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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  
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Massey University  
Manawatū, New Zealand

Eva Maier

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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- $\alpha$ ) treated Caco-2 cells. Live and growing *F. prausnitzii* did not alter the TEER across healthy Caco-2 cells. However, under TNF- $\alpha$  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

## *Abstract*

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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# Table of contents

|   |      |
|---|------|
| Abstract .....  | I    |
| Acknowledgements.....   | III  |
| List of figures.....  | XIII |
| List of tables .....  | XVII |
| Abbreviations.....  | XIX  |
| Introduction .....  | 1    |
| CHAPTER ONE: Review of literature <sup>†</sup> .....                                  | 3    |
| 1.1. Human gastrointestinal microbiota.....   | 4    |
| 1.1.1. Composition.....   | 4    |
| 1.1.2. Establishment and compositional changes associated with ageing .....           | 8    |
| 1.1.3. The role of commensal bacteria in human health .....                           | 9    |
| 1.2. Intestinal barrier function.....   | 10   |
| 1.2.1. Components .....   | 11   |
| 1.2.1.1. Structure and regulation of tight junctions.....                             | 13   |
| 1.2.2. Regulation by commensal and probiotic bacteria .....                           | 15   |
| 1.3. Microbial regulation of immune functions in the gastrointestinal tract .....     | 17   |
| 1.3.1. Microbial influence on development and function of the immune system .....     | 18   |
| 1.3.2. The role of innate signalling in maintaining immune homeostasis .....          | 19   |
| 1.3.3. Microbial modulation of the function of epithelial cells and immune cells..... | 26   |
| 1.4. Approaches to manipulate the gastrointestinal microbiota to promote health.....  | 28   |
| 1.4.1. Classical probiotic concept.....   | 28   |
| 1.4.2. Commensal obligate anaerobes as next-generation probiotics .....               | 29   |
| 1.4.2.1. <i>Faecalibacterium prausnitzii</i> .....                                    | 31   |

## Table of contents

|   |    |
|---|----|
| 1.4.2.2. <i>Bacteroides thetaiotaomicron</i> .....  | 38 |
| 1.4.2.3. <i>Bacteroides fragilis</i> .....  | 40 |
| 1.4.2.4. <i>Akkermansia muciniphila</i> .....   | 41 |
| 1.4.2.5. <i>Bacteroides uniformis</i> .....   | 44 |
| 1.4.2.6. Segmented filamentous bacteria .....   | 45 |
| 1.5. Dual-environment co-culture models to study host-microbe interactions .....  | 46 |
| 1.6. Aims and structure of the dissertation .....   | 52 |
| CHAPTER TWO: <i>Faecalibacterium prausnitzii</i> strain characterisation and growth optimisation in the co-culture model† ..... | 57 |
| 2.1. Introduction .....   | 58 |
| 2.2. Hypothesis and aims .....  | 59 |
| 2.3. Methods .....  | 60 |
| 2.3.1. <i>F. prausnitzii</i> cell culture .....   | 60 |
| 2.3.2. Bacterial culture medium .....   | 60 |
| 2.3.2.1. Anaerobic yeast extract, casitone, fatty acid, glucose broth .....   | 62 |
| 2.3.2.2. Anaerobic brain-heart infusion broth .....   | 64 |
| 2.3.2.3. Anaerobic brain-heart infusion agar .....  | 64 |
| 2.3.3. Long term storage of bacterial cultures .....  | 66 |
| 2.3.4. 16S rRNA gene sequencing .....   | 66 |
| 2.3.4.1. Bacterial DNA extraction .....   | 66 |
| 2.3.4.2. Polymerase chain reaction of the 16S rRNA genes .....  | 67 |
| 2.3.4.3. Sanger sequencing and database comparison .....  | 67 |
| 2.3.5. Enumeration of bacteria .....  | 70 |
| 2.3.5.1. Petroff-Hausser counting chamber .....   | 70 |
| 2.3.5.2. Titration of <i>F. prausnitzii</i> viable cells .....  | 71 |

