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Studies on the impact of probiotic bacteria on enteric microbial diversity and immune response

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STUDIES ON THE IMPACT OF PROBIOTIC BACTERIA ON ENTERIC MICROBIAL DIVERSITY AND IMMUNE RESPONSE

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Abstract

The mechanism of action of probiotics is based on competitive exclusion and immune modulation. However, the literature is scant on supporting data because of the failure to adopt a systems approach to probiotic functionality. This has been partially addressed in this thesis by taking into consideration the tripartite interaction between bacteria and bacteria in the enteric community; between bacteria and the host animal and finally, between the host immune response (innate or acquired) on the plethora of microbes that inhabit the gastrointestinal tract.

A trial involving newly inducted cattle in a feedlot, formed the basis of initial attempts to assess the benefits of a commercial probiotic formulation – Protexin on intestinal health by enumeration of a select subset of cultivable bacteria species and by assessment of immune modulation. The results failed to demonstrate a significant change in the population dynamics of cultured faecal microbes but did show that Protexin stimulated immune responsiveness in T cells. Carcass analysis demonstrated a significant reduction in marbling or intramuscular fat deposition.

In the course of examining the faecal microflora from feedlot cattle, the presence of high levels of *Bacillus* spores suggested that one possible reason for the lack of a growth benefit may be attributed to a high endogenous level of bacilli. Since there were no reliable methodologies for identifying *Bacillus* species, an alternative procedure was developed involving amplified ribosomal DNA restriction analysis (ARDRA). With this protocol, we were able to show that cattle faeces contained large numbers of *Bacillus* spores representing different mesophilic species, where *B. subtilis*, *B. licheniformis* and *B. clausii* dominated.

The presence of a stable population of coliforms in cattle faeces that was not altered by probiotic feeding highlighted the importance of developing better techniques to characterise diversity in *E. coli*, a potential food-borne pathogen of economic significance to the cattle industry. The use of virulence genes to genotype coliforms provided a method for differentiating between pathogenic, clinical and commensal

isolates of *E. coli.* Altogether, a combination of uni- and multiplex PCR assays was developed to screen for 50 virulence genes (VGs) from 8 pathotypes of *E. coli.* There was a significant association between phylogroupings and VG ownership. This result showed clearly that the lack of or possession of VGs in member isolates of each phylogenetic group can be used to assess diversity and potential pathogenesis of *E. coli*.

To understand better the importance of pathogenic enteric coliforms, an alternative animal model involving pigs with post-weaning diarrhoea was used to investigate the relationship between pathogenicity and commensalism by VG profiling. Porcine enterotoxigenic *E. coli* (ETEC) were found to carry VGs identified in *E. coli* that cause extraintestinal infection. Furthermore, by using the appropriate methods of statistical analysis, VG profiling had the capacity to predict the pathogenic and commensal status of individual clones. By developing the capacity to rapidly characterise and genotype virulence and commensalism in *E. coli*, it is now feasible to examine how probiotic feeding can modulate the population dynamics of different community members in pigs with enteric disease, as well as changes in the coliform populations.

Finally, another arm of the tripartite interaction involving bacteria and host interaction was modelled *in vitro* by examining the primary signalling events between bacteria and intestinal epithelial cells. These investigations focused on the judicious selection of T84 as the reporter intestinal epithelial cell line because of low level expression of inflammatory transcripts from 6 other epithelial cell lines. Using a panel of coliforms genotyped for virulence or lack of virulence, the signalling events that followed on from the primary interaction between bacterium and cell, showed there was a lack of correlation between VGs and gene activation. Nonetheless, all the coliform strains tested varied in their capacity to signal transduce T84, confirming that this differential bioactivity can be exploited in the ranking of candidate probiotic strains. The differential responses seen with different *E. coli* strains and the lower and more consistent activation patterns recorded by LABs for both cytokine and chemokine gene activation, demonstrate that a semi-quantitative ranking of microbial bioactivity can be obtained. Such an approach if adopted in conjunction with an even wider panel of genes in a standardised *in vitro* environment can provide invaluable information on the selection of appropriate strains to be further tested *in vivo*.

