UNIVERSITY OF BIRMINGHAM | Microbes, Infection and Microbiomes

# Unmasking the mechanisms behind the Immunomodulatory effects of *Bifidobacterium breve* UCC2003


Contact email: c.n.l.cook@bham.ac.uk

**Charlotte Jones[1], Ian O'Neill[2], Sree Gowrinadh Javvadi[2], Douwe van Sinderen[3] and Lindsay J Hall[1, 2]**

1. Department of Microbes, Infection and Microbiomes, School of Infection, Inflammation and Immunology, College of Medicine Health, University of Birmingham, Birmingham, UK
2. Food, Microbiome and Health, Quadram Institute Bioscience, Norwich Research Park, Norwich, UK
3. APC Microbiome Ireland & School of Microbiology, University College Cork, Ireland


wellcome

don whitley scientific culturing innovation

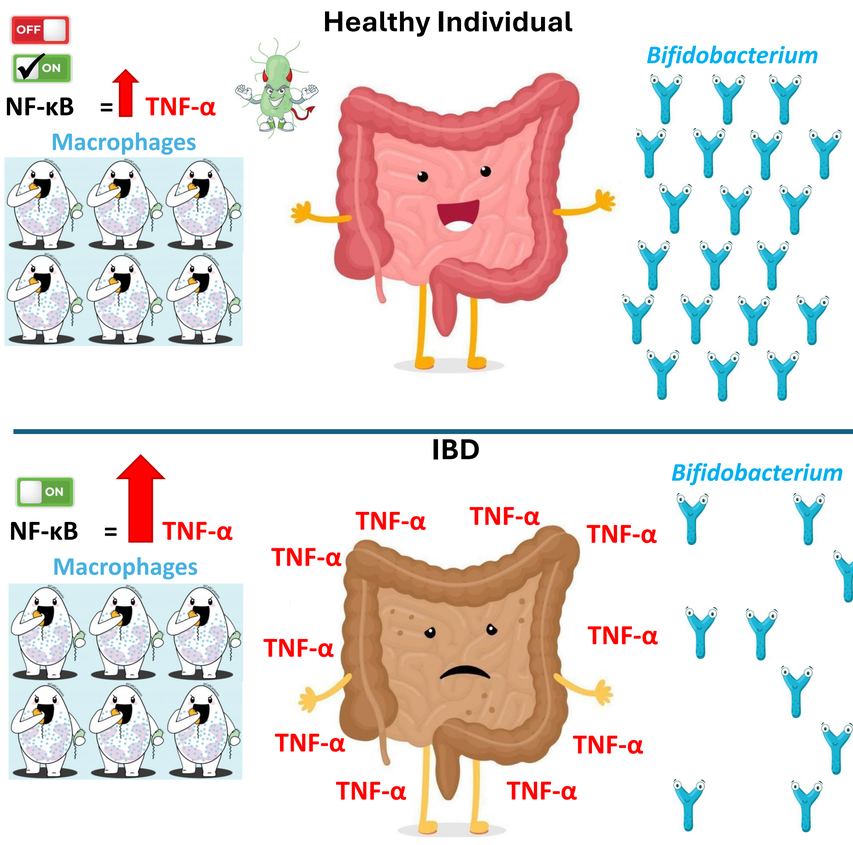
## 1. Introduction:

- *Bifidobacterium* species play a vital role in early life gut development, promote gut health and are used as probiotics.
- Inflammatory Bowel Disease (IBD) patients often have over-activated macrophages, increased inflammatory cytokine levels, especially TNF-α and reduced *Bifidobacterium* levels.
- *B. breve* UCC2003, isolated from a breastfed infant, shows protective effects and improves gut barrier function[1,2,3], but its role in IBD treatment is unclear.

## 2. Aim:

1. Investigate the gene-specific effects of *B. breve* UCC2003 on inflammation using a genome-wide mutagenesis approach – Tn5 insertion[4].
2. Assess the potential of *B. breve* UCC2003 as a probiotic or therapeutic for IBD treatment.

## 3. What is Inflammatory Bowel Disease?




- NF-κB in macrophages activates upon contact with pathogens.
- NF-κB activation triggers TNF-α production, causing inflammation.
- After pathogen clearance, NF-κB is deactivated, and TNF-α production stops.
- NF-κB in macrophages activated constantly.
- Constant activation of NF-κB results in high levels of TNF-α production = chronic inflammation.
- *Bifidobacterium* species = low relative abundance.