|  |    |
|--|----|
| 2.3.6. Gram staining.....  | 71 |
| 2.3.7. Growth curves.....  | 72 |
| 2.3.8. Culture of intestinal epithelial cells (Caco-2).....  | 73 |
| 2.3.8.1. Maintenance of Caco-2 cells .....   | 74 |
| 2.3.8.2. Passaging of Caco-2 cells.....  | 74 |
| 2.3.8.3. Long term storage of Caco-2 cells.....  | 76 |
| 2.3.8.4. Recovering Caco-2 cells from liquid nitrogen .....  | 76 |
| 2.3.8.5. Counting Caco-2 cells.....  | 77 |
| 2.3.8.6. Growing Caco-2 cells on Transwell inserts .....   | 77 |
| 2.3.8.7. Measuring TEER to assess differentiation of Caco-2 monolayers.....  | 78 |
| 2.3.9. Apical anaerobic co-culture model .....   | 79 |
| 2.3.9.1. Setting up the apical anaerobic co-culture model.....   | 81 |
| 2.3.10. Viability of <i>F. prausnitzii</i> in different culture media .....  | 83 |
| 2.3.11. TEER and viability of Caco-2 cells using aerobic media.....  | 84 |
| 2.3.12. TEER and viability of Caco-2 cells using anaerobic media .....   | 85 |
| 2.3.13. Viability of the <i>F. prausnitzii</i> strains in the apical anaerobic co-culture model using mixtures of cell and bacterial culture media ..... | 86 |
| 2.3.14. Trypan blue viability test of Caco-2 cells grown on Transwell inserts.....   | 87 |
| 2.3.15. Neutral red uptake assay.....  | 87 |
| 2.3.16. Statistical analysis .....   | 89 |
| 2.4. Results.....  | 90 |
| 2.4.1. 16S rRNA gene sequencing.....   | 90 |
| 2.4.2. Gram staining.....  | 91 |
| 2.4.3. Growth curves.....  | 91 |
| 2.4.4. Viability of the <i>F. prausnitzii</i> strains in cell culture medium.....  | 94 |

## Table of contents

|   |     |
|---|-----|
| 2.4.5. Viability of the <i>F. prausnitzii</i> strains in cell culture medium supplemented with acetate .....  | 94  |
| 2.4.6. Supplementation of cell culture medium with bacterial culture medium .....   | 96  |
| 2.4.6.1. Effect of ratios of cell and bacterial culture medium on TEER and viability of Caco-2 cells in conventional conditions .....                     | 99  |
| 2.4.6.2. Effect of ratios of cell and bacterial culture medium on TEER and viability of Caco-2 cells in the apical anaerobic co-culture model .....       | 102 |
| 2.4.6.3. Viability of the <i>F. prausnitzii</i> strains in a mixture of cell and bacterial culture medium .....   | 105 |
| 2.4.6.4. Viability of the <i>F. prausnitzii</i> strains in the apical anaerobic co-culture model using mixtures of cell and bacterial culture media ..... | 107 |
| 2.5. Discussion .....   | 107 |
| CHAPTER THREE: Effect of <i>F. prausnitzii</i> on intestinal barrier function <sup>†</sup> .....  | 115 |
| 3.1. Introduction .....   | 116 |
| 3.2. Hypothesis and aims .....  | 117 |
| 3.3. Methods .....  | 117 |
| 3.3.1. Culture of <i>F. prausnitzii</i> .....   | 117 |
| 3.3.2. Caco-2 cell culture .....  | 118 |
| 3.3.3. Trans-epithelial electrical resistance assay using different apical media .....  | 119 |
| 3.3.4. Trans-epithelial electrical resistance assay with TNF- $\alpha$ -treated Caco-2 cells ..   | 120 |
| 3.3.4.1. Viability of <i>F. prausnitzii</i> in co-culture with TNF- $\alpha$ -treated Caco-2 cells  | 121 |
| 3.3.5. Statistical analysis .....   | 122 |
| 3.4. Results .....  | 123 |
| 3.4.1. Trans-epithelial electrical resistance assay with different apical media .....   | 123 |
| 3.4.2. Trans-epithelial electrical resistance assay with TNF- $\alpha$ -treated Caco-2 cells using 50% BHI as apical medium .....                         | 123 |
| 3.4.3. Trans-epithelial electrical resistance assay with TNF- $\alpha$ -treated Caco-2 cells using M199 TEER as apical medium .....                       | 127 |

