Identification and characterisation of gut bacterial strains able to modulate neuroinflammation and neurodegeneration

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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

Parkinson's disease (PD) is a neurodegenerative disorder affecting around 10 million people worldwide. PD is characterised by the degeneration of dopaminergic neurons in the *substantia nigra* of the brain. PD is a multifactorial disease where genetic and environmental factors contribute to disease aetiology in a so-called "**multiple hit hypothesis**" model. Many studies have demonstrated an interplay between the brain and the gut in PD and highlighted the role played by the gut microbiota in this process (Westfall *et al.*, 2017). Moreover, analysis of mucosal and faecal samples have highlighted dysbiosis in PD compared to healthy donors (Minato *et al.*, 2017). Identification of bacterial strains that can potentially ameliorate the neuroinflammation and/or the neurodegenerative processes associated with PD, may lead in the near future to the development of new therapeutic approaches for the clinical management of the disease.

MRx Screening Platform

As a microbiome company with a proprietary culture collection consisting of over 6,500 commensal bacterial isolates from healthy donors, we have developed the multi-disciplinary MicroRx functional screening platform enabling us to target specific biological functions.

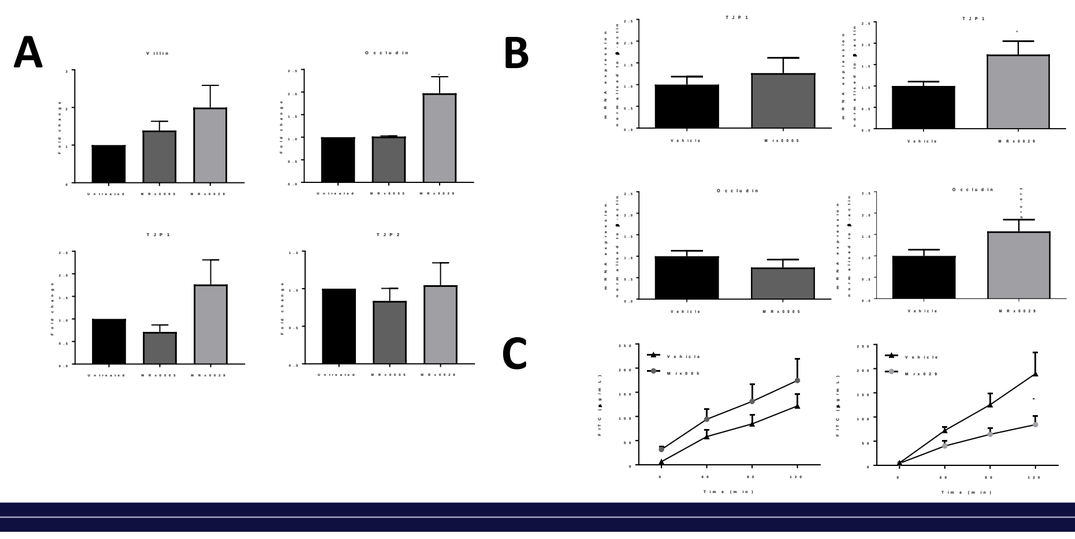
A panel of 69 bacterial strains from our culture collection was screened during our PD Discovery Campaign on different *in vitro* neuro-immune cell models.

We have identified two bacterial strains, *Parabacteroides distasonis* **MRx0005** and *Megasphaera massiliensis* **MRx0029,** with different and complementary cell responses to the stimuli used to mimic *in vitro* the different features of PD pathology, namely neurodegeneration and neuroinflammation induced by both environmental and familiar triggers, neurodifferentiation and effects on the gut barrier function.