## 4. Identification of Tn5 insertion sites in *B. breve* UCC2003

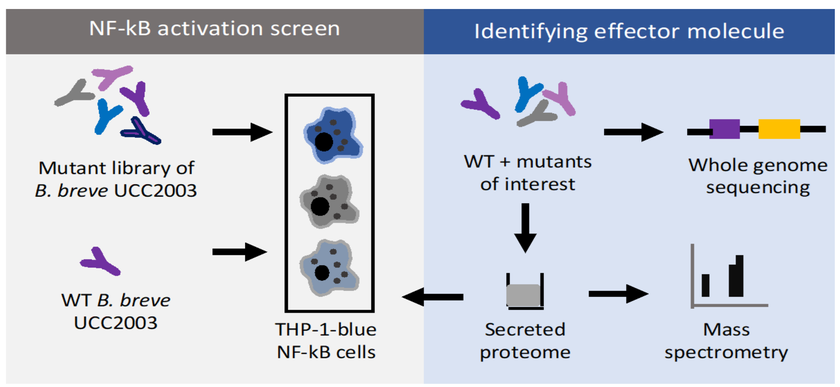



**Figure 1. Overview of pipeline**

| B. breve Tn5 Mutant | Gene KO | Gene Function |
|---|---|---|
| 10:D9 | Intergenic region Bbr_RS11910 - Bbr_0311 | Bbr_RS11910 – hypothetical protein/Beta glucuronidase, Bbr_0311 – Transposase IS605 OrfB family |
| 41:B1 | Bbr_1651 | Conserved hypothetical protein with leucine rich repeat variant |
| 43:C10 | Bbr_1280 | hisA:– involved in L-histidine biosynthesis. |
| 48:D12 | Bbr_1299 lspA | Lipoprotein signal peptidase |
| 24:H1 | Bbr_RS11760 | Integrase |
| 20:A11 | Bbr_0250 | Nonfunctional predicted LacI-type transcriptional regulator |

**Table 1. *B. breve* UCC2003 Tn5 mutants of interest and their disrupted genes**

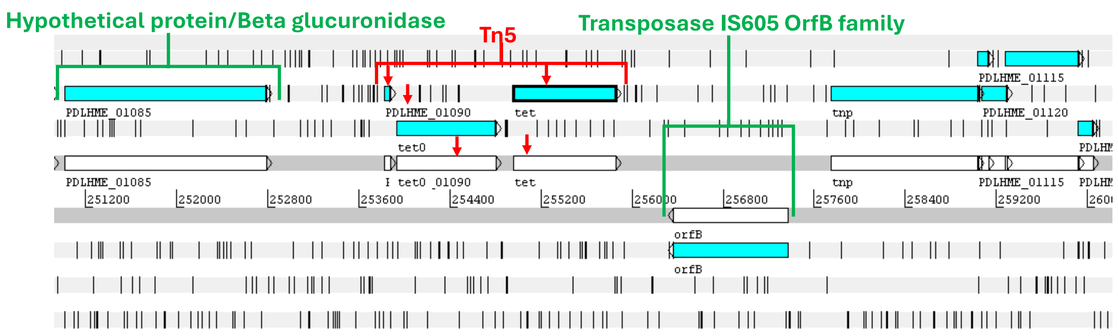



**Figure 2. Example of Tn5 insertion and location within *B. breve* UCC2003 10:D9 mutant genome using Artemis**

## 5. Immunomodulatory effects observed due to secreted molecule

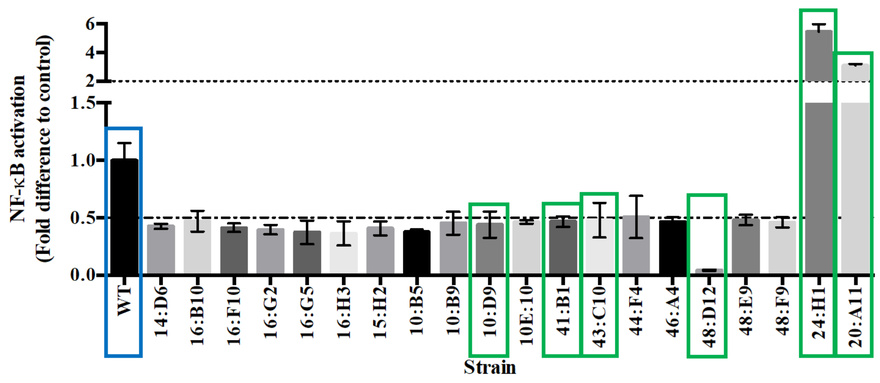



**Figure 3. NF-κB activation of *B. breve* UCC2003 mutants with conditioned media is reduced in some Tn5 mutants and increased in 24:H1 and 20:A11 compared to the WT. WT shown in blue and Tn5 mutants of interest shown in green.**

