From screening to pre-clinical efficacy of microbiome-derived bacterial strains that modulate neuroinflammatory and neurodegenerative processes in Parkinson's disease

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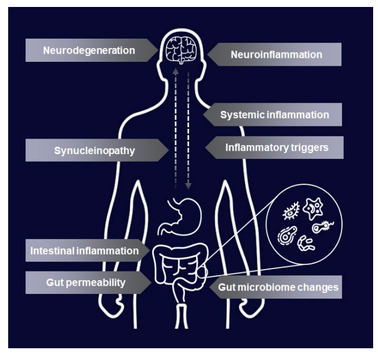
4D pharma is a pharmaceutical company focussed on developing live biotherapeutic products (LBPs) from the human gut microbiome. LBPs represent a new class of drugs that contain live organisms for the prevention, treatment or cure of disease. 4D pharma currently has clinical stage programmes in IBS, IBD, asthma and cancer, and a strong pipeline of pre-clinical programmes in autoimmunity, inflammation and CNS disease. 4D pharma is a microbiome company with a large proprietary culture collection of commensal bacterial isolates from the gut microbiome of healthy human donors. We have developed the multi-disciplinary MicroRx® functional screening platform which enables us to target specific biological functions.

Screening for commensal bacteria with neuroprotective properties

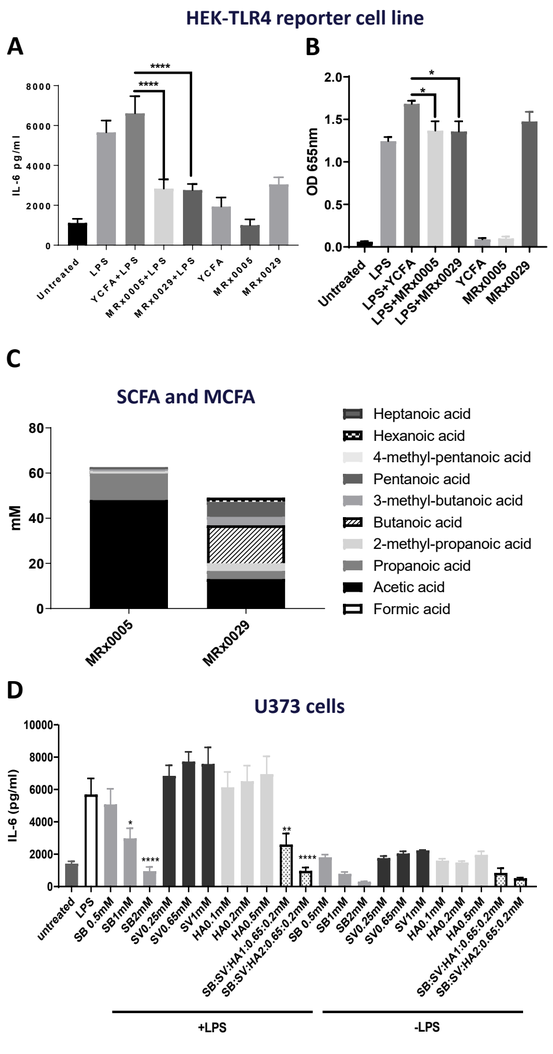
Parkinson's disease (PD) is a neurodegenerative disorder affecting around 10 million people worldwide. PD is characterised by the degeneration of dopaminergic neurons in the *substantia nigra* of the brain. It is a multifactorial disease where genetic and environmental factors contribute to disease aetiology in a so-called "**multiple hit hypothesis**" model. Studies have demonstrated an interplay between the gut and the brain in PD and indicated the role of the gut microbiota in this process (Westfall *et al.*, 2017). Analysis of PD patient gut samples has shown that the microbiome is different compared to healthy donors (Minato *et al.*, 2017). We therefore sought to investigate if specific microbiome-derived bacterial strains could positively influence the neuroinflammatory and/or neurodegenerative processes associated with PD.

