The Effects of New Zealand Manuka-Type Honeys on Bacterial Growth and Morphology, Biofilm Formation and Biofilm Eradication

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledge within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abbreviations

%	Percentage
AGPs	Arabinogalactan proteins
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
a_w	Water activity
C	Clover honey
CA-MRSA	Community-acquired methicillin resistant *Staphylococcus aureus*
CAMHB	Cation-adjusted Mueller-Hinton broth
CFU	Colony Formation Unit
CLSI	Clinical Laboratory Standards Institute
CLSM	Confocal Laser Scanning Microscopy
DHA	Dihydroxyacetone
DNA	Deoxyribonucleic acid
e.g.	Exempli gratia
FDA	Food and Drug Administration
FISH	Fluorescence *in situ* hybridization
g	Gram(s)
GlxI	Glyoxalase I
h	Hour(s)
H_2O_2	Hydrogen peroxide
HA-MRSA	Hopital-acquired methicillin resistant *Staphylococcus aureus*
HPLC	High-performance liquid chromatography
HS-LBA	High-salt Luria-Bertani agar
K	Kanuka honey
kg	Kilogram(s)
LB	Luria-Bertani
M	Manuka honey
MBIC(s)	Minimum biofilm inhibitory concentration(s)
mg	Milligram(s)

MGO	Methylglyoxal
MIC(s)	Minimum Inhibitory Concentration(s)
min	Minute(s)
MK	Manuka-kanuka blends
mL	Millilitre
mm	Millimetre
mM	Millimolar
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSCRAMMs	Microbial surface componenets recognising adhesive matrix molecules
MSSA	Methicillin-Sensitive *Staphylococcus aureus*
NMR	Nuclear magnetic resonance
NZ	New Zealand
^{o}C	Degree Celsius
OD_{595}	Optical Density at 595 nm
PBS	Phosphate buffered saline
PI	Propidium iodine
PIA	Polysaccharide intercellular adhesion
qPCR	Quantitative polymerase chain reaction
QS	Quorum sensing
ROW	Reverse osmosis water
rpm	Revolutions per minute
SD	Standard deviation
SEM	Standard error of the mean
TGA	Therapeutic Goods Administration
TNF- α	Tumour necrosis factor-alpha
TSB	Tryptone soya broth
TSBG	Tryptone soya broth plus 1% glucose
UMF	Unique Manuka Factor
USA	United States of America
USD	United States dollar
UV	Ultra violet

v	Volume
w	Weight
w/v	Weight per volume
µL	Microlitre
µm	Micrometer
μm^2	Square micrometer
μm^3	Cubic micrometer
µmol	Micromole

Abstract

Bacterial pathogenesis is a major threat to human health due to the increase antibiotic resistance among disease-causing bacteria. Effective and alternative therapeutics are urgently required to combat this problem. Honey is a natural product that has been used for over 2,000 years, as an effective topical chronic wound treatment. Numerous studies in the last 30 years have revealed its potent antibacterial properties (due to high sugar content, low pH and hydrogen peroxide production upon dilution). Honeys sourced from the *Leptospermum scoparium* bush in New Zealand (NZ), also referred to as manuka-type honeys, have been known to contain additional 'non-peroxide' antibacterial components (including methylglyoxal (MGO) and various phenolic compounds).

However, for honey to be considered as a mainstream wound treatment by medical professionals, the mechanism behind its antibacterial activity needs to be determined. Moreover, bacteria produce biofilms that is a matrix of extracellular polymeric substance and allow cells to adhere to a surface such as a wound. Biofilms are the preferred mode of life in wounds because it also offers protection from antibiotic treatment. It is therefore essential to evaluate honeys' effects on bacterial biofilms. Unfortunately, almost all previous studies have utilized honeys that are ill-defined chemically. Thus, the objectives of this work were to use a range of well-defined NZ manuka-type honeys and their specific antibacterial components (such as methylglyoxal and sugars) to firstly examine their antibacterial effects on bacterial cell growth and cellular morphology, across a range of different bacteria. Subsequently, the antibiofilm activities on different strains of the same organism were also investigated on preventing biofilm formation and eradicating the pre-established biofilms.

The bacterial cell growth and cellular morphology of three clinically relevant bacteria; the Gram-positive *Staphylococcus aureus*, and, the Gram-negative organisms *Escherichia coli* and *Pseudomonas aeruginosa* were examined against the selected range of NZ honeys, by cell growth assays and fluorescent microscopy.

In addition, a Gram-positive organism, *Bacillus subtilis*, was also studied because it is a model organism where the functions of many genes associated with cellular growth and morphology have been documented. Moreover, *B. subtilis* is often used as a Gram-positive representative organism, typically in drug discovery studies in the industry. Results presented in this work indicate that different bacterial species are susceptible to different components or concentrations of honey and therefore respond in different ways. It is proposed that the complexity of honey makes it hard for bacteria to become resistant to honey's antibacterial effects.

The second and third parts of this work examined the effectiveness of manuka-type honeys in preventing and eradicating preformed bacterial biofilms in *S. aureus* and *P. aeruginosa*. This was performed by using a crystal violate based static biofilm formation assay in combination with Confocal Laser Scanning Microscopy (CLSM) to visualise the integrity of the biofilms after honey treatment. It was found that very low levels of NZ manuka-honey enhanced both *S. aureus* and *P. aeruginosa* biofilm formation, which could possibly due to the evoke of a stress response similar to that seen with some conventional antibiotics. When higher concentrations of honey were used, NZ manuka-honeys were able to prevent or eliminate biofilms. This appears to be influenced by MGO levels and the presence of sugar. However, MGO and sugar content alone does not account for all of the antibiofilm properties observed. Finally, an ATP-based viability assay suggested that both *S. aureus* and *P. aeruginosa* planktonic cells, which were released after honey treatment of pre-formed biofilms were significantly reduced. The development of resistance or tolerance from these recovered planktonic cells was also determined by exposing these cells to the same previously exposed honey agents. Results indicated that the recovered *S. aureus* planktonic cells did not display any resistance to honey. However, the recovered *P. aeruginosa* planktonic cells had an increased tolerance to the same honey treatment. Altogether, these results show that at an appropriate level of manuka-type honey as a whole agent, can be used to kill *P. aeruginosa* and *S. aureus* when present in the biofilm, thereby supporting the use of this honey as an effective topical treatment for chronic

wound infections. Lastly, this work also provided guidelines and strategies for new formulation of wound treatment managements and products, respectively.