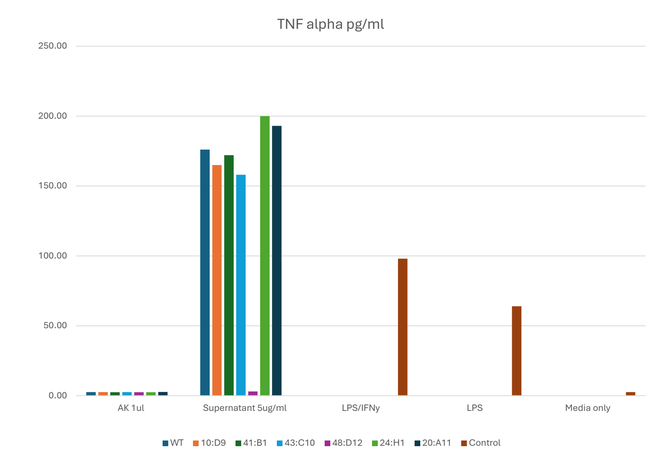



**Figure 4. Levels of secreted TNF-α (pg/ml) produced by THP-1 cells stimulated with acid killed and conditioned media of *B. breve* UCC2003 WT and mutants .**

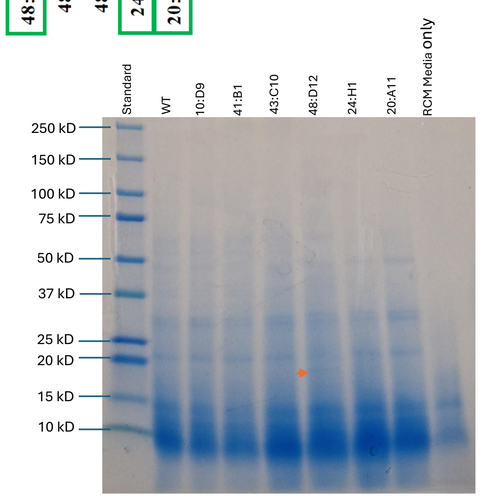
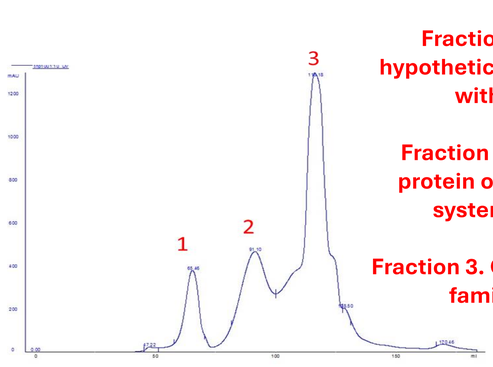



**Figure 5. SDS-PAGE gel showing proteome extractions of condition media. Orange arrow shows band present only in 10:D9 proteome**

## 6. The molecule responsible for anti-inflammatory affects is >50kDa




**Fraction 1. Conserved hypothetical secreted protein with G5 domain**

**Fraction 2. Solute-binding protein of ABC transporter system for peptides**

**Fraction 3. Glycosyl hydrolases family 25 protein**

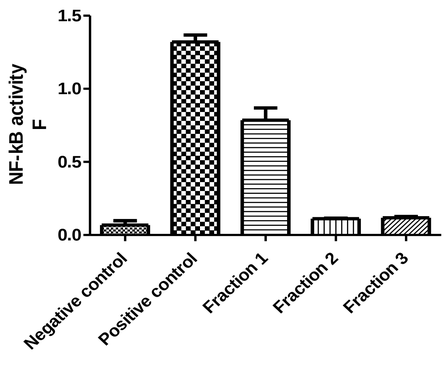



**Figure 6. Mass Spectrometry of >50kDa HPLC fraction of conditioned media showing 3 main peaks.**

**Figure 7. Fraction 2 and 3 show reduced NF-kB activity.**

**7. Discussion:** By pinpointing specific genes/molecules involved in the anti-inflammatory effects on *B. breve* UCC2003 WT and mutants observed, this research could pave the way for an alternative therapeutic approaches to manage chronic inflammation in IBD patients.