A panel of bacterial strains from our culture collection was screened during our PD Discovery Campaign on different *in vitro* neuronal cell models. We identified two bacterial strains, ***Parabacteroides distasonis* MRx0005** and ***Megasphaera massiliensis* MRx0029,** which induce different and complementary cell responses to the stimuli used to mimic the different features of PD pathology *in vitro*, namely neurodegeneration and neuroinflammation induced by both environmental and familial triggers, neurodifferentiation and effects on gut barrier function.



Results

Neuroinflammation



TLR activation by microbe-associated molecular patterns (such as LPS) and damage-associated molecular patterns (e.g., alpha-synuclein in PD) is a dynamic process. TLR activation triggers a series of downstream molecular pathways leading to the translocation of NF-κβ to the nucleus, culminating in upregulation of pro-inflammatory cytokine expression. Therefore, therapeutic interventions aimed at interfering with TLR signalling could decrease pro-inflammatory cytokine responses and lead to an overall reduction of neuroinflammation, oxidative stress and neuronal death (Fellner *et al*., 2013; Rietdijk *et al*., 2016).

Both MRx0005 and MRx0029 decreased secretion of IL-6 in glioblastoma cells (U373) **(A)** and reduced TLR4 activation in HEK-TLR4 reporter cells **(B)**.

Bacterial metabolites can directly influence the host response to oxidative stress and cell-to-cell communication. MRx0005 and MRx0029 have distinct metabolite signatures **(C)**: MRx0005 produces C1-C3 SCFA (acetic acid, propionic acid), while MRx0029 produces C4-C6 SCFA (butanoic, valeric, hexanoic acid).

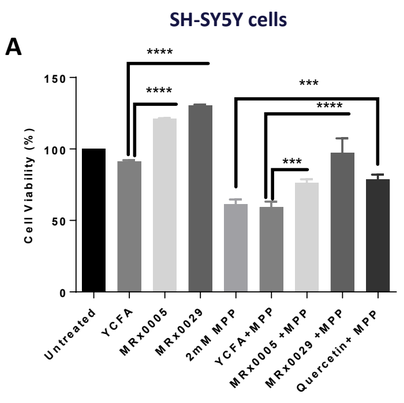
U373 cells were treated with the individual SCFAs sodium butyrate (SB), sodium valerate (SV) and hexanoic acid (HA) as well as concentrations corresponding to MRx0029 production. Butyrate appeared to be mostly responsible for the IL-6 decrease in U373 cells after challenge with LPS **(D)**.

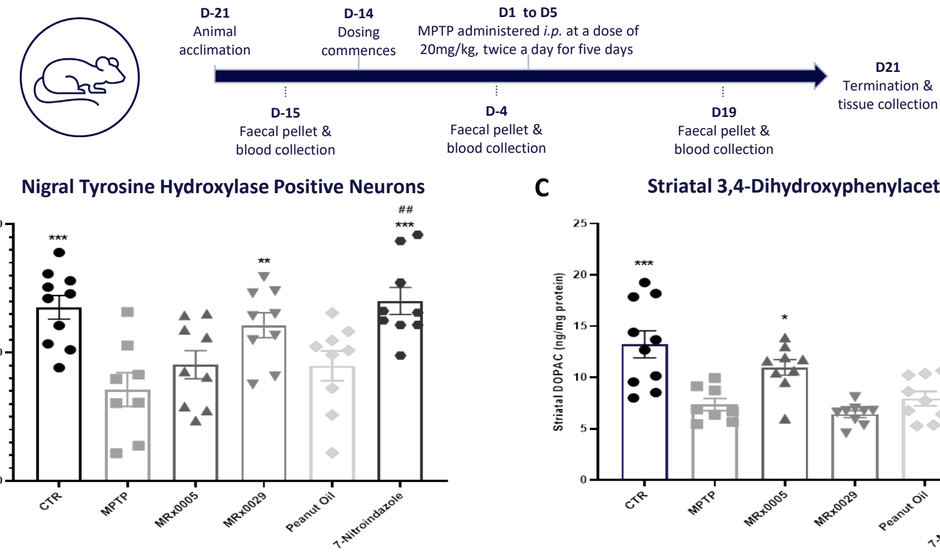
Neuroprotection

MPP+ is the toxic metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which is useful for assessing nigrostriatal dopaminergic deficits, one of the characteristics of PD pathology.

