Overseas Travel Report

Seafood CRC Research Travel Grant

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

National Institute of Health Sciences

Japan

October 2011

Damian May

South Australian Research & Development Institute

Government of South Australia

CRC AUSTRALIA

Australian Government
Fisheries Research and Development Corporation

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Contents

ISBN: 978-1-925982-48-0

Executive Summary

International limits for vibrios in seafood are increasingly being mandated. This means that Australian seafood will be subjected to increased testing regimes to meet market access requirements. Furthermore, the FAO/WHO are currently in the process of deciding on what methods are suitable for this purpose. Professor Mitsuaki Nishibuchi is an integral part of this process within Codex.

The objective of this travel was to visit and learn from Professor Mitsuaki Nishibuchi at Kyoto University, Kyoto, Japan. The information gathered will be useful across a range of projects funded by the Australian Seafood CRC (AS CRC). In particular, the use of immuno-magnetic separation (IMS) and Loop-mediated Isothermal Amplification (LAMP) for the sensitive detection of pathogenic *Vibrio parahaemolyticus* in seafood products was demonstrated and practised. Professor Nishibuchi has also kindly offered to provide various *V. parahaemolyticus* isolates and a novel natural food sanitiser for use in AS CRC projects. A number of potential issues were identified and discussed to develop an on-going long term collaborative link with Prof. Nishibuchi's laboratory. This is strategically important for Australia due to the large volume of seafood Japan imports annually (world's second largest importer of seafood).

Outcomes Achieved to Date

- Knowledge of novel methods for the sensitive detection of pathogenic *V. parahaemolyticus* in seafood will be a direct benefit to the completion of AS CRC project 2009-787.
- Dr Damian May has been trained in the use and application of these novel methods and has had key discussions with a member of the Codex Vibrio Working Group.
- A professional network has been developed with a world leader in seafood safety, particularly with respect to *V. parahaemolyticus* contamination.
- Awareness of the AS CRC in international research groups has been increased,

Outputs Developed as a Result of Travel

- Training in a novel method for the sensitive detection of pathogenic *V. parahaemolyticus*, K-antigen screening and identification of pandemic clones of *V. parahaemolyticus* will be beneficial for current and future AS CRC projects.
- The provision of various *V. parahaemolyticus* isolates and a novel natural food sanitiser will be beneficial for current and future AS CRC projects.

Travel Objectives

Currently in Australia there is significant expertise and capability for microbial modelling in seafood (e.g. UTas). However, technical method development expertise to assist Australian producers to meet future changes to national and international regulations is lacking. The purpose of this travel was to assist SARDI to develop this capability.

Background and Need

Technical method development expertise to assist seafood producers to meet future changes to national and international microbial limits regulations is currently lacking in Australia. The travel to meet with Professor Mitsuaki Nishibuchi and learn new methods being developed in his laboratory to detect potentially pathogenic strains of *Vibrio parahaemolyticus* has assisted SARDI to develop this capability. This capability will be beneficial for the successful completion of AS-CRC funded project number 2009-787. Such knowledge could also potentially assist in future projects, including a project application currently being considered by the FRDC and work being considered involving rock lobsters.

Results

A travel itinerary is provided in Appendix 1. Dr May was trained in immuno-magnetic separation (IMS) and Loop Mediated Isothermal Amplification Assay (LAMP) method for the detection of *V. parahaemolyticus*. Discussions were also held regarding potential collaboration opportunities between SARDI Food Safety and Prof. Nishibuchi's laboratory. Prof. Nishibuchi has agreed to provide type strains to use as controls and for method validation for AS CRC project number 2009-787. This is essential to fully validate methods and gain regulatory acceptance of them.

Professor Nishibuchi stated that he is very interested in collaborating with the AS CRC if the opportunity arose. Particular areas of interest were determining whether the IMS/LAMP assay is useful for detecting pathogenic *V. parahaemolyticus* in Australian seafood and working to map the distribution and prevalence patterns of pathogenic *Vibrio* spp. This relates particularly to *V. parahaemolyticus* in Australian seafood production areas, especially molluscan bivalve species. Results from the Centre for South East Asian Studies (CSEAS) at Kyoto University have shown that the method can be at least 10-fold more sensitive than traditional molecular detection methods. Consequently, this method has been put forward by Prof. Nishibuchi at the FAO/WHO Vibrio working group meeting 17-21 October 2011, as an improved method for the detection and enumeration of toxigenic *V. parahaemolyticus*.

