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Studies on the manipulation of gastrointestinal tract bacteria

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Studies on the Manipulation of Gastrointestinal Tract Bacteria

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ABSTRACT

Increasing awareness that the human intestinal flora is a major factor in health and disease has led to different strategies to manipulate the flora to promote health. These approaches include changes to the diet by inclusion of prebiotics and probiotics. Prebiotics are non-digestible polysaccharide food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gastrointestinal tract (GIT). Probiotics on the other hand are viable culture of bacteria, which applied to animals or humans, beneficially affect the host by improving the properties of the indigenous microflora. One mechanism of action of probiotics is the production of antimicrobial substances called bacteriocins, such as colicins, that inhibit the growth of their competitors. The experiments described in this thesis examined the potential use of prebiotics and probiotics to manipulate GIT bacteria.

A crude polysaccharide extract (HW) from the medicinal mushroom *Ganoderma lucidium* was prepared by extracting the fruiting body with boiling water. The extract was then purified by ethanol precipitation resulting in the hot water-ethanol (HWE) extract. Groups of mice were fed these extracts over a period of three weeks at a concentration of 150 μg/ml in sterile drinking water and the mice then euthanised after three weeks. Changes in population dynamics of lumen bacteria were determined in the duodenum, ileum, colon and faeces by rigorous washing of excised segments while adherent bacteria were released with the non-ionic detergent Triton X100, which does not affect the viability of the bacteria. The prevalence of haemolytic colonies was assessed by plating washouts onto blood agar. Total colony forming units were enumerated on bacteriological media selective for *Enterobacteriaceae, Streptococci, Enterococci* and Lactic acid Bacteria (LAB). Results showed that there was little change in population dynamics elicited by extract feeding. The exception was a significant reduction in haemolytic lumen bacteria and increase in LAB lumen bacteria recovered from the colon of HWE treated mice.

A multiplex PCR was optimized and applied to survey the prevalence of eight common colicin genes (Colicins A, D, E1, E2, E6, E7, Ia and V) in *Escherichia coli* (*E. coli)* isolates. The study focused on 39 clinical isolates from

humans and 68 isolates from pigs with post-weaning diarrhoea. In addition, 152 porcine commensal *E. coli* isolates obtained from different compartments of the GIT (duodenum, ileum, colon and faeces) were included in the PCR analysis. Six individual colicins (E1, E2, E6, E7, Ia and V) and four dual colicin combinations (E1/E2, E1/E7, E2/E7, & E2/Ia) were detected. Approximately 28.2 % of the human pathogenic isolates had at least one colicin gene with colicins D, E1, E7 and V occurring at frequencies of 5.1 % each. Colicins E6, Ia and the dual colicin, E2/Ia, were less frequent and were found in about 2.6 % of clones. Only 4 % of the porcine pathogenic isolates possessed a colicin gene and these were exclusively E1 and V. In contrast, there was a significantly higher carriage (36.2%) of colicin genes in commensal porcine *E.* coli. Of these, E1, E7 and Ia accounted for 87 % of all colicin genes detected. Six of the commensal strains possessed multiple types of colicins with the most common being the E2/E7 combination. Furthermore, there appeared to be differences in the type of colicins found in commensal *E. coli* isolates recovered from different intestinal compartments.

Seven porcine commensal *E. coli* strains producing standard colicins were evaluated for inhibitory activity against five pathogenic *E. coli* of human and porcine origin. The experiment utilized a kinetic inhibitory microtitre assay (KIMA) to assess inhibition using non-induced supernatants and supernatants induced with 0.2 μg/ml of mitomycin C to stimulate colicin production. The level of inhibition was found to be variable with most of the commensal porcine *E. coli* strains showing little or no inhibitory action against the five pathogenic strains. However, two commensal strains, ILC33 and CC89 were found to highly inhibitory to three porcine pathogenic *E. coli* strains of serotypes O141:K85, 0141:K88 and 0149:K88.

The findings of this thesis suggest that purified polysaccharide extracts (HWE) from *Ganoderma lucidium* have the potential to be used in further studies as prebiotics in view of their positive effects on beneficial LAB. In addition, the use of colicin-bearing strains as probiotic bacteria is justifiable because of the low incidence of colicin genes in pathogenic *E. coli* compared to commensals. Finally, these findings indicate that potential probiotic bacterial strains have to be scrutinised for their inhibitory activity against individual pathogenic strains prior to being subjected to further assessments.

CERTIFICATION

I, Peter Njuguna, declare that this thesis, submitted in partial fulfilment of the requirements for the award of the degree of Master of Science (Research), in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualification at any other academic institution.

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PUBLICATIONS AND PRESENTATIONS

Njuguna, P., Wu, K., Chapman T., Chao, R., Zhang, R., Gordon, D., Bettelheim, K., and Chin, J. (2004). *E. coli* at War: Commensals versus Pathogens. PP12.3. Australian Society for Microbiology Conference, Sydney.

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

LIST OF TABLES

Chapter 1

Chapter 3

LIST OF ABBREVIATIONS

BA	**Blood agar**
BP	**Base pairs**
CATC	**Citric Azide Tween Carbonate agar**
CC	**Colon commensal**
CFU	**Colony forming units**
DC	**Duodenum commensal**
E. coli	**Escherichia coli**
EHEC	**Enterohemorrhagic *E. coli***
ETEC	**Enterotoxigenic *E. coli***
FC	**Faecal commensal**
GIT	**Gastrointestinal tract**
HW	**Hot water extract**
HWE	**Hot water-ethanol extract**
ILC	**Ileum commensal**
KDa	**Kilodaltons**
KEA	**Kanamycin Esculin Azide agar**
LA	**Luria agar**
LAB	**Lactic acid bacteria**
LB	**Luria agar broth**
MAC	**MacConkeys agar**
ML	**Milliliter**
MRS	**Deman, Rogosa and Sharpe agar**
NDGOS	**Non-digestible galacto-oligosaccharides**
O.D.	**Optical density**
PBS	**Phosphate Buffer Saline**
PCR	**Polymerase chain reaction**
PWD	**Post-weaning diarrhoea**
RPM	**Rotations per minute**
SCFA	**Short-chain fatty acids**
μg	**Micro-gram**
TX	**Triton-X 100**