MRx0029 induced complete neuroprotection from MPP+ (1-methyl-4-phenylpyridinium) induced cytotoxicity in differentiated SH-SY5Y neuroblastoma cells **(A)**.

In accordance with our *in vitro* findings, MRx0005 and MRx0029 pre-treatment was efficacious in the MPTP mouse model of PD. Oral delivery of MRx0029 successfully protected tyrosine hydroxylase (TH)-positive dopaminergic neurons from MPTP-induced cell death **(B)**, while MRx0005 reduced MPTP-induced deficit of dopamine metabolite (DOPAC) in striatal **(C)**.

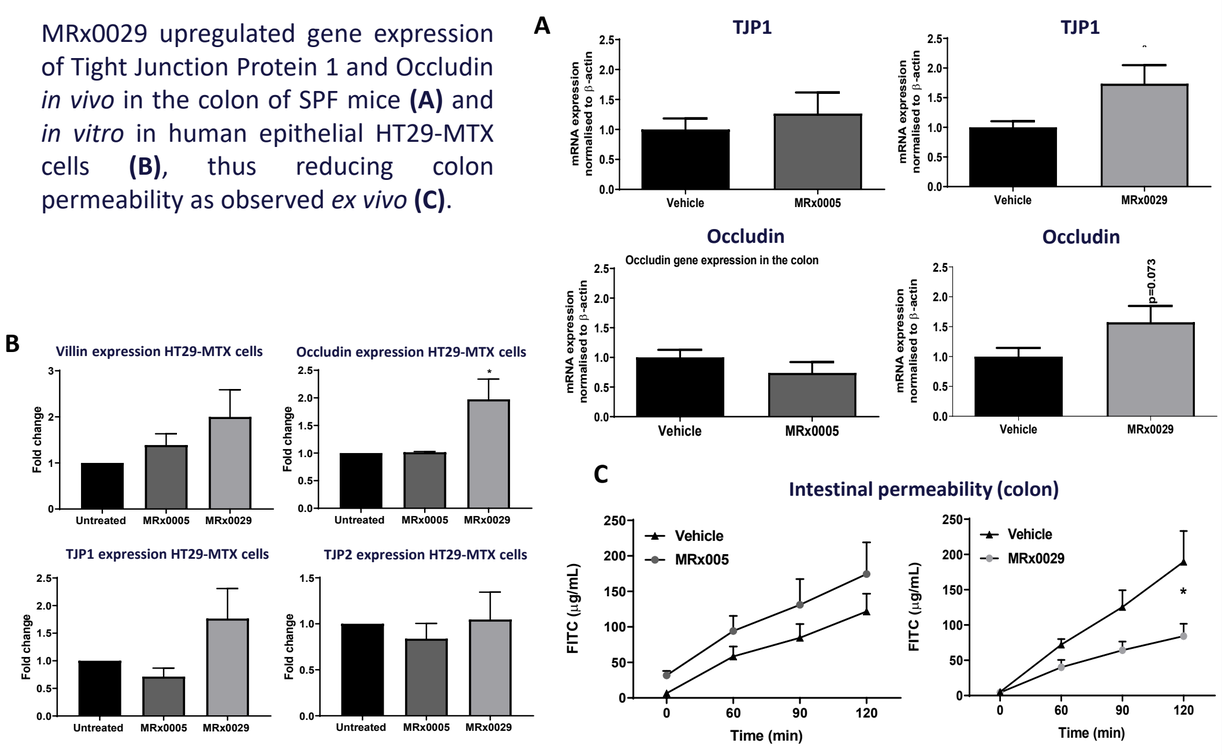




Intestinal barrier function

Intestinal problems can manifest in PD patients long before any clinical motor deficits are evident. So called "leaky gut" is associated with PD, thus contributing to systemic circulation of inflammatory triggers (such as LPS) that are linked to neuroinflammation.

