The interaction of probiotic bacteria and an oligosaccharide-enriched fraction from goat whey on *in vitro* intestinal barrier function and mucin production

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Abstract

Multiple interactions occur in the human large intestine between the host, the intestinal microbiota and fermentable carbohydrates which transit relatively intact through the small intestine. A major site at which many of these interactions occur is the intestinal epithelium, which is formed from a single layer of epithelial cells. The cellular composition of the epithelial layer in the human small and large intestine varies in respect to the numbers of absorptive enterocytes and mucus-secreting goblet cells. For the human intestine the proportion of goblet cells among epithelial cell types is thought to increase from the duodenum (4%) to the distal colon (16-24%).

Epithelial cell co-culture models were developed containing absorptive enterocytes (Caco-2 cells) and mucus-secreting goblet cells (HT29-MTX cells) that more closely simulate the cell proportions found in the small (90:10) and large intestine (75:25). Trans-epithelial electrical resistance (TEER) of the co-cultures was more similar to reported values of *ex vivo* intestinal tissue of human small and large intestine than either of the two mono-cultures. Additionally, the mucus layer thickness present at the apical surface of 75:25 co-cultures (cellular composition representative of the large intestine) was similar to the reported thickness of the inner mucus layer of human large intestine. Introduction of an oligosaccharide-enriched fraction (OEF) from goat whey to the epithelial co-culture models was shown to modulate barrier integrity as measured by TEER, in a dose-dependent manner. Oligosaccharides (1 mg/mL) increased TEER and mucin gene/protein expression of epithelial co-cultures. Finally, the interaction between probiotic bacteria and the OEF and their individual or combined effects on intestinal epithelial barrier integrity and mucin gene/protein expression was investigated.

The OEF supported the growth of selected probiotic strains, and enhanced the adhesion of defined strains to the epithelial co-cultures. When in combination with the OEF, *Lactobacillus plantarum* 299v enhanced TEER and mucin gene/protein expression, the increase of which was

greater than that for either component alone. This suggests that an interaction between *Lactobacillus plantarum* 299v and the OEF exists which enhances barrier integrity through increased TEER and mucin gene/protein expression, all of which are essential components of the intestinal barrier.

The research presented in this dissertation has indicated that *in vitro* epithelial co-cultures can be used as a model to improve our understanding of the mechanisms through which probiotic bacteria/food components and intestinal epithelial cells interact, and these key findings will assist in the development of strategies to improve intestinal barrier function using novel dietary components.

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Abs	**Absorbance**
ADAM10	**A disintegrin and metalloproteinase 10**
A-GM1	**Asialo-GM1**
AI-2	**Autoinducer-2**
ANOVA	**Analysis of variance**
ATCC	**American Type Culture Collection**
ATP	**Adenosine triphosphate**
BSA	**Bovine serum albumin**
cDNA	**Complimentary DNA**
CFU (cfu)	**Colony forming unit**
CK	**Cysteine knot**
CL	**Cell lysate**
DMEM	**Dulbecco's modified eagle's medium**
DMSO	**Dimethylsulfoxide**
DNA	**Deoxyribonucleic acid**
DP	**Degree of polymerisation**
DSM	**Deutsche Sammlung von Mikrooganismen (German Collection of Microorganisms and Cell Cultures)**
DSS	**Dextran-sodium-sulphate**
EDTA	**Ethylenediamine tetra-acetic acid**
EGF	**Epidermal Growth Factor**
EGFR	**Epidermal Growth Factor Receptor**
EPEC	**Enteropathogenic *Escherichia coli***
ERK	**Extracellular regulated kinase**
FBS	**Foetal bovine serum**

FOS	**Fructo-oligosaccharide**
G	**G protein**
GlcNAc	***N*-acetylglucosamine**
GOS	**Galacto-oligosaccharide**
GPCR	**G-protein-coupled-receptor**
HBEFG	**Heparin-binding epidermal growth factor**
HMO	**Human milk oligosaccharide**
IBD	**Inflammatory bowel disease**
Ig	**Immunoglobulin**
IL	**Interleukin**
LPS	**Lipopolysaccharides**
LTA	**Lipoteichoic acids**
M199	**Medium 199**
MAD	**Median absolute deviation**
MAPK	**Mitogen activated protein kinases**
MAPKK	**MAPK kinase**
MAPKK K	**MAPK kinase kinase**
MEK1	**MAPK ERK Kinase**
mL	**Millilitre**
MTX	**Methotrexate**
***MUC*/MUC**	**Mucin (gene/protein)**
mRNA	**Messenger RNA**
MRS	**de Man, Rogosa and Sharpe**
Neu5Ac	***N*-acetyl-neuraminic acid**
Neu5Gc	***N*-glycolylneuraminic acid**
NF-κB	**Nuclear factor kappa B**

OD	**Optical density**
OEF	**Oligosaccharide-enriched fraction**
OF	**Oligo-fructose**
PAS	**Periodic acid Schiff**
PBS	**Phosphate buffered saline**
PCR	**Polymerase chain reaction**
PET	**Polyester (Transwell cell culture inserts)**
PKC	**Protein kinase C**
PLC	**Phospholipase C**
qPCR	**quantitative real time PCR**
RNA	**Ribonucleic acid**
ROS	**Reactive oxygen species**
SCFAs	**Short chain fatty acids**
SEA	**Sea urchin sperm protein, Enterokinase, and Agrin**
SEM	**Standard error of the mean**
SM	**Spent media**
STP	**Serine, threonine and proline**
TEER	**Transepithelial electrical resistance**
TGF-α	**Transforming Growth Factor-α**
TLR4	**Toll-like receptor 4**
TMB	**Tetramethylbenzidine solution**
TNF	**Tumour necrosis factor**
TNBS	**Trinitrobenzenesulphonic acid**
µm	**Micrometre (micron)**
VNTR	**Variable number tandem repeat**
vWF	**von Willebrand Factor**