Prof. Nishibuchi has also developed and patented a natural food sanitiser based on powdered scallop shell. He has agreed to supply SARDI Food Safety with a bottle of this sanitiser which may be trialled in ongoing SARDI Food Safety projects. This has potential applications in extending the shelf life of packaged seafood and sanitation of food preparation surfaces.

Overview of the Immunomagnetic Separation and LoopAmp Method

Professor Nishibuchi has many years experience investigating the safety of seafood, especially with regard to *Vibrio* species. He currently works at the CSEAS and sits on the Codex Alimentarius Working Group on Pathogenic Marine *Vibrio* spp. His recent research has involved the development of a new technique involving a combination of IMS and LAMP analysis to detect pathogenic strains of *V. parahaemolyticus* in seafood products (i.e. those strains that are either tdh^+ or trh^+).

The IMS/LAMP method involves a number of steps that improve the specificity and sensitivity of detection for toxigenic *V. parahaemolyticus* isolates in molluscan bivalves. However, it is likely to be just as effective in detecting and enumerating these organisms in other seafood products (e.g. prawns). The method uses a combination of selective growth

media and IMS to minimise growth inhibition from competing organisms (e.g. *V. alginolyticus*) within the growth media. LAMP is then used to detect the presence of tdh and/or trh genes within the MPN tubes (Figure 1).

LAMP is a newly developed molecular method that is highly specific and has the potential to be easily applied, especially in developing countries with limited resources. The only equipment required is a pipette and a heating block and the reagents can potentially be lyophilized and transported at room temperature. Furthermore, although being based on a DNA amplification system, it is not necessary to use agarose gel electrophoresis to visualise the results as a positive reaction is indicated by precipitate within the reaction tube. Real-time amplification can also be visualised by measuring the turbidity of the reaction mix or by changing the reaction conditions to include fluorescent dyes.

