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Neurogenic and anti-inflammatory effects of probiotics in Parkinson's disease: a systematic review of preclinical and clinical evidence

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Highlights (3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point)

- Probiotics can reduce markers of peripheral inflammation in PD
- Preclinical evidence suggests neuroprotective properties of probiotics in PD
- Probiotics can activate the GLP-1 and PPAR-γ pathway in PD
- Future research should further explore the effects of probiotics on neuropsychiatric aspects of PD

Abstract

There is increasing evidence highlighting the potential role of the gut-brain axis in the pathogenesis of Parkinson's disease (PD) and on the use of probiotics as a therapeutic strategy for this neurodegenerative disorder. While several studies have been published on the topic in recent years, there is still a lack of a comprehensive understanding of the effects of probiotics in PD and their possible underlying mechanisms. Through this systematic review, we collected a total of 17 articles, consisting of preclinical and clinical models of PD investigating the effect of probiotics on (1) energy metabolism, (2) inflammation and oxidative stress, (3) neurodegeneration, as well as (4) motor and (5) non-motor function. Articles were obtained from PubMed/Medline, Scopus, Web of Science and EMBASE databases. Findings from preclinical studies suggest that treatment with probiotics increased glucose metabolism (increased secretion of glucagon-like peptide-1), reduced peripheral and central inflammation (reduced interleukin-6 and tumor necrosis factor-α (TNF-α)), reduced peripheral and central oxidative stress (reduced peripheral superoxide anion levels and increased central antioxidant glutathione levels), decreased neurodegeneration (increased numbers of tyrosine hydroxylase dopaminergic neurons and levels of brain-derived neurotrophic factor), increased motor function (increased motor agility) and non-motor function (decreased memory deficits). Similarly, findings from clinical studies suggest that probiotics increased glucose metabolism (reduced insulin resistance), reduced peripheral inflammation (reduced peripheral TNF-α expression and C-reactive protein levels), and increased motor and non-motor function (decreased overall PD symptomatology and constipation); however, findings on oxidative stress were inconclusive across studies. Overall, this review is the first one to systematically report evidence for the putative beneficial effects of probiotics on molecular and cellular mechanisms, as well as behavioural phenotypes, in either preclinical or clinical studies in PD. However, additional and more robust studies are still needed to confirm these outcomes, and

should aim to focus more on bench-to-bedside investigations, in order to address the existing gaps between preclinical and clinical findings in this field.

1. Introduction

Over the last decade, there has been increasing attention on the role of the gut microbiota in human health and diseases. The gut microbiota consists of a community of bacteria, viruses, protozoa, and fungi that dwell in the human gastrointestinal tract and intimately communicate with the human host, modulating intestinal barrier function, metabolism, and immune as well as nervous system activity (Hollister et al., 2014). The gut microbiota represents, indeed, one of the main characters of the gut-brain axis, a bidirectional network between the gastrointestinal tract and the central nervous system (CNS), and is able to regulate multiple biological mechanisms and, ultimately, brain activity. Different are the pathways of communication between the gut and the CNS which include the autonomic nervous system (mainly the enteric nervous system and the vagus nerve), the neuroendocrine system, the hypothalamic-pituitary-adrenal axis, metabolic pathways, and the immune system (Morais et al., 2021).

As the gastrointestinal tract represents a gateway to the environment, the cross-talk between the human host and the gut microbiota becomes particularly relevant in diseases where environmental factors, including diet and exposure to toxins, seem to contribute to or protect against the development of specific pathological conditions, including idiopathic Parkinson's disease (PD) (Klingelhoefer and Reichmann, 2015). PD is a neurodegenerative condition commonly regarded as a movement disorder where motor dysfunction, including tremor, rigidity, and bradykinesia, mainly result from loss of dopaminergic neurons in the substantia nigra; however, it is well known now that a variety of non-motor features, including symptoms of depression and anxiety, cognitive impairments, pain and constipation, can also occur across all stages of PD (Kalia and Lang, 2015; Schapira et al., 2017).

