoilage bacteria
    - defining properties of bacterial strains in Australian beef that extend shelf-life
- provide industry with tools (models) to monitor and manage distribution conditions
- provide foundation-knowledge for innovation.

3.2.2 Sub-Project 2: Where we are in 2016

<u>Sub-Project 2: Outputs</u>

Sub-program 2 started in September 2013. The work plan and research emphasis underwent several variations during the life of the PROMEP project reflecting industry needs and consultative committee's advice. Detailed description of the work and results achieved in this program are presented in milestone reports, and publications (*see* Appendix 1), resulting from the project. Major achievements are presented below:

- Shelf-life predictive tools have been developed for vacuum packaged beef and lamb primals (Milestone Reports 5 & 6)
- A β–version of shelf-life predictive tool interface has been developed, including graphical displays and outputs predicting remaining shelf-life at nominal storage temperatures, and is being used to provide decision-support to processors with atypical temperature history data for shipments. Additionally, the tool is being applied and refined in a complementary project with AMPC analysing commercial shipment temperature history data (Milestone Report 6).
- Models for the prediction of total viable counts and sensorially-detectable odour were developed using NIR and RAMAN spectroscopy for both vacuum packaged lamb and beef primals. The detectable odour correlated with spectral data similarly to TVCs, which indicated that model/prediction using either RAMAN or NIR analyses of these

criteria should be possible. The level of error derived from the validation curves strongly suggests that both the RAMAN and NIR models will need to be improved with greater sample numbers in future work. This would account for much of variability in the results during the model building stage thereby generating more accurate predictions (Milestone Report 6).

- Shelf-life trials of vacuum-packaged beef striploins and boneless lamb shoulders reinforced the longer reported shelf-life of Australian beef (around 200 days) and lamb (around 100 days) at low storage temperature (around -0.5°C). Shelf-lives of both lamb and beef decreased with increase in storage temperatures as expected. Differences among abattoirs were found with regard to TVC growth rates and shelf-lives of vacuum packaged beef and lamb (Milestone Report 5).

- Shelf-life trials of vacuum packaged bone-in lamb hind shank and boneless lamb shoulders processed at the same abattoir showed similar microbial (TVC and LAB) growth patterns and shelf-life at 8°C storage. Trials involving hind shank stored at lower temperatures are still underway at the time of reporting. These trials are assessing the shelf-life and microbial community structure of hind shank in comparison with boneless lamb shoulder (Milestone Report 6).

- Analyses of bacterial communities on product showed differences in community composition associated with beef (and lamb) from different abattoirs especially at the commencement of the storage period. Further, storage temperature greatly influenced the development of the climax bacterial communities and thus the time to onset of meat spoilage. Higher proportions and faster growth of bacterial species belonging to the family *Enterobacteriaceae* occurred at 8°C storage as compared to the Lactic Acid Bacteria (LAB). *Carnobacterium* spp. (a LAB) always dominated at lower temperatures (-0.5, 2, 4°C). Differences in microbial communities on lamb and beef processed at the same abattoir were also observed with lamb having a greater microbial diversity as compared to beef. Notable differences in community composition during storage at different temperatures were defined that were further investigated in the VP hind shank study mentioned above as well (Milestone Report 6).

- Preliminary models using ^{1}H-NMR spectral data on meat (lamb and beef) metabolites were able to discriminate lamb, beef fat, and beef lean, based on storage temperature and duration. In addition, the analyses could discriminate between abattoirs for the same meat type. The work on specific spectral features allowing identification of key discriminatory metabolites is now underway. Potential exists to identify specific metabolites across sample groups as rapid and objective indicators of quality loss (Milestone Report 6).

- Nitrite actidione polymyxin agar (at pH 5.5) as an alternative medium for lactic acid bacteria enumeration for vacuum packaged red meat showed comparable results to MRS medium with regard to selectivity and efficacy (Milestone Report 4).

