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Antimicrobial Activity of Functional Food Ingredients Focusing on Manuka Honey
Action against *Escherichia coli*.

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
in
Engineering and Technology

at Massey University, Auckland
New Zealand

Douglas Ian Rosendale
2009
Abstract

The goal of this research was to identify functional food ingredients/ingredient combinations able to manage the growth of intestinal microorganisms, and to elucidate the mechanisms of action of the ingredient(s).

By developing a high-throughput *in vitro* microbial growth assay, a variety of pre-selected ingredients were screened against a panel of bacteria. Manuka honey UMF(TM) 20+ and BroccoSprouts(R) were identified as the most effective at managing microbial growth, alone and in combination. Manuka honey was particularly effective at increasing probiotic growth and decreasing pathogen growth. Testing of these two ingredients progressed to an animal feeding trial. Here, contrary to the *in vitro* results, it was found that no significant *in vivo* effects were observed.

All honeys are known to be antimicrobial by virtue of bee-derived hydrogen peroxide, honey sugar-derived osmotic effects, and the contribution of low pH and the other bioactive compounds present, hence their historical usage as an antiseptic wound dressing. The *in vitro* antimicrobial effect of manuka honey has currently been the subject of much investigation, primarily focusing on the Unique Manuka Factor (UMF), recently identified as methylglyoxal, a known antimicrobial agent. This work has taken the novel approach of examining the effects of all of the manuka honey antimicrobial constituents together against *Escherichia coli*, in order to fully establish the contribution of these factors to the observed *in vitro* antimicrobial effects.

For the first time, it has been demonstrated that the *in vitro* antimicrobial activity of manuka honey is primarily due to a combination of osmotically active sugars and methylglyoxal, both in a dose-dependent manner, in a complex relationship with pH, aeration and other factors. Interestingly, the manuka honey was revealed to prevent the antimicrobial action of peroxide, and that whilst methylglyoxal prevented *E. coli* growth at the highest honey doses tested, at low concentrations the osmotically active sugars were the dominant growth-limiting factors.

Contrary to the literature, it was discovered that methylglyoxal does not kill *E. coli*, but merely extended the lag phase of the organism. In conjunction with the lack of antimicrobial activity *in vivo*, this is a landmark discovery in the field of manuka honey research, as it implies that the value of manuka honey lies more towards wound dressing applications and gastric health than as a dietary supplement for intestinal health.
Acknowledgments

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CHAPTER ONE. INTRODUCTION

Overview
1.1 The Gastrointestinal Tract
1.2 Gastrointestinal Microflora
1.3 The Gastrointestinal Defences
   1.3.1 Gastrointestinal Physical Defences
      1.3.1a Mucins
      1.3.1b Epithelial Glycocalyx
      1.3.1c Defensins
1.4 Breakdown of Gut Defensive Function
   1.4.1 Bacterial Pathogens
      1.4.1a Helicobacter pylori
      1.4.1b Escherichia coli
      1.4.1c Salmonella and Yersinia
      1.4.1d Listeria and Shigella
      1.4.1e Staphylococcus
      1.4.1f Clostridia
1.4.2 Parasites 13
1.4.3 Dietary Compounds 14
1.4.4 Antibiotics 15
1.4.5 Alterations in Immune Competency 15

1.5 Promoting Gut Health 15

1.5.1 Probiotics 15
  1.5.1a Lactic Acid Bacteria 16
  1.5.1b Health Benefits from Administered LAB 18
  1.5.1c Immunomodulation by LAB 18
  1.5.1d Antagonisation of Pathogens by LAB 19

1.5.2 Prebiotics 21

1.5.3 Synbiotics 21

1.5.4 Functional Foods 22
  1.5.4a Marketing functional foods 22
  1.5.4b Regulating functional food claims 22
  1.5.4c Functional foods programme of which this thesis forms a part 23
  1.5.4d Functional Food Ingredients Used in this Study 25

1.6 Mechanisms of action of natural antimicrobial agents 25

1.7 Aims of this thesis 29

CHAPTER TWO. MATERIALS AND METHODS 30

2.1 Materials 30
  2.1.1 Chemicals and media 30
  2.1.2 Enzymes 31
  2.1.3 Organisms 31
    2.1.3.1 Animals 31
    2.1.3.2 Bacteria 32
    2.1.3.3 Mammalian Cell Culture 32
2.1.4 Reagent kits 33
2.1.5 Gases 33
2.1.6 Other materials 34