Several studies have shown alterations in the gut microbiota of PD patients when compared with healthy controls (Heintz-Buschart et al., 2018; Scheperjans et al., 2015; Wallen et al., 2020) and associations between faecal levels of specific bacteria and motor and non-

motor features, such as tremor, postural instability and constipation (Scheperjans et al., 2015). Although results of these studies are extremely heterogeneous, mainly due to differences in study methodology and presence of confounding factors, including diet, geographical background and medication (Boertien et al., 2019), two recent meta-analyses reported a pro-inflammatory gut microbiota in PD characterised by depletion of short chain fatty acids (SCFAs) producing bacteria (Nishiwaki et al., 2020; Romano et al., 2020). SCFAs are metabolites deriving from intestinal microbial fermentation and exert multiple beneficial effects on human health, being able to decrease intestinal and systemic inflammation and promote normal neuronal as well as microglia maturation (Keshavarzian et al., 2020). Notably, both the level of SCFAs producing bacteria and faecal levels of these metabolites are reduced in PD patients when compared with healthy controls (Unger et al., 2016; Wallen et al., 2020).

According to recent preclinical and clinical evidence, a new and debated gut-originating pathogenesis model of PD has been postulated, whereby, in some susceptible individuals, PD might be initiated by the ingestion of inflammatory triggers, such as pesticides or pollutants, which can alter the gut microbiota, increase intestinal permeability as well as inflammation, and lead to misfolded α-synuclein (one of the pathological hallmarks of PD) (Metta et al., 2021). The latter could then access the CNS via the gut-brain axis, ultimately stimulating central inflammation, and neurodegeneration (Houser and Tansey, 2017). Indeed, on one side α-synuclein peptides may act as antigenic epitopes and drive immune response in PD (Sulzer et al., 2017); on the other side, proinflammatory immune activity can increase levels of α-synuclein both in the gut and brain (Griffin et al., 2006; Kelly et al., 2014) and its aggregation (Shults, 2006). This positive inflammatory loop can eventually contribute to neuronal death (Rocha et al., 2015).

The identification of a proinflammatory alteration of the gut microbiota in PD, and its potential role in the pathogenesis of this neurogenerative disorder, has driven the interest in

investigating the use of gut microbiota-modulating interventions, such as probiotics, as possible novel therapeutic strategies for PD. Probiotics are defined by the World Health Organization as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (https://www.who.int/foodsafety/probiotic_guidelines.pdf), and evidence supports their beneficial effects on a variety of human diseases, including metabolic and gastrointestinal disorders (Markowiak and Śliżewska, 2017), as well as neurological and neuropsychiatric conditions (Bermúdez-Humarán et al., 2019). Probiotics can exert their beneficial effects via several mechanisms of action, including: 1) colonisation and normalisation of a perturbed intestinal microbial community; 2) improvement of intestinal barrier function; and 3) activation of enzymatic activities and subsequent production of metabolites that positively regulate peripheral and central energy metabolism and inflammation, as well as neurogenesis, neurotransmission and, ultimately, behaviour (Barcelo et al., 2000).

Existing literature has demonstrated that probiotics can affect brain activity by reducing neuroinflammation and, consequently, increasing neurogenesis. In particular, preclinical models of rodents have shown that probiotics can decrease peripheral levels of inflammatory cytokines, such as interleukin-1beta (IL-1β) and IL-6 (Mohammadi et al., 2019; Xin et al., 2020), and ultimately prevent neuroinflammation (Shahbazi et al., 2020). In fact, these peripheral cytokines are often able to cross the blood-brain barrier and access brain regions relevant for neurogenesis, especially the hippocampus, where they can exert detrimental effects on cell viability, proliferation, and differentiation (Borsini et al., 2017; Borsini et al., 2018; Borsini et al., 2020). Considering that a proinflammatory status and neurodegeneration are well-known characteristics of the PD pathology (Lim et al., 2018), clinical intervention with probiotics seems a promising therapeutic approach for this condition.

While evidence like this has suggested a beneficial role of probiotics in PD, there still is a lack of a comprehensive understanding concerning how they exert their properties. Therefore, in this work, we aimed to systematically review all available findings generated from both *preclinical* and *clinical* studies investigating the role of probiotics on candidate mechanisms underlying the PD pathology, and in particular: (1) energy metabolism, (2) inflammation and oxidative stress, (3) neurodegeneration, (4) motor and (5) non-motor function. Ultimately, this review will provide a better understanding of the existing gaps between preclinical and clinical research on the topic and possibly provide future research directions.

