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**Interactions between commensal obligate anaerobes  
and human intestinal cells**

A thesis presented in partial fulfilment of the requirements for the degree of  
**Doctor of Philosophy**

Massey University  
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**Dulantha Ulluwishewa**

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## ABSTRACT

The human intestinal epithelium is formed by a single layer of epithelial cells which regulates intestinal barrier permeability. Increased permeability can result in the entry of potentially harmful compounds into the body, and is implicated in autoimmune, inflammatory and atopic diseases. The intestinal tract is inhabited by an estimated  $10^{14}$  microbes and it is increasingly evident that they affect intestinal barrier function. However, over 90% of commensal intestinal bacteria are obligate anaerobes, making it difficult to co-culture them with oxygen-requiring mammalian cells *in vitro*.

To investigate the interactions between obligate anaerobes and epithelial cells that regulate the intestinal barrier, an apical anaerobic model of the human intestinal epithelium, which utilises a dual-environment co-culture chamber, was developed and validated. The chamber allowed for polarised monolayers of the intestinal cell line Caco-2 to be grown such that the apical (luminal) side was exposed to an anaerobic environment, while maintaining an aerobic basal side. The cell viability and barrier function of Caco-2 monolayers was unaffected by culture in the apical anaerobic model for at least 12 hours. Global gene expression analysis predicted upregulation of cell survival and proliferation in Caco-2 cells cultured in the apical anaerobic model, compared to Caco-2 cells grown under conventional conditions, suggesting an adaptation of the Caco-2 cells to a lower supply of oxygen.

The apical anaerobic model was used to co-culture the commensal obligate anaerobe *Faecalibacterium prausnitzii* with Caco-2 cells. The survival of *F. prausnitzii* was improved in the anaerobic apical environment compared to when cultured in an aerobic atmosphere. Live *F. prausnitzii*, but not non-viable (UV-killed) *F. prausnitzii*, were shown to increase permeability across Caco-2 monolayers. Furthermore, global gene expression analysis suggested that live *F. prausnitzii* cells have more profound effects on Caco-2 cells than non-viable *F. prausnitzii*, illustrating the importance of maintaining viability of obligate anaerobes in an *in vitro* co-culture system.

The apical anaerobic model can be used to gain insights into the mechanisms of crosstalk between commensal obligate anaerobic bacteria and intestinal cells, and new knowledge generated using this model will assist in the development of strategies to improve intestinal barrier function.

*Dedicated to aththamma, seeya, and 'big' aththamma.*

*My greatest inspirations. Love always.*

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## LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BHI	Brain-heart infusion
BLASTN	NCBI nucleotide Basic Local Alignment Search
CAR	Coxsackie and adenovirus receptor
cDNA	Complementary DNA
CFU	Colony-forming units
cIAP	Cellular inhibitor of apoptosis protein
CLA	Conjugated linoleic acids
CRE	cAMP response element
CREB	CRE-binding protein
cRNA	Complementary RNA
DC	Dendritic cells
ddNTPs	Dideoxynucleotides
DGGE	Denaturing gradient gel electrophoresis
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
DO	Dissolved oxygen
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)
EDTA	Ethylenediamine tetra-acetic acid
EGCG	Epigallocatechin gallate

EHEC	Enterohemorrhagic <i>Escherichia coli</i>
EIEC	Enteroinvasive <i>Escherichia coli</i>
EPA	Eicosapentaenoic acid
EPEC	Enteropathogenic <i>Escherichia coli</i>
ERK	Extracellular signal regulated kinases
FBS	Foetal bovine serum
FSH	Follicle stimulating hormone
GFP	Green fluorescent protein
GLA	$\gamma$ -linolenic acid
GO	Gene ontology
hCG $\Delta$	Choriogonadotropin
HIF	Hypoxia-inducible factor
IBD	Inflammatory bowel disease
IEC6	Intestinal epithelial cell line 6
IFN	Interferon
Ig	Immunoglobulin
IKK	I $\kappa$ B kinase
IL	Interleukin
IPA	Ingenuity Pathway Analysis
IRAK	IL-1-receptor-associated kinase
IRF	IFN-regulatory factor
I $\kappa$ B	Inhibitor of NF- $\kappa$ B
JAM	Junctional adhesion molecules
KEGG	Kyoto Encyclopaedia of Genes and Genomes
Lh	Luteinising hormone
LOESS	Locally weighted smoothing spline



LSD	Least significant difference
M199	Medium 199
MAMPs	Microbe-associated molecular patterns
MAPK	Mitogen activated protein kinases
MDCK	Madin Darby canine kidney
MLC	Myosin II regulatory light chain
MLCK	Myosin light chain kinase
MOI	Multiplicity of infection
mRNA	Messenger RNA
MRS	Man, Rogosa and Sharpe
MyD88	Myeloid differentiation primary response gene
NCBI	National Center for Biotechnology Information
NEAA	Non-essential amino acids
NF- $\kappa$ B	Nuclear factor kappa B
NOD	Nucleotide-binding and oligomerisation-domain
NRF2	NF-E2-Related Factor 2
OD	Optical density
PAMPs	Pathogen-associated molecular patterns
pamr	Prediction Analysis for Microarrays
PC	Polycarbonate (Transwell® cell culture inserts)
PCA	Principal component analysis
PCR	Polymerase chain reaction
PDZ	PSD95–DlgA–ZO-1 homology
PEPT1	H <sup>+</sup> /di-tripeptide transporter
PET	Polyester (Transwell® cell culture inserts)
PGE2	Prostaglandin E2

PI	Propidium iodide
PKC	Protein kinase C
PLA2	Group IIA phospholipase A2
PPAR	Peroxisome-proliferator-activated receptor
PRRs	Pathogen recognition receptors
PTFE	Polytetrafluoroethylene (Transwell® cell culture inserts)
<i>q</i>	False discovery rate
REML	Restricted maximum likelihood
RICK	Receptor-interacting serine/threonine kinase
RNA	Ribonucleic acid
RNase	Ribonuclease A
ROCK	Rho kinases
ROS	Reactive oxygen species
rRNA	Ribosomal RNA
SCFAs	Short chain fatty acids
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
SULTs	Sulfotransferases
TAK-1	Transforming growth factor- $\beta$ -activated kinase-1
TEER	Transepithelial electrical resistance
TGF	Transforming growth factor
TJ	Tight junction
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TNFR	Tumour necrosis factor receptor
TRAF6	TNF-receptor-associated factor 6

TREM	Triggering receptor expressed on myeloid cells
TSLP	Thymic stromal lymphopoietin
UV	Ultraviolet
VegF	Vascular endothelial growth factor
ZO	Zonula occludens