Unravelling the molecular mechanisms underlying the therapeutic effects of *Bifidobacterium breve* MRx0004

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4D Pharma PLC is a pharmaceutical company focussed on developing live biotherapeutic products (LBPs) from the human gut microbiome. LBPs represent a new class of drugs that contain live organisms for the prevention, treatment or cure of disease. 4D Pharma currently has two clinical stage programmes (in IBS and IBD) and a strong pipeline of pre-clinical programmes in autoimmunity, inflammation, oncology and CNS disease.

Introduction

Members of the genus *Bifidobacterium* are associated with health-promoting effects. The effects of bifidobacteria on the host are largely mediated through surface components including exopolysaccharide (EPS) and surface-associated proteins[1]. EPS has functional roles in bacterial survival[2] and persistence[3], and modulates the host immune response in a strain-specific manner[4].

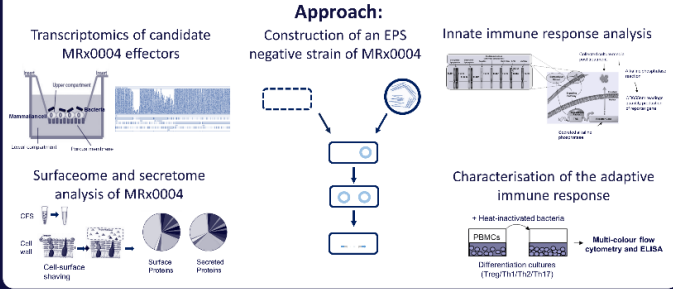
Bifidobacterium breve MRx0004 was isolated from the intestinal tract of a healthy human donor and is a promising next generation live biotherapeutic for the treatment of asthma. MRx0004 has demonstrated efficacy in a murine model of severe asthma, by reducing pulmonary infiltration of neutrophils and eosinophils and increasing levels of FoxP3$^+$ T cells[5].

[1]Ruiz *et al.*, 2016, Front. Microbiol. **7**, 1193; [2]Lebeer *et al.*, 2011, Microb. Biotechnol. **4**, 368-374; [3]Fanning *et al.*, 2012, PNAS **109**, 2108-2113; [4]Delgado *et al.*, 2018, Evidence of the in vitro and in vivo immunological relevance of Bifidobacteria," in The Bifidobacteria and Related Organisms. 295-305; [5]Raftis *et al.*, 2018. Sci. Rep. **8**, 12024

Study Design

Objective: To investigate the immunoregulatory capacity of MRx0004, by targeting its EPS as a potential immunogen

Approach:



Results

Identification of candidate host response effectors in MRx0004

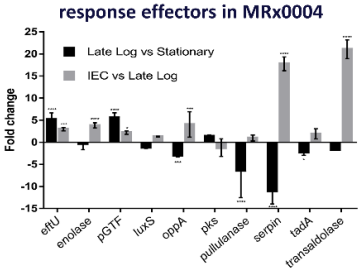


Fig. 1: Six out of ten predicted MRx0004 effector genes (including the priming glycosyltransferase (*pGTF*) of the EPS locus) were significantly upregulated between late log phase growth *in vitro* and contact with intestinal epithelial cells.

Table 1: Predicted moonlighting proteins and adhesins were detected in MRx0004 cell surface shavings and culture supernatants using nanoLC-MS/MS.

	Cell shavings	Supernatant
DnaK	✓	✓
EfTu	✓	×
Enolase	✓	✓
GAPDH	✓	✓
GroEL	✓	✓
Pullulanase	✓	✓
Transaldolase	✓	✓

Properties of an EPS-negative strain of MRx0004

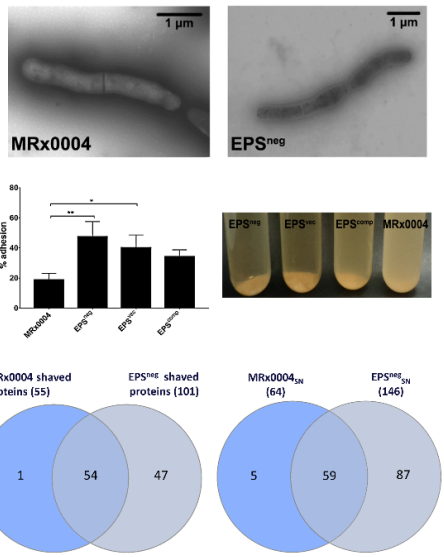


Fig. 2: Absence of EPS in EPS^{neg} was confirmed by TEM. Absence of EPS was associated with increased adhesion and autoaggregation, increased detection of surface-associated proteins post-IEC contact, and increased detection of proteins in culture supernatant.

Host response to MRx0004 and EPS^{neg}

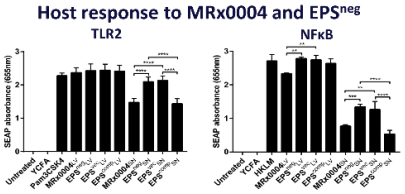


Fig. 3: Unshielding of the cell surface in EPS^{neg} resulted in increased TLR2 activation by culture supernatants, and increased NFκB activation by both live bacteria and supernatants, in comparison to MRx0004.

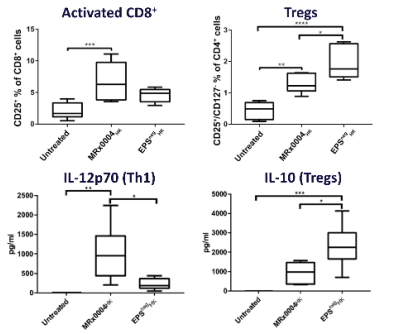


Fig. 4: MRx0004 EPS was directly associated with CD8$^+$ and Th1 (IL-12p70) responses from PBMCs, but did not impact activation of CD4$^+$ cells. EPS^{neg} treatment increased Tregs and IL-10 secretion to a greater extent than MRx0004, possibly due to increased cell surface exposure as a result of EPS deficiency.

Key Findings

- MRx0004 mediated the host response via modulation of TLR2/NFκB signalling, and activation of CD8$^+$, Th1 and Treg responses
- Regulation of CD8$^+$ and Th1 responses was directly associated with the presence of EPS
- EPS^{neg} treatment resulted in an increased Treg response, which may be attributable to unshielding of surface components

Future Direction

- Further studies are underway to determine the roles of additional MRx0004 predicted effectors and surface components
- MRx0004 is currently in development for a first-in-man clinical trial in asthma patients