2. Methods

The review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Liberati et al., 2009; Moher et al., 2009). We searched for both preclinical and clinical articles published until January 2021 in the following databases: PubMed/Medline, Scopus, Web of Science and EMBASE, and used the following search terms: Parkinson's disease AND probiotics AND ((metabolism) OR (inflammation) OR (neurodegeneration OR neurogenesis) OR (motor OR parkinsonism) OR (non-motor OR behaviour OR cognition OR anxiety OR depression OR pain OR constipation)). Two independent authors (VL and OM) conducted the literature search, the initial analysis of titles and abstracts, and retrieved full-text articles for detailed review. Included articles were original research studies published or in-press and written in English. For studies conducted on animals, inclusion required the use of established animal models of PD (Konnova and Swanberg, 2018). For human studies, inclusion required the diagnosis of PD according to Movement Disorder Society (Postuma et al., 2015) or UK Parkinson's Disease Society Brain Bank diagnostic criteria (Hughes et al., 1992). A total of 251 articles were retrieved (Figure 1), of which only

17 fit the eligibility criteria of our search, after exclusion of duplicates and non-relevant papers. Out of the 17 articles retained, 12 were preclinical studies and 5 were clinical studies. Risk of bias was assessed using the SYRCLE guidelines (Hooijmans et al., 2014) or the Rob 2 tool (Sterne et al., 2019), respectively for studies conducted in animals and humans. The overall risk of bias was low (Supplementary Materials).

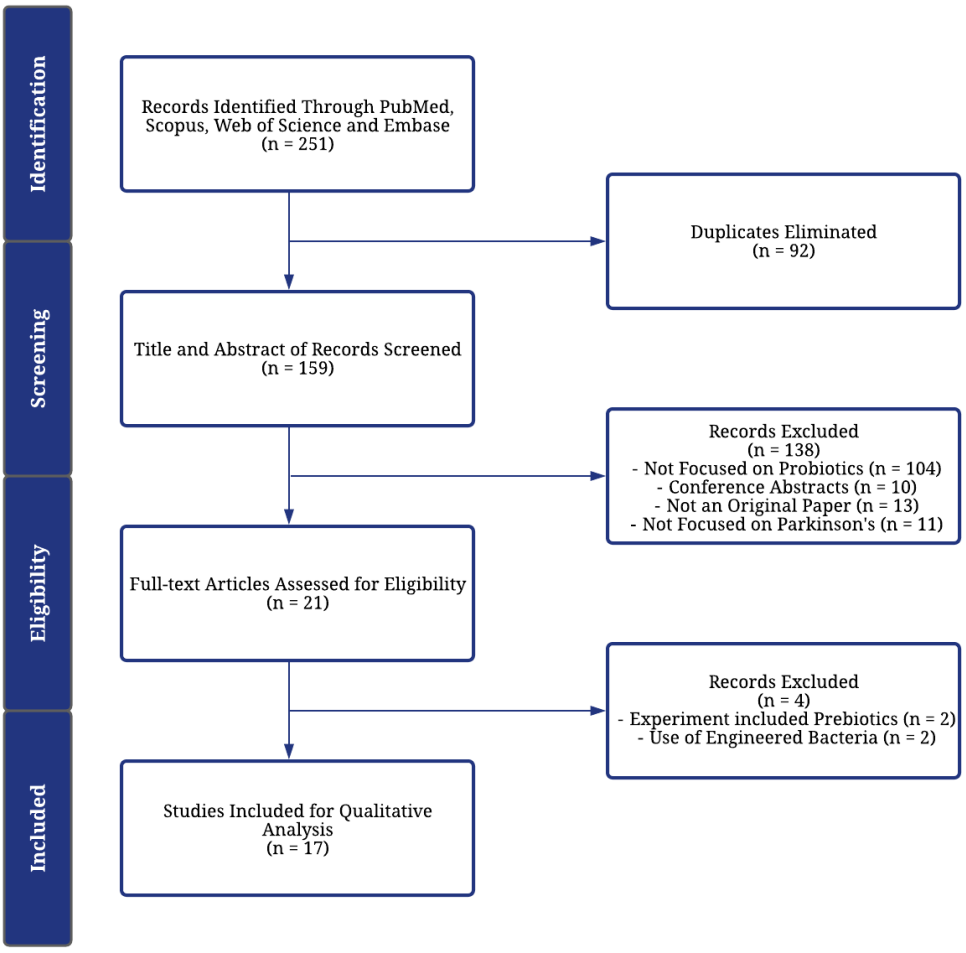

Figure 1. *Flow diagram of the search and selection process*. Seventeen studies met the eligibility criteria and investigated the effects of probiotics on either energy metabolism, peripheral and central inflammation, oxidative stress, neurodegeneration, motor or non-motor function or a combination of them in Parkinson's disease (12 preclinical studies and 5 clinical studies).