- Comparison of alternative methods of TVC enumeration showed non-significant differences among methods studied for chilled vacuum packaged meat except in the early stages of trials where low temperature and longer incubation of TSA plates (-

1°C/21 days) resulted in significantly lower counts than TSA (25°C/4 days), TSA (20°C/5 days) and Petrifilms (25°C/4 days), indicating a relatively low proportion of psychrotrophs (i.e., that are able to grow at - 1°C) are present on freshly slaughtered meat. The comparable results observed for different enumeration methods in this study will provide industry with a choice for TVC enumeration protocol as well as an assurance for resultant TVC data being suitable for shelf-life predictive tool use (Milestone Report 6).

- Investigations on interactions among representative species of bacteria isolated from vacuum packaged beef were studied to understand mechanisms of bacterial community formation in vacuum packaged beef. Thirty-nine effectors and 20 target isolates were selected, representing 10 bacterial genera: *Carnobacterium*, *Pseudomonas*, *Hafnia*, *Serratia*, *Yersinia*, *Rahnella*, *Brochothrix*, *Bacillus*, *Leuconostoc* and *Staphylococcus*. Effector isolates were those that could either inhibit or stimulate the growth of a given target isolate. A number of inhibitory and stimulatory interactions were observed, with 28.6% of isolates inhibiting growth while 4.2% of effector strains promoted growth. The majority of *Pseudomonas* isolates antagonised growth of approximately one-half of target isolates. Two *Bacillus* spp. each inhibited most (80%) targets. Among lactic acid bacteria (LAB), *Carnobacterium maltaromaticum* inhibited a wider range of isolates compared to other LAB. The majority of effector isolates enhancing target isolate growth were Gram-negative, including *Pseudomonas* spp. and *Enterobacteriaceae* (Milestone Report 6).

- Studies to characterize factors mediating growth inhibition and promotion showed that bacterial interactions were mediated by diverse mechanisms. The inhibitory effect of two isolates of *Carnobacterium maltaromaticum* and one isolate of *Bacillus subtilis* was mediated by heat- and pH-stable proteinaceous substances, likely bacteriocins. In contrast, the inhibitory effect of three isolates of *Bacillus* sp., *Pseudomonas putida*, and *Pseudomonas* sp., on corresponding isolates of *Yersinia enterocolitica*, *C. maltaromaticum*, and *B. subtilis*, occurred only in the presence of live effector cells, yet was not contact-dependent. Compounds produced by *B. subtilis* and *Serratia* sp. that promoted the growth of *P. lundensis* were non-proteinaceous, and heat- and pH-stable (Milestone Report 6).

- The putative bacteriocin-mediated intraspecific inhibitory activity of two *Carnobacterium maltaromaticum* strains (D0h & D8c), was greatest at 15°C, followed by 7, -1, and 25°C, and higher under aerobic than anaerobic conditions. Agar supplemented with lactic acid and glucose increased inhibition. Inhibition was less at pH 6.5, compared to pH 5.5 and 6 (Milestone Report 6).

- pH was the factor most clearly associated with growth inhibition by *Carnobacterium maltaromaticum* D0h, compared to other factors including lactic acid, glucose, and atmosphere (aerobic/anaerobic). Lactic acid significantly reduced D0h inhibitor production at lower pH (5.5), possibly due to the relatively high concentration of undissociated lactic acid reducing cell growth and activity. Production was also influenced by levels of glucose and pH, where relatively higher concentration of glucose (5.55 mM) enhanced production at pH 6.5, whereas it decreased production at pH 5.5. Storage atmosphere composition did not significantly affect production of inhibitory compounds (Milestone Report 6).

Sub-Project 2: Outcomes

Predictive mathematical models to assess the influence of storage and transport temperatures actually experienced by products on their shelf-life, in comparison to the expected shelf life (*i.e.*, without sensorially detectable deterioration, either visual or olfactory) at -0.5°C, have been developed for Australian vacuum packaged beef and lamb primals. A β–version of the model interface is now being used to provide decision-support to processors with atypical temperature history data for shipments.

