# The biotransformation of glucosinolates

## A bacterial perspective

A thesis presented in partial fulfilment of the

requirements for the degree of

PhD

in Food Technology

At Massey University, Manawatu

New Zealand

Jane Adair Mullaney

2013

## Abstract

Epidemiological studies have shown an association between the consumption of cruciferous vegetables and a reduced risk of certain types of cancers, in particular, colon, bladder and bowel. This is thought to be due to the conversion of glucosinolates present in the vegetables into bioactive isothiocyanates which in turn stimulate a host response involving detoxification pathways. Conversion of glucosinolates is catalysed by the enzyme myrosinase, which is co-produced by the plant but stored in separate tissue compartments and brought together when the tissue is damaged. Myrosinase activity can be reduced or lost during storage of vegetables and is often inactivated by cooking. However, in the absence of active plant myrosinase, bacteria are capable of carrying out a myrosinase-like activity on glucosinolates producing isothiocyanates or nitriles.

This thesis examined the bacterial biotransformation of glucosinolates by two lactic acid bacteria and *Escherichia coli* Nissle 1917, all three considered beneficial bacteria. They were compared with a known glucosinolate-metabolising gut bacterium *Enterobacter cloacae in vitro, in vivo* and *ex vivo* to determine the bacterial responses to glucosinolates and what the products of their glucosinolate metabolism might be. Exposure of the host to beneficial bacteria and glucosinolates resulted in induction of the host detoxification enzyme quinone reductase which was elevated in bladder tissue for all dietary intervention groups consuming glucosinolates and beneficial bacteria, alone or combined.

*In vitro*, Nissle reduced alkylsulfinyl glucosinolates and their hydrolysis products through redox to alkylthiols and *in vivo*, the host microbiota responded similarly. *In vivo*, the host response to alkylthiol nitriles was to oxidise these back again to alkylsulfinyl nitriles and oxidise further resulting in some nitriles being irreversibly oxidised to the sulfone.

The association between consumption of cruciferous vegetables and reduced cancer of the colon, bladder and bowel is only that; an association. However, the results of this thesis demonstrated that bladder tissue was affected by beneficial bacteria and glucosinolates alone or together, which suggests that both exert a protective effect that could be measured by elevated quinone reductase, a biomarker for cancer chemoprevention.

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There is a saying that “once you know something you cant not know it any more”. I believe in the health benefits of combining broccoli with beneficial bacteria.

I would like to express my gratitude to Massey University who awarded me a doctoral scholarship and also to Riddet Institute who in collaboration with AgResearch and Plant and Food Research chose me for this project.

I also wish to express my heartfelt thanks and appreciation to my supervisors Julian Heyes (Massey), Juliet Ansell (Plant & Food) and Bill Kelly (AgResearch).

During my PhD, I was part of the Food and Innovation Portfolio at Plant and Food Research Institute and I would like to express my appreciation to Doug Rosendale in this group who supported and mentored me as a colleague throughout and was nearly always available for discussions on experimental design and being a sounding board for ideas and concepts (see the last figure in the Appendix 4X). Another member of this group I wish to thank personally is Halina Stoklosinski for all her technical assistance with GC, (short chain fatty acids analysis) and for her help with the discriminant analyses in chapter 8.

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This work is dedicated to

**Graeme and Daphne Brockelbank**

and I did it all because of Paul

# Abbreviations

| | |
|---|---|
| Allyl isothiocyanate | AITC |
| Antioxidant response element | ARE |
| Benzyl isothiocyanate | BITC |
| Broccoli seed powder | BSP |
| Cytochrome P450 | Cyp450 |
| de Man Rogosa and Sharpe media | MRS |
| Dichloromethane | DCM |
| Glucose 6-phosphate dehydrogenase | G6PH |
| Glucosinolate | GSL |
| Glutathione | GSH |
| Glutathione S Transferase | GST |
| Histone deacetylases | HDAC |
| Glycoside family 1 | GH1 |
| Isothoicyanate | ITC |
| Kelch-like ECH-associated Protein 1 | Keap1 |
| Nicotinamide adenine dinucleotide phosphate | NADP |
| Nicotinamide adenine dinucleotide phosphate-oxidase | NADPH |
| Nuclear Magnetic Resonance spectroscopy | NMR |
| Nuclear response factor 2 | Nrf2 |
| Quinone reductase | QR |
| Reactive oxygen species | ROS |
| Reinforced clostridia media | RCM |

# Table of Contents

## Chapter One

## Chapter Two

## Chapter Three



## Chapter Four

## Chapter Five

## Chapter Six



## Chapter Seven

## Chapter Eight

# Appendix B

DRC16 Statement of Contribution (2)

1. Lactic acid bacteria convert glucosinolates to nitriles efficiently yet differently to Enterobacteriaceae (Journal of Agricultural and Food Chemistry, DOI: 10.1021/jf305442j)
2. The biotransformation of glucosinolates – a bacterial perspective (CAB Reviews in revision as at March 10 2013)

# List of Tables

# List of Figures

# APPENDIX A Figures and Tables

Ethics and ERMA approval was obtained for work within this thesis as follows:

- Gene cloning and expression: ERMA No. 200814
- Animal trial: Animal ethics approval No. AE12354