3. Results

3.1 Preclinical studies

A total of 12 preclinical studies were identified, 10 *in vivo* (Alipour Nosrani et al., 2020; Dwyer et al., 2021; Goya et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019; Sun et al., 2020; Xie and Prasad, 2020), 1 both *in vitro* and *in vivo* (Castelli et al., 2020), and 1 *ex vivo* (Magistrelli et al., 2019) (Table 1).

3.1.1 Energy Metabolism

Five preclinical studies, 4 *in vivo* (Alipour Nosrani et al., 2020; Goya et al., 2020; Liao et al., 2020; Sun et al., 2020) and 1 both *in vitro* and *in vivo* (Castelli et al., 2020), investigated the effects of probiotics on energy metabolism in PD (Table 1), and, overall, found that probiotics may prevent the decrease in glucose metabolism in PD models.

In particular, 1-16 day-treatment with *Bacillus subtilis PXN21* increased the expression of ceramide synthase (lagr-1/ceramide synthase 1 (CERS1)) and acid sphingomyelinase (acid sphingomyelinase 3 (asm-3)/sphingomyelin phosphodiesterase 1 (SMPD1))) genes, all involved in the metabolism of the sphingolipids, in a *C. elegans* model of synucleinopathy (overexpression of α-synuclein) (Goya et al., 2020). Similarly, 2-week treatment with a combination of probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum prevented midbrain increase in malondialdehyde (MDA), a metabolite resulting from lipid peroxidation, in a 6-hydroxydopamine (6-OHDA) model of PD (Alipour Nosrani et al., 2020).

Four-week treatment with *Clostridium butyricum* WZMC1016 prevented the decrease in colonic levels of SCFAs receptors (G-protein-coupled receptor (GPR41/43)) and hormone GLP-1, as well as cerebral expression of GLP-1 receptors, known to regulate glucose metabolism, in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD (Sun et

al., 2020). In contrast, 4-week treatment with *Lactobacillus plantarum PS128* did not prevent the decrease in SCFAs faecal levels in an MPTP model of PD (Liao et al., 2020).

Five-week administration of a mixture of probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum prevented the decrease in protein levels of peroxisome proliferator-activated receptor-gamma (PPAR-γ), a transcription factor activated by GLP-1 and involved in lipid storage and glucose metabolism, in the substantia nigra of a 6-OHDA model of PD (Castelli et al., 2020). In the same study, 2-hour treatment with the same mixture of probiotics prevented the decrease in PPAR-γ protein levels, this time in an *in vitro* 6-OHDA model of PD using dopaminergic-like SH-SY5Y neuro-blastoma cells (Castelli et al., 2020).

In conclusion, evidence suggests that probiotics treatment can stimulate the GLP-1 pathway leading to activation of PPAR-γ, increase sphingolipid metabolism, and reduce lipid peroxidation; however, there is inconclusive data on their ability to increase intestinal levels of SCFAs.

3.1.2 Inflammation and oxidative stress

Six preclinical studies, 5 *in vivo* (Castelli et al., 2020; Dwyer et al., 2021; Liao et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019) and 1 *ex vivo* (Magistrelli et al., 2019) investigated the effects of probiotics on inflammation and oxidative stress in PD (Table 1). Three of the abovementioned studies were previously introduced in section 3.1.1 (Castelli et al., 2020; Goya et al., 2020; Liao et al., 2020). Overall, these studies found that probiotics may prevent the increase in central and peripheral inflammation and oxidative stress in PD models.