Certification

I, Xi-Yang Wu, declare that this thesis, submitted in fulfilment of the requirement for the award of Doctor of Philosophy, 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

Xi-Yang Wu

Signature: …….

Date: ……...

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List of Publications

This thesis is based on the following articles

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Xi-Yang Wu, James Chin, Aida Ghalayini and Michael Hornitzky (2005): Pulsed-field gel electrophoresis typing and oxytetracycline sensitivity of *Paenibacillus larvae* subsp. *larvae* isolates of Australian origin and those recovered from honey imported from Argentina. Journal of Apiculture Research 44(2): 87-92

Xi-Yang Wu, Mark Walker, Barbara Vanselow, Ri-Liang Chao and James Chin (2006): Characterization of mesophilic bacilli in faeces of feedlot cattle. Journal of Applied Microbiology (Published article online: 15-Aug-2006)

Toni Chapman, **Xi-Yang Wu**, Idris Barchia, Karl Bettelheim, Steven Driesen, Darren Trott, Mark Wilson and James J-C Chin (2006): A comparison of virulence gene profile between *E. coli* strains isolated from healthy and diarrheic swines. Applied & Environmental Microbiology 72(7):4782-95

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X-Y. Wu, T. Chapman, D. Trott, D.N. Thuy, S. Driesen, M. Walker and J. Chin (2006): Virulence gene profiling of enterotoxigenic *Escherichia coli* isolates associated with post-weaning diarrhoea in pigs. Applied & Environmental Microbiology (In press)

X-Y. Wu, T. Chapman, D. Gordon, K. Bettelheim, M. Walker and J. Chin (2006): Validation of *Escherichia coli* phylogenetic assignments based on virulence gene ownership (submitted to Microbiology)

James Chin, Toni Chapman and **Xi-Yang Wu** (2006): A re-appraisal of early signalling events and gene activation in a human intestinal epithelial cell line *in vitro* by probiotic and enteric pathogenic bacteria species (Manuscript)

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Abbreviations

ARDRA	Amplified ribosomal DNA restriction analysis
bp	Basepair(s)
BSA	Bovine serum albumin
cDNA	Complementary DNA
CE	Competitive exclusion
CFU	Colony forming units
Da	Dalton(s)
DFM	Direct-fed microbial
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleic triphosphate
DTT	Dithiothreitol, threo-1,4-Dimercapto-2,3-butandiol
EaggEC	Enteroaggregative *E. coli*
EDTA	Ethylendiamin-N,N,N',N'-tetra acid
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EMSA	Electrophoretic mobility shift assay
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
ExPEC	Extraintestinal pathogenic *E. coli*
FCS	Fetal calf serum
GIT	Gastrointestinal tract
h	Hour
IEC	Intestinal epithelial cell
IL	Interleukin
IPEC	Intestinal pathogenic *E. coli*
kb	Kilobasepair(s)
LAB	Lactic acid bacteria
M	Molar
Mb	Megabase

min	Minutes
MLEE	Multilocus enzyme electrophoresis
MOI	Multiplicity of infection
MQ-H_2O	Milli-Q filtered deionised water
mRNA	Messager RNA
NMEC	Neonatal Meningitis *E. coli*
O.D.	Optic density
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PWD	Post-weaning diarrhoea
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolution per minute
RT	Room temperature
RT-PCR	Reverse transcriptase polymerase chain reaction
sec	Second(s)
TBE	Tris-borate-EDTA
TE	Tris-EDTA
TEMED	N,N,N',N'-tetramethylethylendiamin
µg	Microgram
UPEC	Urinary pathogenic *E. coli*
V	Voltages
v/v	Volume for volume
VG	Virulence gene
Vol	Volume