|   |     |
|---|-----|
| 3.4.4. Viability of <i>F. prausnitzii</i> in co-culture with TNF- $\alpha$ -treated Caco-2 cells .....                          | 131 |
| 3.5. Discussion .....   | 136 |
| CHAPTER FOUR: Adaptation of the Toll-like receptor activation assay to the apical anaerobic co-culture model <sup>†</sup> ..... | 145 |
| 4.1. Introduction .....   | 146 |
| 4.2. Hypothesis and aims .....  | 147 |
| 4.3. Methods.....   | 148 |
| 4.3.1. Culture of HEK293-TLR cells.....   | 148 |
| 4.3.1.1. Maintenance of HEK293-TLR cells .....  | 148 |
| 4.3.1.2. Passaging of HEK293-TLR cells .....  | 150 |
| 4.3.1.3. Long term storage of HEK293-TLR cells.....   | 152 |
| 4.3.1.4. Recovering HEK293-TLR cells from liquid nitrogen .....   | 152 |
| 4.3.2. Stable transfection of HEK293-TLR cells.....   | 153 |
| 4.3.2.1. Growth of pNiFty2-Luc-transformed bacteria .....   | 153 |
| 4.3.2.2. Extraction of pNiFty2-Luc plasmid DNA.....   | 153 |
| 4.3.2.3. Determining zeocin sensitivity of the HEK293-TLR cells .....   | 155 |
| 4.3.2.4. Stable transfection and selection of stable clones .....   | 156 |
| 4.3.3. Culture of HEK293-TLR-Luc cells .....  | 158 |
| 4.3.3.1. Passaging of HEK293-TLR-Luc cells using trypsin .....  | 158 |
| 4.3.3.2. Coating of Transwell inserts with collagen I .....   | 159 |
| 4.3.4. Toll-like receptor activation assay in conventional conditions .....   | 159 |
| 4.3.5. Growth optimisation of <i>F. prausnitzii</i> in cell culture medium .....  | 162 |
| 4.3.5.1. Culture of the <i>F. prausnitzii</i> strains .....   | 162 |
| 4.3.5.2. Viability of the <i>F. prausnitzii</i> strains in different culture media.....   | 162 |
| 4.3.6. Adaptation of the Toll-like receptor activation assay to the apical anaerobic co-culture model.....                      | 163 |

## Table of contents

|   |     |
|---|-----|
| 4.3.6.1. Toll-like receptor activation assay using Transwell inserts.....   | 166 |
| 4.3.6.2. Determining the viability and attachment of HEK293-TLR2-Luc cells under different assay conditions.....                          | 167 |
| 4.3.6.3. Toll-like receptor activation assay in apical anaerobic conditions .....   | 168 |
| 4.3.6.4. Dissolved oxygen concentration in the apical and basal compartments ...  | 168 |
| 4.3.7. Viability of <i>F. prausnitzii</i> A2-165 in co-culture with HEK293-TLR2-Luc cells in the apical anaerobic co-culture model.....   | 170 |
| 4.3.8. Statistical analysis.....  | 172 |
| 4.4. Results .....  | 173 |
| 4.4.1. Zeocin sensitivity of HEK293-TLR2 cells .....  | 173 |
| 4.4.2. Toll-like receptor activation assay in conventional conditions.....  | 175 |
| 4.4.3. Viability of the three <i>F. prausnitzii</i> strains in cell culture medium.....   | 175 |
| 4.4.4. Viability of the <i>F. prausnitzii</i> strains in cell culture medium supplemented with acetate .....                              | 178 |
| 4.4.5. Viability of the <i>F. prausnitzii</i> strains in cell culture medium supplemented with bacterial culture medium .....             | 178 |
| 4.4.6. Toll-like receptor activation assay with Transwell inserts.....  | 181 |
| 4.4.7. Approaches to improve the attachment of HEK293-TLR2-Luc cells in the apical anaerobic co-culture model.....                        | 183 |
| 4.4.8. Toll-like receptor activation assay in apical anaerobic conditions.....  | 191 |
| 4.4.9. Apical and basal dissolved oxygen concentrations .....   | 194 |
| 4.4.10. Viability of <i>F. prausnitzii</i> A2-165 in co-culture with HEK293-TLR2-Luc cells with different apical medium compositions..... | 195 |
| 4.5. Discussion.....  | 199 |
| CHAPTER FIVE: TLR activation by <i>F. prausnitzii</i> in conventional and apical anaerobic conditions <sup>†</sup> .....                  | 207 |
| 5.1. Introduction.....  | 208 |
| 5.2. Hypothesis and aims.....   | 209 |