A better understanding of the factors affecting shelf-life of vacuum packaged red meat was obtained and reinforced that storage temperature and meat type (beef, lamb) have the clearest influence on the shelf life achieved. The basis of the unusually long shelf-life of Australian vacuum packaged meat, compared to that produced in other nations, appears to have a complex origin involving interactions amongst both abiotic (pH, glucose/lactic acid, storage temperature and packaging atmosphere) and biotic (microbial communities) factors. From the project those biotic factors relevant to vacuum packaged lamb and beef that influence their shelf-life were more clearly defined with the findings providing a foundation for manipulation of the microbiology (species present, interactions) to more consistently achieve longer shelf-life, or to extend it further, and allow development of more objective and rapid quality assessment approaches.

3.2.3 Sub-Project 2: Where to from here?

The benefits of having a science-based mathematical model that provides reliable predictions of shelf life, or remaining shelf life, based on product type and the product's actual, or expected, time-temperature history, has the potential to provide enormous financial benefits to the Australian export red meat industry. These benefits arise, for example, by being able to:

- identify and divert or discard product that has begun to spoil, due to mishandling before its expected shelf life has expired, without the need for microbiological testing;
- evaluate the quality status of product that has reduced shelf-life due to loss of temperature control at some point in the supply chain, and determine its fitness for sale to different markets;
- negotiate new science-based regulations for assessment of remaining shelf life of Australian product based on knowledge of the expected (demonstrated) shelf life at some reference temperature, and details of actual temperature histories of shipments, as interpreted by the model, and
- rationally design supply chain conditions (times and temperature) or products (e.g. with manipulated microbiology – *see below*) to reach and exploit new markets.

The results from Sub-Project 2 have suggested that it is feasible to have single growth models for vacuum packaged beef and lamb, respectively, and that can be used by any meat processor. Further, the results have suggested that those models could deliver the

above benefits if the models are accepted as reliable by Australian processors, and international customers and regulators.

The models, however, have not been extensively tested and, while based on rigorously gathered experimental data, also rely on several credible, but simplifying, assumptions. While the very limited number of independent datasets we have used to 'test' the models to date have suggested that the model provides predictions that agree with the observed quality of the product (within the expected variability range), the models require much more extensive evaluation before they can be used confidently by the Australian industry and its customers.

Due to the cost and time required to conduct shelf life/storage trials that simulate the time and temperature conditions for international shipments of Australian red meats (*i.e*., up to 6 – 8 months), two alternatives sets of studies are proposed as being necessary. Firstly, the initiatives currently underway to collect genuine industry data, both for good and for faulty transport chains and events, should be continued and expanded because they offer potential to cost-effectively, and in a much shorter amount of time, assess the performance of the models. Secondly targeted studies to monitor temperature and microbial loads through real international shipping routes relevant to the Australian export meat industry should be undertaken. This would, however, probably require high-level facilitation/co-ordination to obtain the needed co-operation between shipping services and international customers and regulators in relevant international markets to recover the temperature data but also to undertake microbiological testing after receipt of the shipment. The benefits of improvement of the model (if necessary) and eventual rigorous demonstration of its validity and limits of reliability would, as described above, greatly enhance market access and would justify the effort required. It would also enable innovation in those markets currently adhering to simplistic quality assessment based on microbial counts. Such criteria are typically irrelevant to vacuum-packed product (*i.e.*, due to the rapid initial growth of benign lactic acid bacteria that do not cause spoilage or significant product deterioration). Equally, the model's performance should be evaluated for a wider range of types of beef and lamb products.

Technologies for rapid detection of markers of eventual or incipient spoilage, whether specific spoilage organisms or specific metabolites produced by microbes present on the products, could also greatly assist in the management of product quality and its rapid evaluation. New technologies (*e.g*., NMR, and Raman and NIR spectroscopy) trialled in Sub-Project 2 have the potential to achieve this goal of rapid detection and before spoilage is evident. These approaches should be critically evaluated and further developed if the initial results are promising.

