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Effect of *Faecalibacterium prausnitzii* on intestinal
barrier function and immune homeostasis

A dissertation presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy in Nutritional Science

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Abstract

Various gastrointestinal (GI) diseases, for example inflammatory bowel disease, are linked to impaired barrier function, chronic inflammation and dysbiosis of the resident microbiota. *Faecalibacterium prausnitzii*, an abundant obligate anaerobe of the healthy human microbiota, has reduced abundance in the GI tract of people with these diseases, and has been suggested to exert beneficial effects. Only a few studies have investigated its mechanisms of action, partly due to the difficulty of co-culturing live obligate anaerobes with oxygen-requiring human cells. The novel apical anaerobic co-culture model used in this study allows this co-culture through the separation of anaerobic and aerobic compartments. This model was used to investigate the effects of live *F. prausnitzii* (strains A2-165, ATCC 27768 and HTF-F) on intestinal barrier integrity, measured by trans-epithelial electrical resistance (TEER) of the intestinal epithelial cell line Caco-2, and on immune homeostasis, specifically on Toll-like receptor (TLR) activation. Method development was required to adapt these assays to the novel model and to optimise the growth of *F. prausnitzii* co-cultured with Caco-2 cells and TLR-expressing cell lines while maintaining their viabilities. Firstly, the optimised co-culture conditions were used to determine the effect of the three *F. prausnitzii* strains on barrier integrity of healthy and tumour necrosis factor alpha (TNF- α) treated Caco-2 cells. Live and growing *F. prausnitzii* did not alter the TEER across healthy Caco-2 cells. However, under TNF- α mediated inflammatory conditions, dead *F. prausnitzii* decreased TEER, whereas live bacteria maintained TEER. Secondly, the TLR activation assay was adapted to be carried out in the novel model. Using the adapted assay conditions it was determined that live *F. prausnitzii* induced greater TLR2 and TLR2/6 activation than dead *F. prausnitzii*. Collectively, these results indicate greater immuno-stimulatory effects of live *F. prausnitzii*, via TLR2

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activation, and this effect is potentially linked to its barrier maintaining properties, because previous research showed enhancement of barrier integrity induced by TLR2 signalling. This new knowledge contributes to the understanding of how *F. prausnitzii* may maintain immune homeostasis in the GI tract. Unravelling the biological mechanisms used by prevalent species of the human microbiota, such as *F. prausnitzii*, will ultimately allow better comprehension of microbial regulation of GI function.

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Abbreviations

| | |
|-------|---|
| ANOVA | Analysis of variance |
| ATCC | American Type Culture Collection |
| BHI | Brain-heart infusion |
| CD | Cluster of differentiation |
| CFU | Colony-forming unit |
| DAMP | Damage-associated molecular pattern |
| DC | Dendritic cell |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMSO | Dimethyl sulphoxide |
| DNA | Deoxyribonucleic acid |
| DO | Dissolved oxygen |
| DSM | Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms) |
| DSS | Dextran sodium sulphate |
| FBS | Foetal bovine serum |
| GALT | Gut-associated lymphoid tissue |
| GI | Gastrointestinal |
| HEK | Human embryonic kidney |

Abbreviations

| | |
|----------------|---|
| HKLM | Heat-killed <i>Listeria monocytogenes</i> |
| IBD | Inflammatory bowel disease |
| IBS | Irritable bowel syndrome |
| IEC | Intestinal epithelial cell |
| IFN- γ | Interferon gamma |
| IKK | Inhibitor of kappa B kinase |
| IL | Interleukin |
| IRAK | Interleukin-1 receptor-associated kinase |
| I κ B | Inhibitor of kappa B |
| LPS | Lipopolysaccharide |
| LSD | Least Significant Difference |
| M199 Std | M199 Standard medium |
| MAPK | Mitogen-activated protein kinase |
| MOI | Multiplicity of infection |
| MyD88 | Myeloid differentiation primary response protein 88 |
| NCBI | National Center for Biotechnology Information |
| NEAA | Non-Essential Amino Acid |
| NF- κ B | Nuclear factor-kappa B |
| NOD | Nucleotide-binding and oligomerisation domain |
| XX | |

| | |
|----------------|--|
| OD | Optical density |
| ODS | Output delivery system |
| PAMP | Pathogen-associated molecular pattern |
| PBMC | Peripheral blood mononuclear cell |
| PBS | Phosphate-buffered saline |
| PCR | Polymerase chain reaction |
| PPAR- γ | Peroxisome proliferator-activated receptor gamma |
| PRR | Pattern recognition receptor |
| rRNA | Ribosomal ribonucleic acid |
| SCFA | Short-chain fatty acid |
| SEM | Standard error of the mean |
| SFB | Segmented filamentous bacteria |
| sIgA | Secretory immunoglobulin A |
| TEER | Trans-epithelial electrical resistance |
| TJ | Tight junction |
| TLR | Toll-like receptor |
| TNBS | 2,4,6-Trinitrobenzenesulfonic acid |
| TNF- α | Tumour necrosis factor alpha |
| TOLLIP | Toll-interacting protein |

Abbreviations

| | |
|-------|--|
| TRAF | TNF receptor associated factor |
| TRIF | TIR-domain-containing adapter-inducing interferon beta |
| YCFAG | Yeast extract, casitone, fatty acid, glucose |
| ZO | Zona occludens |