|   |     |
|---|-----|
| 5.3. Methods.....   | 210 |
| 5.3.1. Culture of <i>F. prausnitzii</i> .....   | 210 |
| 5.3.2. Preparation of aerobic brain-heart infusion broth.....   | 210 |
| 5.3.3. Preparation of the HEK293-TLR-Luc cells for the TLR activation assays .....  | 211 |
| 5.3.3.1. Maintenance of HEK293-TLR-Luc cells.....   | 211 |
| 5.3.3.2. Seeding HEK293-TLR-Luc cells on Transwell inserts .....  | 212 |
| 5.3.4. Preparation of the positive controls.....  | 212 |
| 5.3.5. TLR activation assays with live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions..... | 214 |
| 5.3.5.1. Determination of the multiplicity of infection for the TLR activation assays .....                                     | 216 |
| 5.3.6. Phylogenetic analysis of bacterial cell envelope marker genes.....   | 217 |
| 5.3.7. Statistical analysis .....   | 217 |
| 5.4. Results.....   | 218 |
| 5.4.1. TLR2 activation .....  | 219 |
| 5.4.1.1. Determination of the multiplicity of infection .....   | 219 |
| 5.4.1.2. TLR2 activation in conventional and apical anaerobic conditions.....   | 219 |
| 5.4.2. TLR2/6 activation in conventional and apical anaerobic conditions.....   | 222 |
| 5.4.3. TLR4 activation in conventional and apical anaerobic conditions.....   | 227 |
| 5.4.4. Activation of the control cell line in conventional and apical anaerobic conditions .....                                | 230 |
| 5.4.5. Phylogenetic analysis of bacterial cell envelope marker genes.....   | 230 |
| 5.5. Discussion .....   | 234 |
| CHAPTER SIX: General Discussion.....  | 247 |
| 6.1. Background .....   | 248 |
| 6.2. Summary of results.....  | 249 |

*Table of contents*

|  |     |
|--|-----|
| 6.3. General discussion, limitations and future perspectives .....                                 | 252 |
| 6.3.1. Method development .....  | 252 |
| 6.3.2. Effect of <i>F. prausnitzii</i> on intestinal barrier function and immune homeostasis ..... | 254 |
| 6.4. Concluding remarks .....  | 260 |
| References .....   | 263 |

## List of figures

|  |     |
|--|-----|
| Figure 1.1 Components of the intestinal barrier. ....  | 12  |
| Figure 1.2 Tight junction structure. ....  | 14  |
| Figure 1.3 Schematic diagram of TLR distribution and signalling. ....  | 21  |
| Figure 1.4 Effects of probiotic bacteria divided into three levels of action. ....   | 30  |
| Figure 1.5 Proposed immunomodulatory mechanisms of four examples of obligate anaerobic commensals. ....  | 32  |
| Figure 1.6 Comparison of four examples of dual-environment co-culture models. ....   | 48  |
| Figure 1.7 Structure of the dissertation. ....   | 56  |
| Figure 2.1 Schematic diagram of a single well of the apical anaerobic co-culture model. ....   | 80  |
| Figure 2.2 Prototype II co-culture chamber used for the apical anaerobic co-culture model. ....  | 82  |
| Figure 2.3 Gram staining of the three <i>F. prausnitzii</i> strains. ....  | 92  |
| Figure 2.4 Growth curves for the three <i>F. prausnitzii</i> strains in BHI broth. ....  | 93  |
| Figure 2.5 OD <sub>600nm</sub> (A) and normalised change in OD <sub>600nm</sub> (%) (B) of the three <i>F. prausnitzii</i> strains in anaerobic cell culture medium. ....      | 95  |
| Figure 2.6 OD <sub>600nm</sub> of the three <i>F. prausnitzii</i> strains in anaerobic M199+NEAA with or without acetate supplementation. ....                                 | 97  |
| Figure 2.7 Normalised change in OD <sub>600nm</sub> (%) of the three <i>F. prausnitzii</i> strains in anaerobic M199+NEAA with or without acetate supplementation. ....        | 98  |
| Figure 2.8 Viability of Caco-2 cells exposed to cell and bacterial culture medium in conventional conditions (5% CO <sub>2</sub> in air atmosphere). ....                      | 100 |
| Figure 2.9 TEER across Caco-2 cell monolayers exposed to mixtures of cell and bacterial culture medium in conventional conditions (5% CO <sub>2</sub> in air atmosphere). .... | 101 |
| Figure 2.10 Viability of Caco-2 cells exposed to mixtures of cell and bacterial culture medium in the apical anaerobic co-culture model. ....                                  | 103 |
| Figure 2.11 TEER across Caco-2 cell monolayers exposed to mixtures of cell and bacterial culture medium in the apical anaerobic co-culture model. ....                         | 104 |
| Figure 2.12 Viability of the three <i>F. prausnitzii</i> strains in cell culture medium supplemented with bacterial culture medium. ....                                       | 106 |
| Figure 2.13 Viability of the three <i>F. prausnitzii</i> strains in the apical anaerobic co-culture model with Caco-2 cells. ....  | 108 |
| Figure 3.1 Change in TEER across healthy Caco-2 monolayers co-cultured with live <i>F. prausnitzii</i> using 25 and 50% BHI as apical medium. ....                             | 124 |
| Figure 3.2 Change in TEER across Caco-2 monolayers with or without TNF- $\alpha$ treatment using 50% BHI as apical medium. ....  | 125 |
| Figure 3.3 Change in TEER across healthy Caco-2 monolayers co-cultured with live or UV-killed <i>F. prausnitzii</i> using 50% BHI as apical medium. ....                       | 126 |