Studies performed in Sub-Project 2 also aimed to determine factors that influence microbial interactions on meat surfaces and how the growth rates, prevalence/abundance of individual species, and storage conditions lead to the observed spoilage microbiota and time to spoilage. We observed that some bacteria, especially those belonging to the genus *Carnobacterium*, inhibit growth of some other microbes present on vacuum packaged meat. These bacteria could potentially be manipulated to achieve extension of shelf-life. Systematic studies with the aim of identifying and quantifying the significance of these interactions under conditions relevant to vacuum-packed beef and lamb products should be continued and developed into a predictive tool (and software) that explains and quantifies rates and processes of spoilage of vacuum packaged beef and lamb in distribution networks

on the basis of species composition, storage conditions, etc. This would further aid decisions that require evaluation of remaining, or expected, shelf life. The ideal is to allow users to have the means to better manage red meat supply chain(s) so they can improve their market access and develop more agility.

We hypothesise that the long shelf-life of Australian vacuum packaged beef and lamb is due to a complex interaction of factors. Changes to processing practices may affect these factors and thus could affect the shelf-life of exports, especially in long supply chains. Further research, coordinated with studies suggested in Sub-Project 1 (microbial interventions), should be undertaken to assess the effect on shelf life and quality of implementation of new processes, in particular antimicrobial interventions.

Determination and documentation of the basis of the observed long shelf-life of Australian VP meats and knowledge should be made available in a way that enables the manipulation of the microbiology (species present, interactions), to guarantee the existing high quality standard and long shelf life of Australian red meats or to extend that shelf-life further. This will allow processors to better evaluate their own production systems and processes, and to identify where improvements might be achieved.

Finally, the relevant science/observations underpinning the current shelf-life model, its evaluation and any proposed refinements, should be subject to peer-review including, for example, publication in the international peer-reviewed literature. This will facilitate acceptance of the model, and its reliability, by regulatory authorities and customers in export markets.

3.3 Sub-Project 3: Expert Advice

This small Sub-Project was not a discovery-driven program of experiments but, rather, utilised Project staff to provide expertise, opinion, analytical skills and literature-based research to Meat and Livestock Australia and its stakeholders through participation in expert panels, *ad hoc* advice to meat industry stake-holders, etc. As such, a different set of headings and a different format is adopted for this Section.

During the term of the Project:

- Assoc. Prof Tom Ross served on, and provided advice and analysis of data and literature, to the MLA Scientific Risk Assessment panel. That analysis, together with advice from other experts on the Panel, was used to prioritise research needs, to contribute to decisions about research investments, to evaluate and advise on responses to potentially adverse reports affecting the perception of safety of Australian meat products, and to contribute to responses and submissions to regulatory authorities, etc.

- Support was provided by Assoc. Prof. Tom Ross and Dr. Mandeep Kaur to processors/exporters for analysis of temperature history data using the shelf-life model.

- Advice sought from, and provided by Assoc. Prof. Tom Ross, Drs. Mandeep Kaur and Chawalit (‘Jay’) Kocharunchitt, on a range of proposed shelf-life extension

technologies being considered for funding.

- Support provided to parallel AMPC project on interpretation of data records for temperatures during export shipments, and enabling refinement of the shelf-life model software.

- Presentation made as part of a workshop for the visit of Dr. Al Almanza, Deputy Under Secretary for Food Safety of the United States Department of Agriculture, and former Administrator of USDA's Food Safety and Inspection Service.

Ad hoc advice was also provided upon request to MLA staff, its contractors and industry personnel.

3.4 Sub-Project 4: Knowledge Management

This Sub-Project also was not a discovery-driven program of experiments but, rather, utilised Project staff to find, collate, interpret and disseminate to the Australian meat industry news and information about the microbiology of red meat products that had the potential to affect the Australian red meat industry, whether positively or adversely. As such, a different set of headings and a different format is adopted for this Section.

