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

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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.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

µm	micrometer
mm	millimitre
m	metre
µL	microlitre
mL	millilitre
L	litre
µM	micromolar
mM	millimolar
M	molar
APCs	antigen presenting cells
sIgA	secretory immunoglobulin A
℃	degrees Celcius
16S rRNA	16 S subunit of ribosomal polymerase gene
6-MPT	6-methylpterin
ANOVA	analysis of variance
APC	antigen presenting cells
AQ	absolute quantification
atm	atmospheres
ATP	adenosine tri phosphate
a_w	water activity
BCA	bichinchoninic acid
BHI	Brain Heart Infusion
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CD4(+)	immune T cell lineage
CD8(+)	immune T cell lineage
CD86	immune T cell lineage
CO_2	carbon dioxide
Ct	crossing threshold
DC	dendritic cell
DHA	dihydroxyacetone
$DiSC_35$	3,3-dipropylthiacarbocyanine
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethylsulfoxide
DNA	deoxyribose nucleic acid
DPC16	*L. reuteri* strain Drapac # 16
DR10	*B. lactis* HN001
DR20	*L. rhamnosus* HN019
EDTA	ethylene diamine tetraacetic acid
EHEC	enterohaemorrhagic *E. coli*
Em.	emission (wavelength)
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
Ex.	excitation (wavelength)

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
fMLP	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-1ß, 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+	potassiuim 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 histocompatability 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-butyldimethylsilytrifluoracetamide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2HPO_4	disodium hydrogen phosphate
NaCl	sodium chloride salt
NADPH	nicotinamide dinucleotide phosphate
NaH_2PO_4	sodium dihydrogen phosphate
NER	net experimental reading
NF-kB	nuclear factor kappa B
NK	natural killer
NO	nitric oxide
NPR	net positive reading
O_2	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
pIgR	polymeric immunoglobulin receptor
PMNs	polymorphonuclear cells
PPARg	peroxisome proliferator-activated receptor g
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-b	transforming growth factor beta
T_H	T helper cells
T_H1, T_H2, T_H3	T helper cell lineages
TLRs	toll-like surface receptors
TNF-α	tumour necrosis factor alpha
Treg	regulatory T cells

TRI	6-hydroxy-2,4,5-triaminopyrimidine
trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TSB	tryptic soy broth
U	units (enzyme activity)
UMF	unique manuka factor
USFDA	United States Food and Drug Administration
VSPs	variant-specific surface proteins
w/v	weight per volume
Δgrowth	change in growth (relative to control 100% growth)