## List of figures

|  |     |
|--|-----|
| Figure 3.4 Change in TEER across healthy Caco-2 monolayers co-cultured with live or UV-killed <i>F. prausnitzii</i> using 50% BHI as apical medium. ....                         | 128 |
| Figure 3.5 Change in TEER across TNF- $\alpha$ -treated Caco-2 monolayers co-cultured with live or UV-killed <i>F. prausnitzii</i> using 50% BHI as apical medium. ....          | 129 |
| Figure 3.6 Change in TEER across Caco-2 monolayers with or without TNF- $\alpha$ treatment using M199 TEER as apical medium. ....  | 130 |
| Figure 3.7 Change in TEER across healthy Caco-2 monolayers co-cultured with live or UV-killed <i>F. prausnitzii</i> using M199 TEER as apical medium. ....                       | 132 |
| Figure 3.8 Change in TEER across TNF- $\alpha$ -treated Caco-2 monolayers co-cultured with live or UV-killed <i>F. prausnitzii</i> using M199 TEER as apical medium. ....        | 133 |
| Figure 3.9 Change in TEER across TNF- $\alpha$ -treated Caco-2 monolayers co-cultured with live or UV-killed <i>F. prausnitzii</i> using M199 TEER as apical medium. ....        | 134 |
| Figure 3.10 Viability of the three <i>F. prausnitzii</i> strains co-cultured with TNF- $\alpha$ -treated Caco-2 cells using 50% BHI as apical medium. ....                       | 135 |
| Figure 3.11 Viability of the three <i>F. prausnitzii</i> strains co-cultured with healthy or TNF- $\alpha$ -treated Caco2-cells using M199 TEER as apical medium. ....           | 137 |
| Figure 4.1 Overview of the stable transfection of HEK-TLR cell lines with a NF- $\kappa$ B inducible luciferase plasmid. ....  | 154 |
| Figure 4.2 Schematic diagram of the TLR activation assay. ....   | 160 |
| Figure 4.3 Adaptation of the TLR activation assay to the apical anaerobic co-culture model. ....   | 164 |
| Figure 4.4 HEK293-TLR-Luc cells in the apical anaerobic co-culture model. ....   | 165 |
| Figure 4.5 Methods to measure the DO concentrations in the apical and basal compartments of the co-culture chamber. ....   | 169 |
| Figure 4.6 Zeocin sensitivity of HEK293-TLR2 cells. ....   | 174 |
| Figure 4.7 TLR activation assay in conventional conditions. ....   | 176 |
| Figure 4.8 OD <sub>600nm</sub> (A) and normalised change in OD <sub>600nm</sub> (%) (B) of the three <i>F. prausnitzii</i> strains in anaerobic cell culture medium (DMEM). .... | 177 |
| Figure 4.9 OD <sub>600nm</sub> of the three <i>F. prausnitzii</i> strains in anaerobic DMEM with or without acetate supplementation. ....  | 179 |
| Figure 4.10 Normalised change in OD <sub>600nm</sub> (%) of the three <i>F. prausnitzii</i> strains in anaerobic DMEM with or without acetate supplementation. ....              | 180 |
| Figure 4.11 Viability of the three <i>F. prausnitzii</i> strains in cell culture medium (DMEM+FBS) supplemented with bacterial culture medium (BHI). ....                        | 182 |
| Figure 4.12 TLR activation assay using Transwell inserts. ....   | 184 |
| Figure 4.13 TLR activation assay using cells harvested with or without TrypLE. ....  | 188 |
| Figure 4.14 HEK293-TLR2-Luc cells exposed to bacterial culture medium and a mixture of cell and bacterial culture media in the apical anaerobic co-culture model. ....           | 190 |
| Figure 4.15 Viability of HEK293-TLR2-Luc cells exposed to different apical media in apical anaerobic and conventional conditions. ....   | 192 |