Sub-Project 4 aimed to produce and disseminate to the Australian meat industry current and relevant food safety and quality information. These aims were met, primarily by the PROMEP Project's post-doctoral fellows (Drs. Kaur and Kocharunchitt), through:

- Identification of current articles in journal and non-journal sources, reviewed and then distributed via FSC Knowledge Portal subscription service. The target frequency was 2-3 articles per week.

- Production and distribution of the quarterly MLA Meat Safety News Digest.

- Provision of material for the MLA Shelf Life Manual, first published in 2014 and its second edition, published in 2016.

In future, it is proposed that this service be continued but that the format be modified using newly available social media-type platforms to increase communication of Project progress, as well as alerting subscribers to the availability of new postings/information, rather than forwarding information that is unwanted.

3.5 Sub-Project 5: Capability development

This program aimed to increase scientific capabilities in the Australian meat industry through recruitment and training of a new Post-Doctoral shelf-life expert and a new Post-Doctoral red meat microbial safety expert who could support the industry to respond to market challenges and recognise and exploit new opportunities.

3.5.1 Sub-Project 5: Where we were in 2013

There was general awareness in the industry of a loss of scientific/technical expertise in the industry, particularly with the effective disbanding of CSIRO's Meat Industry Services group in Queensland and the retirement of key staff. To address the expertise gap, new positions were created as part of this Project and those staff mentored to engage with the meat industry and to acquire industry-relevant expertise and understanding as well as scientific knowledge and research training, and to improve their industry communication skills. Additionally, the PROMEP Project was to serve as a vehicle to train other young people in meat microbiology, with an industrial application perspective, by exposure to industry personnel and issues complementing their scientific and research training.

3.5.2 Sub-Project 5: Where we are in 2016

<u>Outputs and Outcomes</u>

Through this Project, two young researchers, Drs. Chawalit ('Jay') Kocharunchitt, and Mandeep Kaur, were recruited and received training in generic science research and communication skills, but also developed expertise in the science concerning red meat shelf life and microbiological safety, with particular emphasis on interventions that could be applied in Australian abattoirs to minimise the risk from STEC and related enteric pathogens.

Both Drs. Kaur and Kocharunchitt are increasingly recognised within the Australian meat industry as having relevant expertise in microbial meat safety and shelf life science and its application to industry issues. They enjoyed increasing contact with industry personnel through industry conference presentations, presentation of research results and subsequent discussions with the Project advisory committee, the industry newsletters and Meat Safety Digest, Shelf Life Manual etc., and intervention trials in commercial meat works (Dr. Kocharunchitt) and shelf life interpretation/advisory service (Dr. Kaur). In addition, the project funds have been leveraged to attract University of Tasmania/Australian Postgraduate Award scholarships for postgraduate students, and to support research expenses of other research students and to introduce them to meat microbiology research issues and the industry more broadly.

In summary:

- Two post-doctoral research associates (Kaur, Kocharunchitt), three PhD scholars (Gardner, Porteus and Zhang) and a number of Masters and Honours students have been trained in red meat shelf-life and physiology and ecology of bacterial pathogens on red meat. The PhD scholars' stipends were funded from sources other than MLA, AMPC or MINTRAC.

- Postdoctoral research associates and PhD scholars visited and engaged with processors any times both at industry meetings and conferences, Consultative Group meetings, factory visits, especially with those companies in Tasmania that provided facilities for in-plant studies undertaken as part of the project to date.

- Postdoctoral research associates attended and presented their work at 2014 and 2015 MINTRAC Meat Inspection and Quality Assurance conferences.

- Postdoctoral research associates regularly attended and presented their progress at consultative group meetings.
- Postdoctoral research associates attended the MSA Meat Science Course, Nov 2015.
- Postdoctoral research associate Dr. Kaur attended shelf-life expert panel and industry meetings in 2014 and the Chilled red meat shelf-life tools – workshop in 2015.
- Postdoctoral research associate Dr. Kocharunchitt attended and presented at the STEC symposium, 2015, in Wagga Wagga, NSW.
- Postdoctoral research associate and PhD scholars attended a training course in meat processing for hygiene and quality (delivered by John Sumner), April 2014.
- Postdoctoral research associate Dr. Kocharunchitt has been working closely with a commercial processor to conduct trials of the novel intervention technology under 'industry' conditions.