2.2 Methods 34

2.2.1 Microbial methods 34
  2.2.1.1 Sterilisation 34
  2.2.1.2 Storage of Bacteria 35
  2.2.1.3 Recovery of Bacteria 35
  2.2.1.4 Broth Culture 35
  2.2.1.5 Maintenance of Anaerobic Conditions 35

2.2.2 Mammalian cell culture methods 36
  2.2.2.1 Sterilisation 36
  2.2.2.2 Storage 36
  2.2.2.3 Recovery 36
  2.2.2.4 Growth 36
  2.2.2.5 Isolation of Pig White Blood Cells (pWBCs) 37

2.2.3 General methods 37
  2.2.3.1 Extraction of Functional Food Ingredients 37
  2.2.3.2 Ingredient Extract Concentration 38

2.2.4 Analytical methods 38
  2.2.4.1 Antimicrobial Assays 38
  2.2.4.2 Protein Estimation 39
  2.2.4.3 Measurement of Phagocytosis 40
  2.2.4.4 Determination of Methylglyoxal 41
  2.2.4.5 Determination of Short Chain Fatty Acids 42
  2.2.4.6 Measurement of Water Activity ($a_w$) 42
  2.2.4.7 Assay of Cell Viability or Respiration 43
  2.2.4.8 Statistical Analyses 44
CHAPTER FIVE. MANUKA HONEY ANTIMICROBIAL ACTIVITY: Contributions by Potential Active Factors

5.1 Introduction 79
5.2 Hydrogen peroxide. 81
   5.2.1 Introduction 81
   5.2.2 Methods 82
   5.2.3 Results and discussion 83
5.3 Osmotic effects. 88
   5.3.1 Introduction 88
   5.3.2 Methods 89
   5.3.3 Results 90
      5.3.3.1 Antimicrobial activity and a_w 90
      5.3.3.2 Well Diffusion 91
   5.3.4. Discussion 92
5.4 Methylglyoxal 94
   5.4.1 Introduction 94
   5.4.2 Methods 97
   5.4.3 Results 98
      5.4.3.1 Measurement of MGO standard compound 98
      5.4.3.2 Measurement of MGO in manuka honey 100
      5.4.3.3 Antimicrobial activity of MGO and honey 102
   5.4.4 Discussion 104
5.5 Acidity 105
   5.5.1 Introduction 105
   5.5.2 Methods 106
   5.5.3 Results and discussion 107
5.6 Conclusions 112

CHAPTER SIX. MANUKA HONEY ANTIMICROBIAL ACTIVITY: Effects on rate and extent of E. coli growth

6.1 Introduction 115
6.2 Manuka honey osmotically active solutes 116
   6.2.1 Introduction 116
   6.2.2 Methods 117
   6.2.3 Results and discussion 117
6.2.4 Summary 121

6.3 Manuka honey methylglyoxal 122
   6.3.1 Introduction 122
   6.3.2 Methods 122
   6.3.3 Results and discussion 122
   6.3.4 Summary 124

6.4 Discussion 125

CHAPTER SEVEN. Mechanisms of Manuka Honey Antimicrobial Activity 127

7.1 Introduction 127

7.2 Cellular K+ response of E. coli to manuka honey exposure 128
   7.2.1 Methods 129
   7.2.2 Results and discussion 129
   7.2.3 Summary 130

7.3 Manuka honey effects on membrane integrity and respiration. 131
   7.3.1 Membrane integrity 131
      7.3.1.1 Methods 132
      7.3.1.2 Results and discussion 132
      7.3.1.3 Summary 136
   7.3.2 Respiration (Aerobic vs anaerobic) 136
      7.3.2.1 Methods 136
      7.3.2.2 Results and discussion 136
      7.3.2.4 Summary 139
   7.3.3 Respiration (MTT) 139
      7.3.3.1 Methods 139
      7.3.3.2 Results and discussion 140
      7.3.3.3 Summary 142
   7.3.4. Cellular ATP levels 142
      7.3.4.1 Methods 142
      7.3.4.2 Results and discussion 143
      7.3.4.3 Summary 144

7.4 Conclusions 144
CHAPTER EIGHT. General discussion/conclusion 147
8.1 Summary 147
8.2 Implications 148
8.3 Future Work 149
8.4 Conclusion 150