|  |     |
|--|-----|
| Figure 4.16 TLR activation assay in conventional and apical anaerobic conditions.....  | 193 |
| Figure 4.17 Basal DO levels when incubating HEK293-TLR2-Luc cells in the apical anaerobic co-culture model. ....   | 196 |
| Figure 4.18 Viability of <i>F. prausnitzii</i> A2-165 before and after the co-culture with HEK293-TLR2-Luc cells in the apical anaerobic co-culture model. ....                        | 197 |
| Figure 4.19 Viability of <i>F. prausnitzii</i> A2-165 after 6 h of co-culture with HEK293-TLR2-Luc cells in the apical anaerobic co-culture model.....                                 | 198 |
| Figure 5.1 Determination of the MOI for the TLR activation assays.....   | 220 |
| Figure 5.2 TLR2 activation (observed values) by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....   | 221 |
| Figure 5.3 TLR2 activation (fitted values) by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....   | 223 |
| Figure 5.4 TLR2 activation (fitted values) by <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....  | 224 |
| Figure 5.5 TLR2/6 activation (observed values) by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....                                       | 225 |
| Figure 5.6 TLR2/6 activation (fitted values) by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....   | 226 |
| Figure 5.7 TLR2/6 activation (fitted values) by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....   | 228 |
| Figure 5.8 TLR4 activation by the controls and by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. ....                                       | 229 |
| Figure 5.9 Activation of the control cell line (HEK293-null-Luc) by the controls and by live and UV-killed <i>F. prausnitzii</i> in conventional and apical anaerobic conditions. .... | 231 |
| Figure 5.10 Evolutionary relationships of taxa based on the phylogenetic analysis of the bacterial cell envelope marker gene Hsp60.....  | 232 |
| Figure 5.11 Evolutionary relationships of taxa based on the phylogenetic analysis of the bacterial cell envelope marker gene Hsp70.....  | 233 |
| Figure 5.12 Partial sequence alignment of the Hsp60 protein.....   | 235 |
| Figure 5.13 Partial sequence alignment of the Hsp70 protein.....   | 236 |



## List of tables

|  |     |
|--|-----|
| Table 1.1 Summary of advantages and disadvantages of four examples of dual-environment co-culture models .....                             | 49  |
| Table 2.1 Details of the three <i>F. prausnitzii</i> strains used in this PhD project.....   | 61  |
| Table 2.2 Components used to prepare anaerobic yeast extract, casitone, fatty acid, glucose (YCFAG) broth.....                             | 63  |
| Table 2.3 Components used for the preparation of anaerobic brain-heart infusion broth. ..  | 65  |
| Table 2.4 Oligonucleotide sequences of the forward and reverse primers used for the amplification of the bacterial 16S rRNA gene. ....     | 68  |
| Table 2.5 PCR program used for the amplification of the bacterial 16S rRNA gene. ....  | 69  |
| Table 2.6 Composition of the Medium 199 standard medium (M199 Std) used for culturing Caco-2 cells. ....                                   | 75  |
| Table 4.1 HEK293 cell lines expressing different cell surface TLRs used in this study...   | 149 |
| Table 4.2 Composition of the growth media used to culture HEK293-TLR cells (without selective antibiotics).....                            | 151 |
| Table 4.3 Summary of experiments to improve the attachment of HEK293-TLR2-Luc cells in the co-culture model.....                           | 185 |
| Table 5.1 Details of the positive controls used for the TLR activation assays including concentrations of stock and working solutions..... | 213 |



## 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- $\gamma$  | Interferon gamma                                    |
| IKK            | Inhibitor of kappa B kinase                         |
| IL             | Interleukin   |
| IRAK           | Interleukin-1 receptor-associated kinase            |
| I $\kappa$ 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- $\kappa$ 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- $\gamma$ | 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- $\alpha$  | 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   |