Gut commensal bacteria, associated with clinical remission in ulcerative colitis, protect and heal gut epithelial barrier through three novel pathways

Tim Sharpington[1], Fernanda Schreiber[1], Oliver Rimington[1], Isobel Whitehead[1], Trevor D. Lawley[1,2], Matthew J. Robinson[1] and Ghaith Bakdash[1]

[1] Microbiotica, Cambridge, CB10 1XL, UK

[2] Host-Microbiota Interactions Lab, Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge, CB10 1DR, UK

Introduction

- Precision microbiome profiling of a human fecal microbiota transplantation (FMT) study identified 8 bacteria associated with clinical response in mild-to-moderate Ulcerative Colitis (UC) patients. These 8 strains form **MB310**, a Live Biotherapeutic Product (LBP) for the treatment of UC
- In vitro primary human cell studies with MB310 species showed inflammatory response modulation and regulatory T cell (Treg) induction
- **Aim of study: explore MB310 species impact on barrier function and mechanisms of action behind their protective effect**

Methods

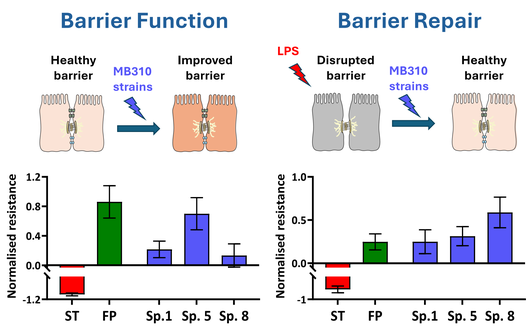
To understand how MB310 drives clinical benefit we tested how the individual species impacted barrier function

- **Barrier assays**: transepithelial electrical resistance on Caco-2 cells, untreated (Barrier Function) or damaged with LPS (Barrier Repair) to mimic inflammation. *Salmonella typhimurium* (ST) and *Fecaelibacterium prausnitzii* (FP) were used as negative and positive controls, respectively
- **Mechanisms of action** were investigated through
  - **Comparative genomics**: comparing strains of same species with positive and negative effect on barrier function
  - **Cloning** of novel protein into *E. coli*
  - **Tight junction protein expression**: measured by qPCR
  - **Metabolomics**: determination of bacterial metabolites in bacterial culture supernatants
  - **Blocking of bacterial metabolites:** determine the role of metabolites in barrier function by blocking receptors

Tim Sharpington CEO

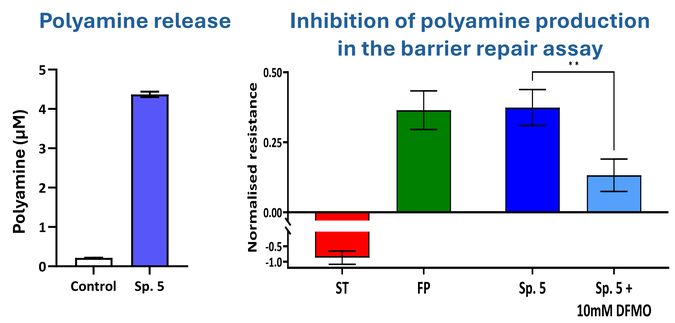
tsharpington@microbiotica.com

MB310 strains improve barrier function and repair



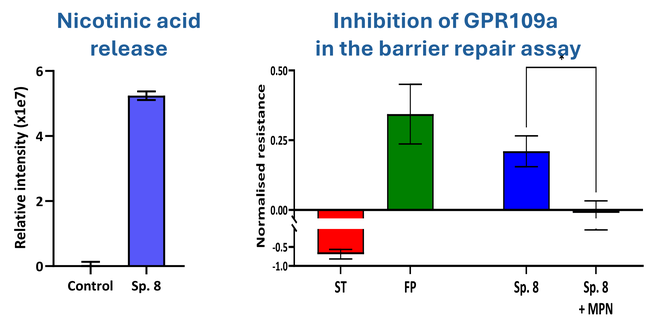
3 of the MB310 strains (species 1 , 5 and 8) enhance epithelial barrier function and repair