APPENDIX A 151
Poster 1: AIFST 40th Annual Convention, Melbourne, Australia, June 2007 151
Research Summary: MacDiarmid Young Scientist of the Year Awards, Auckland, March 2008 152
Poster 2: MacDiarmid Young Scientist of the Year Awards, Auckland, August 2008 154

APPENDIX B. Attached publication. 155

APPENDIX C. Screening food ingredients for their effects on innate immunity 167
C.1 Introduction 167
  C1.1 Gastrointestinal Immune System 167
    C1.1.2 The Follicle-Associated Epithelia 167
    C1.1.3 The gut-associated lymphoid tissue 167
    C1.1.4 Oral Tolerance 170
C.2 Methods 171
  C.2.1 Lymphocyte proliferation 171
  C.2.2 Natural Killer Assay 172
  C.2.3 Phagocytosis 173
C.3 Results and discussion 173
  C3.1 Lymphocyte proliferation 173
  C3.2 Natural Killer Assay 175
  C3.3 Phagocytosis 177
C.4 Conclusions 179

APPENDIX D. Rodent Purified Diet AIN-76A 181

REFERENCES 183
List of Figures

Figure 1. Workflow of the Foods for *H. Pylori* programme. 24

Figure 2.1 Derivatisation of MGO with TRI to form 6-MPT. 41

Figure 3.1 Growth values from bacterial cultures supplemented with increasing doses of functional food extracts. 52

Figure 4.1.1 Log$_{10}$ number of bacteria in the 5 groups pre- and post-treatment with manuka UMF$^®$20 honey or control honey. 64

Figure 4.1.2 Process work flow depicting sample collection and fate of samples during course of animal trial. 65

Figure 4.3.1 Mouse weight gains during course of 28 day feeding Trials. 70

Figure 4.3.2 Average weekly food intake during 28 day feeding trials. 70

Figure 4.3.3 Peritoneal macrophages from mice treated with different functional food dietary supplements. 72

Figure 4.3.4 Macrophage phagocytosis from Trial 1 animals. 73

Figure 4.3.5 SCFA analyses of mice caecal contents. 74

Figure 4.3.6 Bacterial group numbers from the caecum of mice. 76

Figure 5.1 Organisational chart of this chapter. 80

Figure 5.2 Summary (work flow) of the approach used to investigate role of hydrogen peroxide as a potential manuka honey antimicrobial factor. 82

Figure 5.2.1 Microbial assay results measuring effects of manuka honey and clover honey with and without peroxide and catalase. 84

Figure 5.3 Summary of approach (work flow) used to measure the contribution of osmotically active factors to manuka honey antimicrobial activity. 89

Figure 5.3.1 Water activity of manuka honey, artificial honey and NaCl, antimicrobial activity of manuka honey, artificial honey and NaCl against *E. coli*. 91

Figure 5.4 Summary of the approach (work flow) used to measure the contribution of MGO to manuka honey antimicrobial activity 97

Figure 5.4.1 MGO standard curve. 98

Figure 5.4.2 HPLC chromatograms of various MGO derivatisations. 99

Figure 5.4.3 Antimicrobial dose response profile of honeys and MGO standard compound. 102
Figure 5.4.4 Antimicrobial dose-response profile of MGO standard compound.

Figure 5.5 Summary of the approach used to measure the effect of pH on the antimicrobial activity of manuka honey and the contributing antimicrobial factors sugar (artificial honey) and MGO.

Figure 5.5.b Summary of the results obtained during the measurement of the effect of pH on the antimicrobial activity of manuka honey and the contributing antimicrobial factors sugar (artificial honey) and MGO.

Figure 5.5.1 Growth of E. coli on Na$_2$HPO$_4$-NaH$_2$PO$_4$ buffer solutions pH 7.0, in TSB medium, over a range of buffer concentrations.

Figure 5.5.2 Growth of E. coli on Na$_2$HPO$_4$-NaH$_2$PO$_4$ buffer solutions (100 mM), further buffered by the TSB growth medium, over a range of pH.

Figure 5.5.3 E. coli microassay showing growth in the presence of varying pH 7-controlled doses of manuka honey, artificial honey, manuka-honey equivalent dose of MGO, and artificial honey spiked with manuka honey-equivalent MGO.