In particular, 4-week treatment with *Lactobacillus plantarum PS128* prevented the increase in protein levels of cytokines (IL-6, IL-1β and tumor necrosis factor-α (TNF-α)) and glial-related markers (glial fibrillary acidic protein (GFAP) and ionized calcium-binding

adaptor molecule 1 (Iba1)), and the decrease in antioxidants levels (superoxide dismutase, glutathione and catalase), in the striatum of an MPTP model of PD (Liao et al., 2020). Similarly, 3-week treatment with a mixture of probiotics belonging to the *Firmicutes* phylum, including *Lactobacillus plantarum CRL 2130*, prevented the increase in IL-6 and TNF-α and decrease in IL-10 serum levels, as well as the increase in TNF-α levels in brain homogenates, in a similar MPTP model of PD (Perez Visñuk et al., 2020).

Furthermore, 4-5-week treatment with mixtures of probiotics belonging to *Firmicutes* or *Actinobacteria* phylum prevented the increase in GFAP and Iba-1 expression in the substantia nigra of an MPTP, rotenone and 6-OHDA model of PD (Castelli et al., 2020; Srivastav et al., 2019). In one of these studies, 5-week treatment with the same probiotics prevented the increase in NF-κB and the decrease in nuclear factor erythroid 2–related factor 2 (Nrf2) and hemeoxygenase-1 (HO-1) protein levels in the substantia nigra and striatum of a 6-OHDA model of PD (Castelli et al., 2020). In contrast, 4-week treatment with the same mixture of probiotics did not prevent the increase in GFAP and Iba-1 protein levels in the substantia nigra and IL-6, TNF-α, IL-1β plasma levels in a lipopolysaccharide (LPS) and paraquat toxin model of PD (Dwyer et al., 2021).

Similar results were also observed in an *ex vivo* study on peripheral blood mononuclear cells (PBMCs) isolated from PD patients and co-cultured with different strains of probiotics for 24 hours (Magistrelli et al., 2019). In particular, treatment with probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum decreased IL-6 and TNF-α, and increased IL-10 protein levels, and reduced levels of the oxidant superoxide anion, all in cell supernatant (Magistrelli et al., 2019).

In conclusion, evidence suggests that probiotics treatment can reduce peripheral and central levels of pro-inflammatory cytokines (IL-6 and TNF-α), reduce the number of

astrocytes and microglial cells in the substantia nigra, and decrease peripheral and central oxidative stress (reduced peripheral levels of superoxide anion and increased central levels of superoxide dismutase, glutathione and catalase) in models of PD.

3.1.3 Neurodegeneration

Ten preclinical studies, 9 *in vivo* (Alipour Nosrani et al., 2020; Dwyer et al., 2021; Goya et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019; Sun et al., 2020) and 1 both *in vitro* and *in vivo* (Castelli et al., 2020), investigated the effect of probiotics on the loss of dopaminergic neurons in PD (Table 1). Eight of the abovementioned studies were previously introduced in section 3.1.1 (Alipour Nosrani et al., 2020; Castelli et al., 2020; Goya et al., 2020; Liao et al., 2020; Sun et al., 2020) and 3.1.2 (Castelli et al., 2020; Dwyer et al., 2021; Liao et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019). Overall, these studies found that probiotics may prevent the loss of dopaminergic neurons in the substantia nigra in models of PD.

In particular, 4-week treatment with *Lactobacillus plantarum PS128* prevented the decrease in tyrosine hydroxylase positive (TH+) dopaminergic neurons in the substantia nigra of an MPTP model of PD (Liao et al., 2020). Similar results were obtained after 4-week treatment with *Clostridium butyricum WZMC1016*, which prevented the decrease in TH+ neurons in the substantia nigra in the same model of PD (Sun et al., 2020). Likewise, 3-week treatment with *Lactobacillus fermentum U-21* prevented the decrease in TH+ neurons in the substantia nigra in a paraquat toxin model of PD (Marsova et al., 2020). Similarly, 2-16-week treatment with mixtures of probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum prevented the decrease in TH+ neurons in the substantia nigra, in MPTP (Perez Visñuk et al., 2020; Srivastav et al., 2019), 6-OHDA (Alipour Nosrani et al., 2020; Castelli et al., 2020), rotenone (Srivastav et al., 2019) and MitoPark models of PD (Hsieh et al., 2020). In contrast,

4-week treatment with a mixture of probiotics from the *Firmicutes* or *Actinobacteria* phylum did not prevent the decrease in TH+ neurons in the substantia nigra in LPS and paraquat toxin model of PD (Dwyer et al., 2021).

