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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.

From the Chemistry and Bioactives team I would like to thank Tony McGhie (LC-QTOF-HRMS) and Martin Hunt (GC-MS) for all of their analytical chemistry expertise and technical assistance and Daryl Rowan and Adam Matich for their advice, shared knowledge and discussion along the way.

From the biometrician team I would like to thank Duncan Hedderley and Andrew MacKenzie for all of their help with the statistical analyses

Finally I would like to thank everyone from the Gut Nutrition group in Palmerston North and the support staff at FISC who looked after me.

Thanks go out also to my family and friends who got me here this far and a massive thank you to my husband Rory Mullaney and daughter Caitlin Atwood. They are the key people in my life, I love them and thank them for putting up with the demands of doing a PhD. Rory has been fully supportive of me throughout this PhD and just makes me a better person than I am. There is no scientific evidence that I will be easier to live with now but anecdotal evidence suggests I will be. My Mum and Dad Daphne and Graeme Brockelbank of course get the credit for me being me.

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
Isothiocyanate	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

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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)

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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