Figure 5.5.4 E. coli microassay in the presence of varying doses of manuka honey, and the following solutions all buffered to the same pH as the manuka honey: artificial honey, manuka-honey equivalent dose of MGO, and artificial honey spiked with manuka honey-equivalent MGO.

Figure 5.6.1 Proposed summary of degree of contribution of manuka honey antimicrobial components.

Figure 6.2.1 Effect of varying concentrations of either manuka honey or artificial honey combined with betaine on growth of E. coli.

Figure 6.2.2 Growth kinetics of E. coli supplemented with manuka honey, artificial honey or NaCl in the presence and absence of betaine.

Figure 6.3.3 Mean growth vs time for E. coli at 10$^5$ or 10$^3$ cfu/mL initial concentration, supplemented with either MGO or manuka honey.

Figure 7.1 PBFI structure.

Figure 7.2. PBFI determination of intracellular K$^+$ measured in E. coli cells treated with manuka honey, artificial honey or MGO.

Figure 7.3.1 LIVE/DEAD Badlight fluorescent membrane integrity assay.

Figure 7.3.2 LIVE/DEAD Badlight fluorescent membrane integrity assay of E. coli subjected to varying concentrations of manuka honey.

Figure 7.3.3 Effects of a range of concentrations of manuka honey and MGO on E. coli.
Figure 7.3.4 The effect of manuka honey on the growth and respiratory activity of *E. coli* Nissle.

Figure 7.3.5 Respiratory activity of *E. coli* after honey treatment.

Figure 7.3.6 Luminescence due to ATP levels of *E. coli* supplemented with manuka honey, artificial honey and MGO.

Figure C.3.1 Cell viability assay on pBWCs subjected to food extracts.

Figure C.3.2 LDH release from lysed and unlysed cells.

Figure C.3.3 Optimising the effector cell to target cell ratio for best release of LDH.

Figure C.3.4 Comparing target + effector cell LDH release to effector cell leakage under conditions of unchanged target cell numbers and varying effector cell numbers.

Figure C.3.5 Phagocytosis activity of RAW264.7 mouse macrophage cells after exposure to food ingredients.

Figure C.3.6 Phagocytosis activity of RAW264.7 mouse macrophage cells after exposure to various food ingredients after pre-incubation with LPS.
List of Tables

