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**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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**Alicia Barnett**

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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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## List of abbreviations

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

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

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



