Gut microbiome-derived bacterial strains have the ability to modulate neuroinflammation and neurodegeneration in Parkinson's Disease

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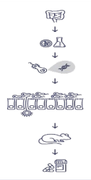


4D Pharma PLC is a pharmaceutical company focussed on developing live biotherapeutic products (LBPs) from the human gut microbiome. LBPs represent a new class of drugs that contain live organisms for the prevention, treatment or cure of disease. 4D Pharma currently has clinical stage programmes in IBS, IBD and cancer, 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 7-10 million people worldwide. PD is characterised by the degeneration of dopaminergic neurons in the *substantia nigra* of the brain. It is a multifactorial disease where genetic and environmental factors contribute to disease aetiology in a "**multiple hit hypothesis**" model. Recent studies have demonstrated an interplay between the brain and the gut in PD (Westfall *et al.*, 2017). Moreover, analysis of mucosal and faecal samples have highlighted microbiome dysbiosis in PD compared to healthy donors (Minato *et al.*, 2017). Identification of bacterial strains that can ameliorate the neuroinflammatory and/or neurodegenerative processes associated with PD, may lead to the development of new therapeutic approaches for the clinical management of the disease.

MRx Screening Platform

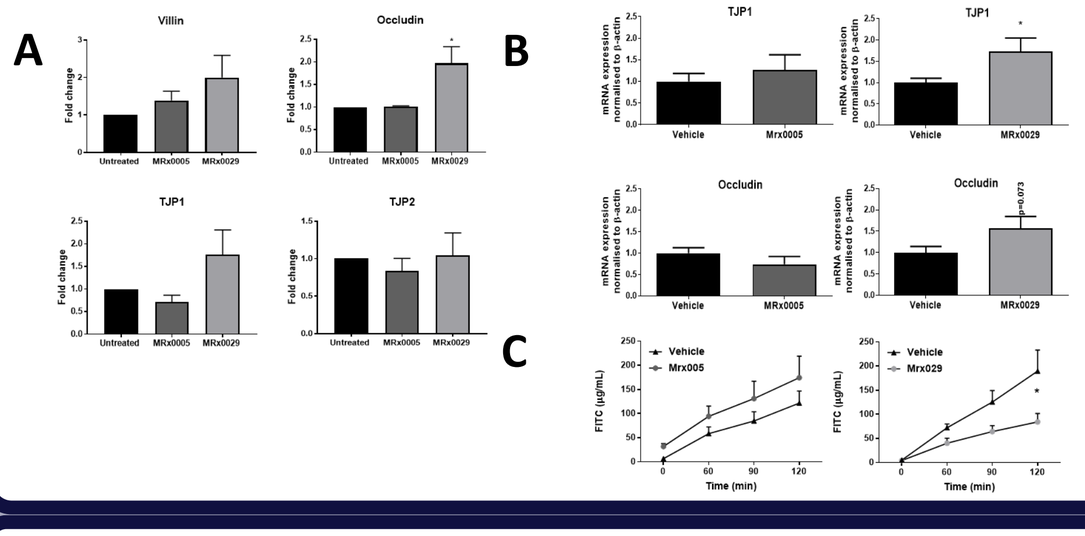
As a microbiome company with a proprietary culture collection of commensal bacterial isolates from healthy donors, we have developed the multi-disciplinary MicroRx® functional screening platform enabling us to target specific biological functions.

A panel of 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 different features of PD pathology, namely neurodegeneration, neuroinflammation and effects on the gut barrier function.

Results

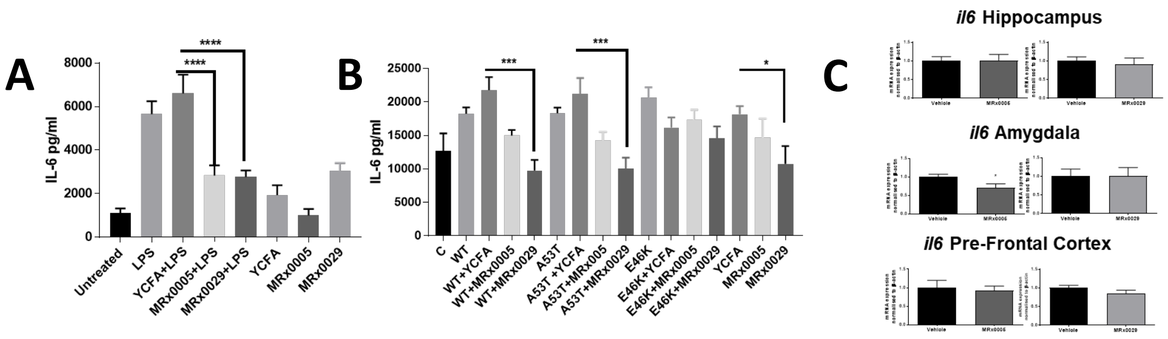
Gut barrier function

Intestinal problems start in PD patients before any clinical neuro-motor deficit symptom is evident. So called "leaky gut" is associated with PD, thus contributing to systemic circulation of LPS linked to neuroinflammation. MRx0029 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)**.