Table 1.1 Commensal microbial elements contributing to maintenance of mucosal integrity and resistance to pathogenic assault. 5
Table 1.2 Host elements contributing to maintenance of mucosal integrity. 5
Table 1.3 Elements of microbe-host interactions. 6
Table 1.4 Examples of the probiotic effects of lactic acid bacteria in human and animal health. 20
Table 1.5 Classes of antimicrobial compounds, modes of action and targets 26
Table 2.1 Probiotic and pathogenic bacteria and culture media. 32
Table 2.2 Mammalian cell lines and culture media 33
Table 3.1 Extracts and concentrations at the highest dose used for the Single Extract Assay. 47
Table 3.2 Microbial synergy assay results. 59
Table 4.1 Animal treatment diets, where the functional food supplement replaced an equivalent weight of the sucrose component of the diet. 67
Table 4.2 Microbial group primers used to quantitatively amplify gut microbial populations from the mouse caecum using RT-PCR with primers for 16S rRNA genes. 69
Table 5.2.1 Well diffusion assay comparing manuka honey and clover honey with addition of peroxide and/or catalase to examine possible contribution of peroxide to antimicrobial activity. 86
Table 5.2.2 Estimation of phenolic content of manuka and clover control honey in Gallic Acid Equivalents. 87
Table 5.2.3 Estimation of FRAP activity in 25% (w/v) manuka and clover control honey as millimolar Trolox Equivalents. 87
Table 5.3.1 Well diffusion assay comparing manuka honey and clover honey with artificial honey (sugar solution) to examine possible contribution of sugars to antimicrobial activity. 92
Table 5.4.1 MGO quantity in honey samples. 101
Table 5.4.2 Effect of alkali treatment on recovery of the MGO standard compound and from manuka honey. 101
Table 5.5.1 pH of manuka honey and artificial honey sugar solutions in TSB medium over the assay range of concentrations. 108
Table 5.5.2 pH of 100 mM Na₂HPO₄-NaH₂PO₄ buffer solutions in TSB. 108
Table 1.3 Toll-like receptor ligands. 169
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
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<tr>
<td>µm</td>
<td>micrometer</td>
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<tr>
<td>mm</td>
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<td>microlitre</td>
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<td>secretory immunoglobulin A</td>
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<td>16S rRNA</td>
<td>16 S subunit of ribosomal polymerase gene</td>
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<td>6-methylpterin</td>
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<td>water activity</td>
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<tr>
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<tr>
<td>BHI</td>
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<td>BSA</td>
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FAE follicle-associated epithelium
FAE follicle-associated epithelia
FAO/WHO World Health Organisation
Fc fraction crystallisable (region of antibody molecule)
FCS fetal calf serum
Fe iron
Fe$^{III}$ TPTZ ferric-tripyridyltriazine
tfMLP formyl-methionine-leucine-phenylalanine
FOSHU foods for specified health uses
FRAP ferric reducing/antioxidant power
FRST Foundation for Research, Science and Technology
FSANZ Food Standards Australia New Zealand
GAE gallic acid equivalents
GALT gut-associated lymphoid tissue
GI gastrointestinal
H$^+$ Hydrogen ion/proton
HBSS Hank’s Buffered Salt Solution
HCl hydrochloric acid
HPLC high performance liquid chromatography
IBD inflammatory bowel disease
IBD inflammatory bowel disease
IELs intraepithelial lymphocytes
IgA immunoglobulin A
IgG immunoglobulin G
IgM immunoglobulin M
IL-1, IL-1$\beta$, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13 interleukins (cytokines)
IFN-γ interferon gamma (cytokine)
IRAK IL-1 receptor-associated kinase
K$^+$ potassium ion
kDa kilo Daltons
Kdp turgor-sensitive transporter
KefB and KefC proton antiporter
LAB lactic-acid producing bacteria
LD lethal dose
LDH lactate dehydrogenase
LP effector lamina propria
LP lamina propria
LPLs LP lymphocytes
LPS lipopolysacharide
LSD least significant difference
M cells microfold cells
MAC membrane attack complex
MALT mucosa-associated lymphoid tissue
MAPK  mitogen-activated protein kinase
MGO  methylglyoxal
MHC II  major histocompatibility complex class II
MICs  minimum inhibitory concentrations
MLN  mesenteric lymph nodes
mRNA  messenger ribose nucleic acid
MRS  de Man Rogosa and Sharp media
MRSA  multidrug resistant *Staphylococcus aureus*
MTBSTFA  N-methyl-N-E-butylidemethylsilyltrifluoracetamide
MTT  3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na₂HPO₄  disodium hydrogen phosphate
NaCl  sodium chloride salt
NADPH  nicotinamide dinucleotide phosphate
NaH₂PO₄  sodium dihydrogen phosphate
NER  net experimental reading
NF-κB  nuclear factor kappa B
NK  natural killer
NO  nitric oxide
NPR  net positive reading
O₂  oxygen
OD  optical density
OTC  over the counter
PAMPs  pathogen-associated molecular patterns
PBFI  potassium-binding benzofuran isopthalate
PBS  phosphate buffered saline
PCR  polymerase chain reaction
PI  propidium iodide
plgR  polymeric immunoglobulin receptor
PMNs  polymorphonuclear cells
PPARg  peroxisome proliferator-activated receptor γ
pWBC  pig white blood cells
RNIs  reactive nitrogen intermediates
ROI  reactive oxygen intermediates
SCFA  short chain fatty acid
SEM  standard error of the means
TBDMSCl  tert-butylmethylsilyl chloride
TGF-β  transforming growth factor beta
Tₜ  T helper cells
Tₜ₁, Tₜ₂, Tₜ₃  T helper cell lineages
TLRs  toll-like surface receptors
TNF-α  tumour necrosis factor alpha
Treg  regulatory T cells
<table>
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<tr>
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<td>TRI</td>
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</tr>
<tr>
<td>trolox</td>
<td>6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid</td>
</tr>
<tr>
<td>TSB</td>
<td>tryptic soy broth</td>
</tr>
<tr>
<td>U</td>
<td>units (enzyme activity)</td>
</tr>
<tr>
<td>UMF</td>
<td>unique manuka factor</td>
</tr>
<tr>
<td>USFDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>VSPs</td>
<td>variant-specific surface proteins</td>
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<tr>
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