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Peter Njuguna
University of Wollongong

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Studies on the Manipulation of Gastrointestinal Tract Bacteria

A thesis submitted in fulfillment of the requirements for the award of the degree

Master of Science (Research)

from



by

Peter Njuguna, MSc

School of Biological Sciences

2005

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 lucidum* 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 be highly inhibitory to three porcine pathogenic *E. coli* strains of serotypes O141:K85, O141:K88 and O149:K88.

The findings of this thesis suggest that purified polysaccharide extracts (HWE) from *Ganoderma lucidum* 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.

Peter Njuguna

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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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
<i>E. coli</i>	<i>Escherichia coli</i>
EHEC	Enterohemorrhagic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
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