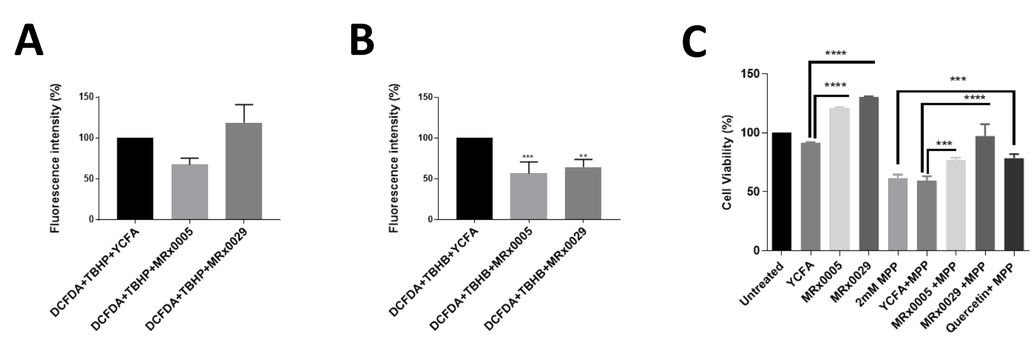
Neuroinflammation

Alongside misfolded α-synuclein proteins, toxins produced by bacteria such as LPS play a role in neuroinflammation. 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)**. MRx0005-fed mice showed a decrease of IL-6 gene expression in the amygdala of the brain **(C)**.

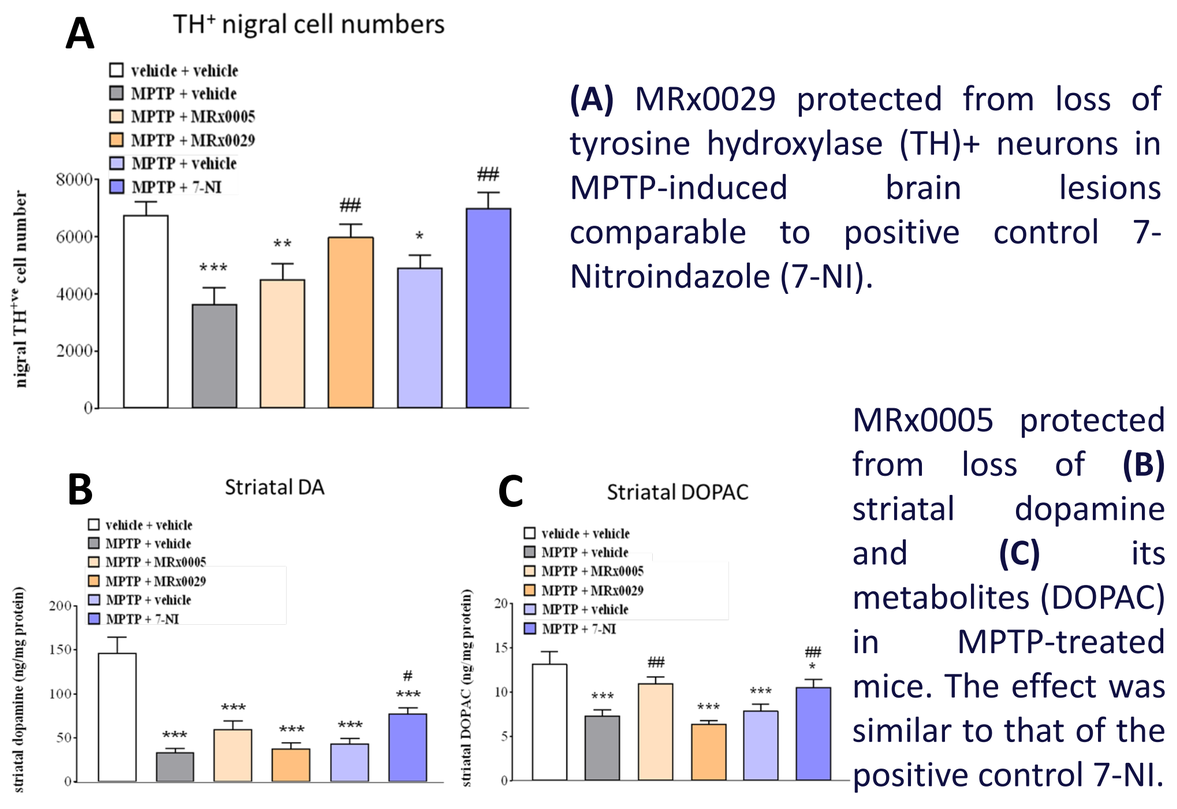


Neuroprotection- *In vitro*

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.



Neuroprotection- *In vivo*



(A) MRx0029 protected from loss of tyrosine hydroxylase (TH)+ neurons in MPTP-induced brain lesions comparable to positive control 7-Nitroindazole (7-NI).

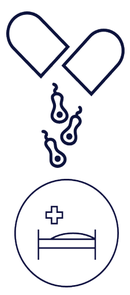
MRx0005 protected from loss of **(B)** striatal dopamine and **(C)** its metabolites (DOPAC) in MPTP-treated mice. The effect was similar to that of the positive control 7-NI.

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 protects neurons from cytotoxicity induced by both environmental and familiar PD triggers, is potentially able to promote neurodifferentiation 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.