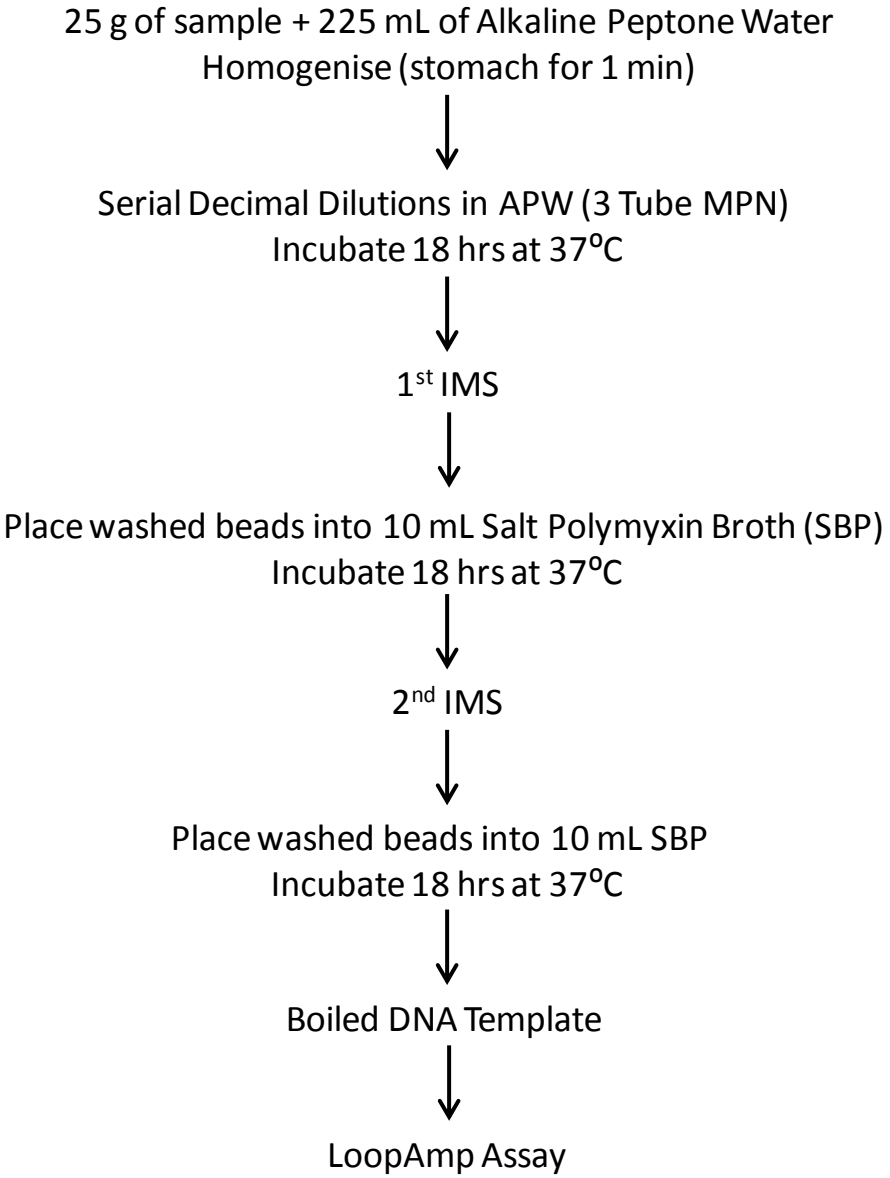

Figure 1: Work flow process of IMS/LAMP method

Outcomes

- Knowledge of novel methods for the sensitive detection of pathogenic *V. parahaemolyticus* in seafood will be a direct benefit to the completion of AS CRC project 2009-787.
- Dr Damian May has been trained in the use and application of these novel methods and has had key discussions with a member of the Codex Vibrio Working Group.
- A professional network has been developed with a world leader in seafood safety, particularly with respect to *V. parahaemolyticus* contamination.
- Awareness of the AS CRC in international research groups has been increased.

Recommendations

It is recommended that a member of Prof. Nishibuchi's staff visit SARDI Food Safety to assist in the set up of the IMS-LoopAmp method for the detection of *V. parahaemolyticus* in Australian seafood products. This is important given that Japan is a key seafood trading partner. Furthermore, the LAMP method has a number of advantages in that it is more sensitive and has the potential to be useful in developing countries due to the limited infrastructure requirements and that it may be lyophilised and transported at room temperature.

It is the author's opinion that if the AS CRC/FRDC are interested in further mapping the prevalence, and hence risk, of pathogenic marine *Vibrio* spp surrounding molluscan bivalve growing and harvesting areas, a collaborative project between CSEAS and the AS CRC would be ideal.

Appendix 1: Itinerary

Friday, 30 September 2011	Depart Adelaide
Saturday, 01 October 2011	Arrive Kyoto, Japan. Initial discussions with Prof Nishibuchi
Monday, 03 October 2011	Discussions with Prof Nishibuchi surrounding use of LoopAmp for detection of toxigenic *V. parahaemolyticus* in bivalves
	Begin processing spiked bloody clam samples using LoopAmp method combined with MPN
	Further reading material and instruction provided
Tuesday, 04 October 2011	Continue processing of spiked bloody clam samples
Wednesday, 05 October 2011	Continue processing of spiked bloody clam samples
Thursday, 06 October 2011	Finish processing of spiked bloody clam samples
	Discussion with Prof Nishibuchi surrounding future collaboration opportunities
Friday, 07 October 2011	Characterisation of Australian *V. parahaemolyticus* isolates provided by SARDI using Prof. Nishibuchi in house methods (PCR for toxR, tdh, trh and pandemic strains, immune-agglutination for K-antigens
Tuesday, 11 October, 2011	Begin processing bloody clams spiked with Australian tdh^+ *V. parahaemolyticus* isolate
	Further discussion with Prof Nishibuchi regarding collaboration opportunities
Wednesday, 12 October 2011	Continue processing of spiked bloody clam samples
Thursday, 13 October 2011	Return to Adelaide