## 8. What next?

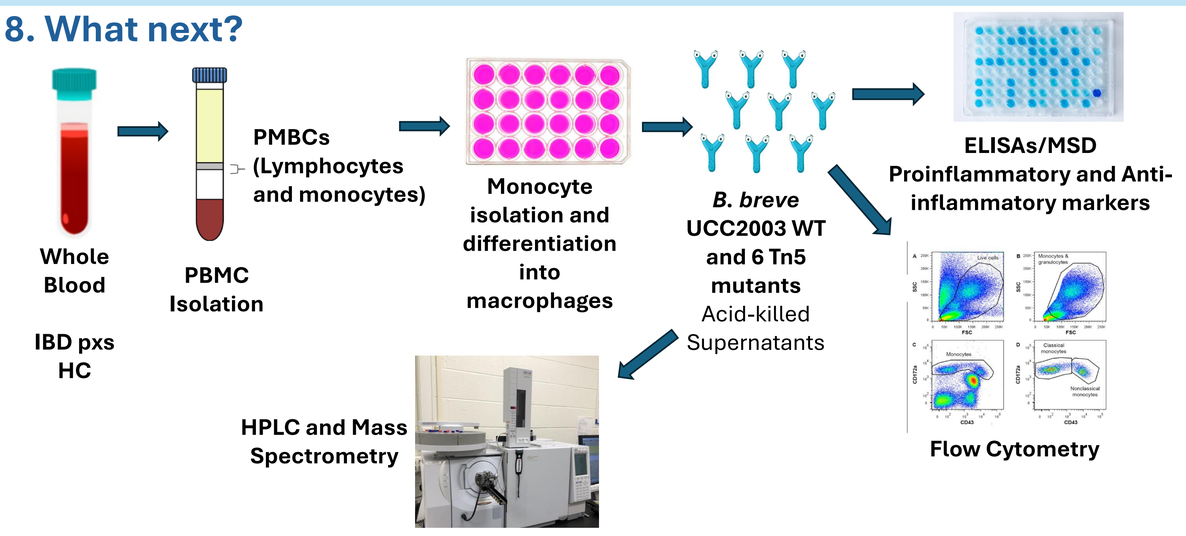



**Figure 7. Pipeline for processing of Peripheral Mononuclear Blood Cells (PBMCs) from Healthy Controls (HCs) and IBD patients and further analysis**

## 9. Future Directions:

- Perform assays with acid-killed *B. breve* UCC2003 WT and Tn5 mutants, and their conditioned media, on macrophages from HC and IBD patients (**Fig. 7**).
- Collect supernatants for cytokine profiling – looking at pro- and anti-inflammatory markers.
- Harvest other immune cells for flow cytometry to assess phenotype changes.
- Isolate and identify secreted molecules using size exclusion chromatography and mass spectrometry.

## References


(1) Fanning S, Hall L.J, Cronin M, Zomer A, MacSharry J, Goulding D, Motherway M.O, Shanahan F, Nally K, Dougan G, et al (2012b) Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. Proc. Natl. Acad. Sci. USA *109*, 2108–2113. (2) Fanning S, Hall L.J, and van Sinderen D (2012a) Bifidobacterium breve UCC2003 surface exopolysaccharide production is a beneficial trait mediating commensal-host interaction through immune modulation and pathogen protection. Gut Microbes *3*, 420–425. (3) Kiu R, Treveil A, Harnisch L.C, Caim S, Leclaire C, van Sinderen D, Korcsmaros T, and Hall L.J (2020) Bifidobacterium breve UCC2003 Induces a Distinct Global Transcriptomic Program in Neonatal Murine Intestinal Epithelial Cells. IScience *23*, 101336. (4) Ruiz L, Motherway MO, Lanigan N, van Sinderen D (2013) Transposon mutagenesis in Bifidobacterium breve: construction and characterization of a Tn5 transposon mutant library for Bifidobacterium breve UCC2003. PLoS One. May 30;8(5):e64699. doi: 10.1371/journal.pone.0064699. PMID: 23737995; PMCID: PMC3667832.