3.5.3 Sub-Project 5: Where to from here?

It is suggested that the research project and training of the two current postgraduate research scholars (Gardner, Porteus) should continue, as should the training and industry mentoring of the two post-doctoral research associates (Drs. Kaur and Kocharunchitt). The University of Tasmania will continue to provide training to these post-doctoral research associates in teaching and communication skills, as well as providing the research infrastructure for future meat microbiology related research programs. Meat industry funding for the research proposed in the previous sections will be sought and, if successful, can be leveraged to attract additional research students, and scholarships/stipends to support them. This will enable human capital development by enabling them to conduct research relevant to the Australian meat industry and to train them to be ready to contribute to the ongoing research and technical needs of the industry. The University of Tasmania has been successful in large Australian Research Council-funded programs aimed specifically at training industry-savvy researchers. The lessons learned from those programs can be extended to enhance the training of research students working on meat industry funded research programs.

4 Conclusions/Recommendations

Conclusions and recommendations for future research and activities to support the Australian red meat industry in understanding, controlling and exploiting the microbiology of meats were described in previous sections. Conclusions from the work undertaken in the PROMEP Project were described in Sections 3.1.2, 3.2.2 and 3.5.2 (under the headings “Where we are in 2016”) while Sections 3.1.3, 3.2.3 and 3.5.3 offer recommendations for further work and mechanisms for industry uptake of the results (under the headings “Where to from here?”)

5 Appendix: Communications resulting from G.MFS.0289

5.1 Industry Publications

- Shelf-life of Australian red meat ("The Shelf-life Manual") - two editions (2014 & 2016).
- Quarterly MLA Meat Safety News Digests.
- Updates on the review of current and emerging interventions in Australian meat processing operations, regularly disseminated *via* email distribution service.

5.2 Theses and Dissertations

- Zhang, P. (2016). Interactions between bacterial strains isolated from vacuum packaged Australian beef primals. PhD Thesis, University of Tasmania, Hobart, Australia.
- Ismael, H. (2014). Response of *Salmonella* to carcase chilling in comparison to *Escherichia coli*, Master by Coursework Thesis, University of Tasmania, Hobart, Australia.
- Phunnathorn, P. (2014). VBNC state of *Escherichia coli* and *Salmonella* under combined chilling and water activity stresses, Master by Coursework Thesis, University of Tasmania, Hobart, Australia.
- Samuel, S. (2014). Comparing low water activity tolerance/persistence of *Salmonella* spp. and related Enterobacteriaceae. BSc (Hons) Thesis, University of Tasmania, Hobart, Australia.

5.3 Journal Articles - Published

- Kocharunchitt, C., King, T., Gobius, K., Bowman, J.P., and Ross, T. (2014). Global genome response of *Escherichia coli* O157:H7 Sakai during dynamic changes in growth kinetics induced by an abrupt downshift in water activity, *PLoS ONE* 9(3), e90422.
- King, T., Kocharunchitt, C., Gobius, K., Bowman, J.P., and Ross, T. (2014). Global genome response of Escherichia coli O157:H7 Sakai during dynamic changes in growth kinetics induced by an abrupt temperature downshift, *PLoS ONE*, 9(6), e99627.
- Zhang P, Baranyi J, Tamplin M. (2015). Inter-strain interactions between bacteria isolated from vacuum-packaged refrigerated beef. *Applied and Environmental Microbiology*, 81(8): 2753-61.
- Mellefont, L.A., Kocharunchitt, C., and Ross, T. (2015). Combined effect of chilling and desiccation on survival of *Escherichia coli* suggests a transient loss of culturability, *International Journal of Food Microbiology,* 208, 1-10.