Results

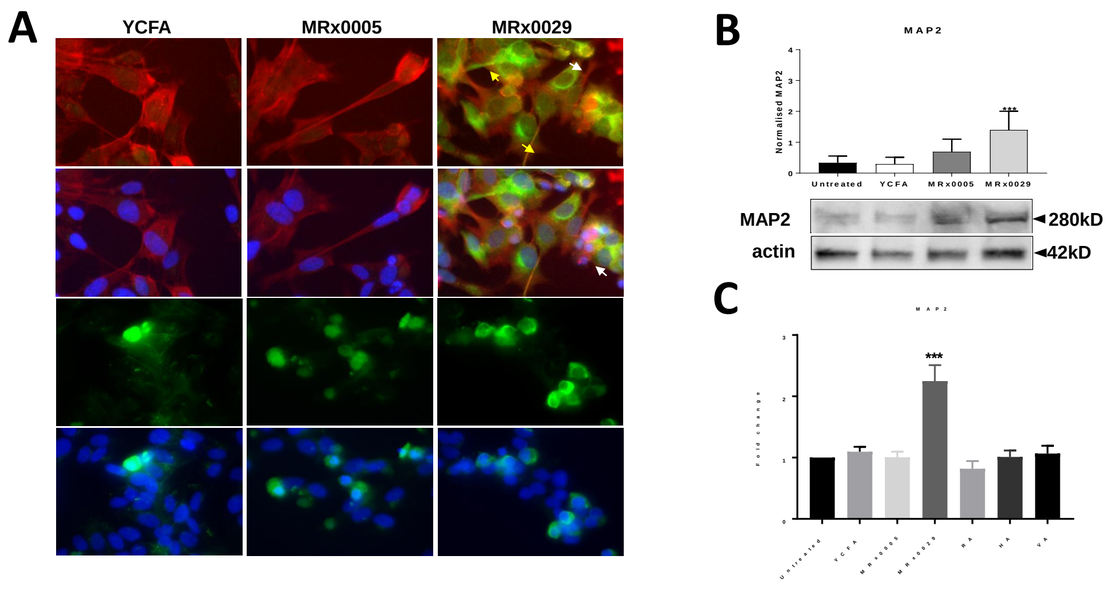
Gut barrier function

Intestinal problems start in PD patients before any clinical neuro-motor deficits are evident. So called "leaky gut" is associated with PD, thus contributing to systemic circulation of LPS linked to neuroinflammation. MRx0029, but not MRx0005, upregulated gene expression of Tight Junction Protein 1 and Occludin *in vitro* in human epithelial HT29-MTX cells **(A)** and *in vivo* in the colon of healthy mice **(B)**, thus reducing colon permeability as observed *ex vivo* **(C)**.



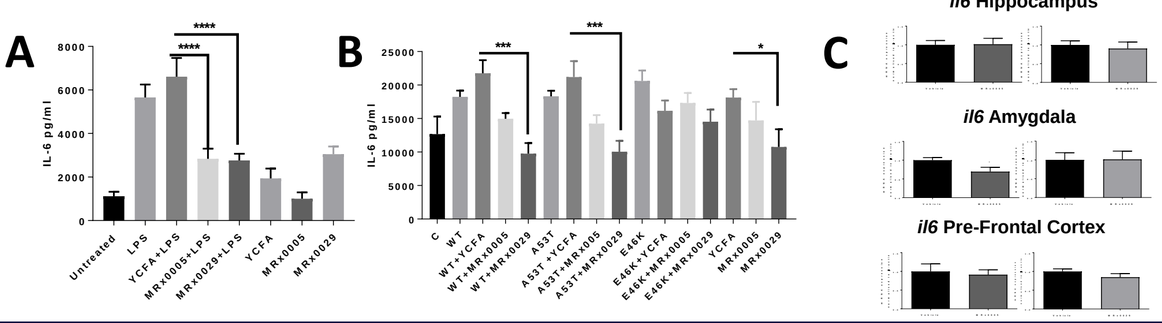
Neurodifferentiation

Interestingly, only MRx0029 induced a differentiated phenotype in neuroblastoma (SH-SY5Y) cells as shown by the expression of neuronal marker microtubule-associated protein 2 (MAP2) by immunofluorescence staining **(A)**, protein **(B)** and gene expression **(C)**.



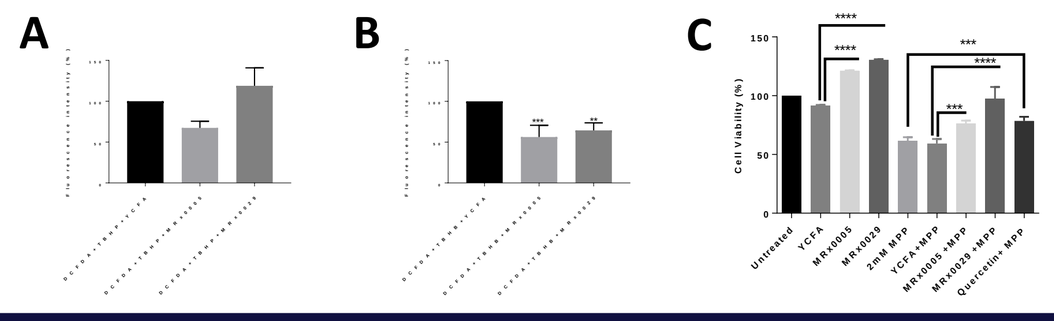
Neuroinflammation

Alongside misfolded α-synuclein proteins, toxins produced by bacteria such as LPS are at the core of neuroinflammation, a co-morbidity factor leading to neurodegeneration in PD. Both MRx0029 and MRx0005 decreased secretion of IL-6 in glioblastoma cells (U373) alone and in co-culture with differentiated neuroblastoma (SH-SY5Y) cells **(A and B)**. However, only MRx0005-fed mice showed a decrease of IL-6 gene expression in the amygdala of the brain **(C)**.



Neuroprotection

Chemical and environmental factors are well-known triggers of oxidative stress, which plays a key role in neurodegeneration. While MRx0005 showed protection from oxidative stress induced by different stimuli in both glioblastoma and neuroblastoma cells **(A, B and C)**, MRx0029 specifically protected differentiated neurons from oxidative stress induced by tert-Butyl hydroperoxide (TBHP) and completely reverted the cytotoxicity induced by MPP+ **(B and C)**, showing a tropism for neuronal-like cells.



Key Findings

We have identified two gut-derived bacteria that can modulate the gut:brain axis. The two strains have complementary characteristics:

- *P. distasonis* **MRx0005** has a predominantly anti-inflammatory signature;
- *M. massiliensis* **MRx0029** is potentially able to promote neurodifferentiation, protects neurons from cytotoxicity induced by both environmental and familiar PD triggers and reduces colon permeability.

Future Studies

- We are currently characterising the phenotype of neuronal-like cells *in vitro* after treatment with **MRx0005** and **MRx0029**;
- **MRx0005** and **MRx0029** are currently in development for future pre-clinical studies;
- *In vivo* studies are underway to test **MRx0005** and **MRx0029** in relevant models for PD.

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