Species 5 induces epithelial barrier repair by release of polyamine



Species 5 produces high levels of polyamines. Blocking polyamine production by species 5 with Difluoromethylornithine (DFMO) inhibits the barrier repair

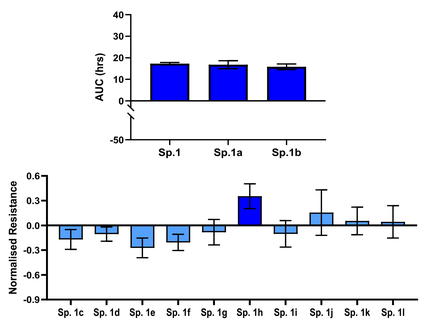
Species 8 induces epithelial barrier repair by release of nicotinic acid



Species 8 releases the metabolite nicotinic acid. The protective effect on barrier repair by species 8 is reversed by blocking the nicotinic acid receptor GPR109a with Mepenzolate (MPN)

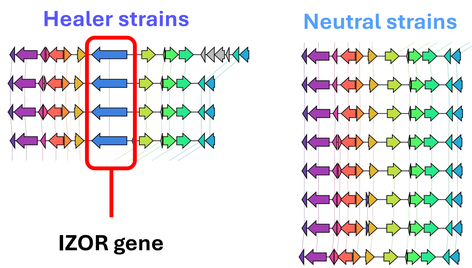
Novel internalin-like ZO1 regulator protein (IZOR) promotes protective barrier function effects in Sp. 1

Screen of 12 strains of Species 1 on barrier function



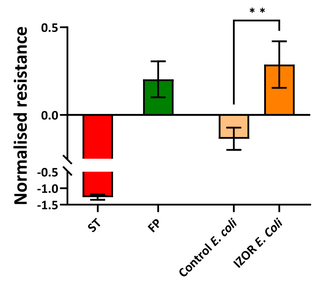
12 strains of Species 1 were tested in the barrier repair assay in 2 batches. 4 beneficial (healer) strains and 10 non-beneficial (neutral) strains were identified

Comparative genomics on Species 1 strains



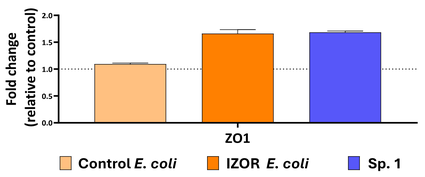
When comparing the genomes of healer strains and neutral strains only 1 gene was differentially present, internalin-like ZO1 regulator protein (IZOR). IZOR is a novel protein only present in a subset of species 1

Regulation of barrier function by IZOR-expressing *E. coli*



Transduction of *E. coli* with IZOR gene confers beneficial outcome in barrier function assay indicating it is sufficient for protective effect

Regulation of tight junction expression by IZOR



IZOR-expressing *E. coli* and species 1 regulate tight junction proteins in Caco-2 cells. Both strongly induce ZO1, which is key for barrier integrity

Conclusions

- The 8 MB310 species, identified as being associated with clinical benefit, interact with the host in different ways to oppose key UC disease pathologies
- 3 of the MB310 species improve gut epithelial barrier integrity, particularly promoting recovery of epithelium post inflammatory damage.
- These species act through 3 distinct mechanisms of action:
  - Species 1 expresses a novel protein, IZOR, which regulates the expression of tight junction proteins and mediates the barrier protective effects of species 1.
  - Species 5 and species 8 promote recovery of epithelium post pro-inflammatory damage by secretion of bacterial metabolites; polyamines and nicotinic acid, respectively
- A combination of anti-inflammatory and barrier repair address key UC disease pathologies, offering therapeutic potential for a broad spectrum of UC patients
- COMPOSER-1 is an ongoing Phase 1b trial testing MB310 in UC patients