5.4 Journal Articles – in Preparation or under Revision

- Zhang, P., Kaur, M., Bowman, J.P. and Tamplin, M. (2016). Effect of related environmental factors of vacuum-packaged beef on antibacterial compound production by *Carnobacterium maltaromaticum* (currently under revision for submission to International Journal of Food Microbiology).
- King, T., Kocharunchitt, C., Gobius, K., Bowman, J.P. and Ross, T. Molecular response of *Escherichia coli* O157:H7 Sakai during dynamic changes in growth kinetics induced by an abrupt downshift in temperature and water activity (currently under revision for resubmission to *Molecular and Cellular Proteomics*).
- Kaur *et al.* Inter-abattoir variation in bacterial community structure of vacuum-packaged beef primals and relationships to meat type and refrigerated storage.
- Porteus, B.F., Kocharunchitt, C., Bowman, J.P., Mellefont, L. and Ross, T. Oxidants targeting the reduction of *Escherichia coli* O157:H7 during carcase chilling.
- Kocharunchitt, C., Gardner, T., Mellefont, L., Bowman, J.P. and Ross, T. Viable but non-culturable state of *Escherichia coli* as induced by combined cold and water activity stresses.
- Kaur *et al*. Core microbial communities of VP Australian red meat, their spatial and temporal dynamics during storage at different temperature.
- Kaur *et al*. Potential and comparison of spectroscopic techniques in meat industry for shelf-life prediction of beef and lamb.
- Kaur *et al*. Monitoring metabolites in Australian VP beef and lamb stored at different temperatures.
- Zhang *et al*. Preliminary characterization of interaction mechanism among bacteria from vacuum-packaged refrigerated beef.

5.5 Conferences Presentations

- Kaur, M., Ross T., Tamplin M. and Bowman J.P. (2015). Bacterial populations - how they change during the storage of VP primals? Annual MINTRAC meat inspection and quality assurance conference, Sunshine Coast, Australia (invited presentation).
- Kocharunchitt, C. and Ross, T. (2015). New intervention during carcase processing, STEC symposium, 9-10 March 2015, Wagga Wagga, Australia (invited presentation).
- Kocharunchitt, C. and Ross, T. (2015). Chilling as an intervention: an update, Annual MINTRAC Meat Inspection and Quality Assurance Conference, 21-22 October 2015, Gold Coast, Australia (invited presentation).
- Tamplin, M., Kaur M., Powell S. and Bowman J.P. (2015). Metagenomics reveals microbial communities in vacuum-packed meats. International Association for Food Protection annual meeting, 25 – 28 July, Portland, USA.
- Kaur, M. and Ross T. (2014). Power failure – what do we do now? A new software tool for predicting shelf-life of vacuum packed primals. Annual MINTRAC meat inspection and quality assurance conference, Sydney, Australia (invited presentation).
- Kocharunchitt, C., Porteus, B.F., Bowman, J.P., Jenson, I. and Ross, T. (2014). Towards development of effective interventions to eliminate Escherichia coli during

carcass chilling, International Association for Food Protection Annual Meeting, 3-6 August 2014, Indiana, USA.

- Kocharunchitt, C., Porteus, B.F. and Ross, T. (2014) Chilling as an intervention, Annual MINTRAC Meat Inspection and Quality Assurance Conference, 17-18 September 2014, Sydney, Australia (invited presentation).
- Zhang, P., Baranyi, J. and Tamplin, M. (2014) Inter-strain interactions among bacteria isolated from Australian vacuum-packaged refrigerated beef. International Association for Food Safety, European Symposium on Food Safety, Budapest, Hungary.
- Zhang, P., Ross T., Bowman, J., Williams, M. and Tamplin, M. (2013) Inter-strain inhibitory activity within the bacterial community of Australian vacuum-packaged beef. University of Tasmania Graduate Research – Sharing Excellence in Research Conference, Hobart, Australia.
- Zhang, P., Williams, M., Ross, T., Bowman, J. and Tamplin, M. (2013) Inter-strain inhibitory activity within the bacterial community of Australian vacuum-packaged beef. Australian Society of Microbiology annual scientific meeting, July, Adelaide, Australia.