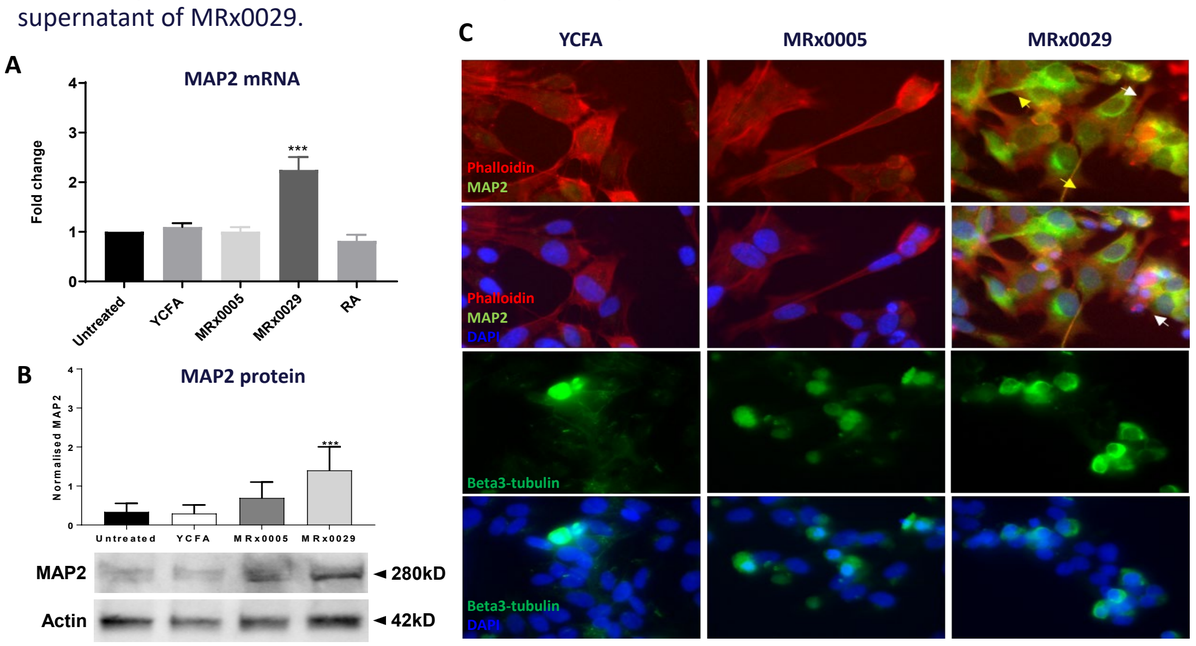
MRx0029 upregulated gene expression of Tight Junction Protein 1 and Occludin *in vivo* in the colon of SPF mice **(A)** and *in vitro* in human epithelial HT29-MTX cells **(B)**, thus reducing colon permeability as observed *ex vivo* **(C)**.



Neurodifferentiation

Microtubule-associated protein 2 (MAP2) is considered a marker of terminal neuronal differentiation, therefore we investigated the gene and protein expression of MAP2 in undifferentiated neuroblastoma (SH-SY5Y) cells.

Interestingly, MRx0029 induced a differentiated phenotype in SH-SY5Y cells, as shown by the expression of MAP2 gene expression **(A)**. We next examined whether this increased expression was reflected at the protein level by Western blot **(B)** and using immunofluorescence labelling **(C)**. Both of these analyses confirmed increased MAP2 protein expression in cells treated with the bacterial cell-free supernatant of MRx0029.



Key findings

We have identified two gut microbiome-derived bacterial strains that can modulate different aspects of the gut-brain axis. The two strains have complementary characteristics:

- ***Parabacteroides distasonis* MRx0005** has strong anti-inflammatory properties and reduces ileal permeability;
- ***Megasphaera massiliensis* MRx0029** protects neurons from cytotoxicity induced by both environmental and familiar PD triggers, potentially promotes neurodifferentiation and reduces colon permeability

Ahmed S, Busetti A, Fotiadou P, Vincy Jose N, Reid S, Georgieva M, Brown S, Dunbar H, Beurket-Ascencio G, Delday MI, Ettorre A, Mulder IE (2019). In vitro Characterization of Gut Microbiota-Derived Bacterial Strains With Neuroprotective Properties. Front. Cell. Neurosci., https://doi.org/10.3389/fncel.2019.00402

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