Three of the aforementioned studies also investigated the effect of probiotics on brain levels of neurotrophic factors (Castelli et al., 2020; Liao et al., 2020; Srivastav et al., 2019). Four-five week treatment with mixtures of probiotics from the *Firmicutes* or *Actinobacteria* phylum prevented the decrease in brain-derived neurotrophic factor (BDNF) levels in the basal ganglia, in 6-OHDA, MPTP and rotenone models of PD (Castelli et al., 2020; Srivastav et al., 2019). In line with previous findings, 4-week treatment with *Lactobacillus plantarum PS128* prevented the decrease in striatal BDNF and nerve growth factor (NGF) levels, in an MPTP model of PD (Liao et al., 2020). Additionally, in one of the aforementioned studies (Castelli et al., 2020), 2-hour treatment with a mixture of probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum prevented the increase in the apoptotic brain-derived neurotrophic factor precursor (pro-BDNF) protein levels, in an *in vitro* 6-OHDA model of PD using dopaminergic-like SH-SY5Y neuro-blastoma cells.

In conclusion, evidence suggests that probiotics treatment can prevent the reduction of dopaminergic neurons in the substantia nigra and the decrease of neurotrophic factors levels, including BDNF, in the basal ganglia of PD models.

3.1.4 Motor function

Ten *in vivo* studies investigated the effects of probiotics on motor function in PD (Alipour Nosrani et al., 2020; Castelli et al., 2020; Dwyer et al., 2021; Goya et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al.,

2019; Sun et al., 2020) (Table 1). All these studies were previously introduced in section 3.1.3, and, overall, they found that probiotics may prevent the decrease in motor agility in models of PD.

In particular, 4-week treatment with *Lactobacillus plantarum PS128* prevented the decrease in motor agility in an MPTP model of PD (Liao et al., 2020). Similarly, 4-week treatment with *Clostridium butyricum WZMC1016* prevented the decrease in motor agility in the same animal model of PD (Sun et al., 2020). Similar results were obtained after 3-week treatment with *Lactobacillus fermentum U-21,* which prevented the decrease in motor agility in a paraquat toxin model of PD (Marsova et al., 2020). Also, 6-day treatment with *Bacillus subtilis PXN21* prevented the decrease in motility in a *C. elegans* model of synucleinopathy (Goya et al., 2020).

Similar results were obtained in 5 other studies, where 2-16-week administration of mixtures of probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum prevented the decrease in motor agility in an MPTP (Perez Visñuk et al., 2020; Srivastav et al., 2019), 6-OHDA (Alipour Nosrani et al., 2020; Castelli et al., 2020), rotenone (Srivastav et al., 2019), and MitoPark model of PD (Hsieh et al., 2020). In contrast, 4-week treatment with a mixture of probiotics from the *Firmicutes* or *Actinobacteria* phylum did not prevent the reduced motility, in an LPS and paraquat toxin model of PD (Dwyer et al., 2021).

In conclusion, evidence suggests that probiotics treatment can prevent the locomotor impairments observed in different models of PD and thus, overall, improve motor function.

3.1.5 Non-motor function

Two *in vivo* studies investigated the effects of probiotics on non-motor function (Alipour Nosrani et al., 2020; Xie and Prasad, 2020) (Table 1), one of which was previously introduced in sections 3.1.1, 3.1.3 and 3.1.4 (Alipour Nosrani et al., 2020). Overall, they found

that probiotics may reduce memory deficits in models of PD, whereas no effect was observed on anxiety-like behaviour.

In particular, 6-week treatment with *Lacticaseibacillus rhamnosus* prevented the decrease in spatial memory deficits, but not anxiety-like behaviour, in a 6-OHDA model of PD (Xie and Prasad, 2020). Similarly, 7-day treatment with a mixture of probiotics from the *Firmicutes* or *Actinobacteria* phylum prevented the decrease in spatial learning and memory loss, in the same model of PD (Alipour Nosrani et al., 2020).

3.2 Clinical studies

A total of 5 clinical studies were identified, all of which were randomised clinical trials (Borzabadi et al., 2018; Georgescu et al., 2016; Tamtaji et al., 2019; Tan et al., 2020), with the exception of 1 which was an open-label study (Cassani et al., 2011) (Table 2).

3.2.1 Energy Metabolism

Two clinical studies investigated the effect of probiotics on energy metabolism (Borzabadi et al., 2018; Tamtaji et al., 2019) (Table 2) and found that probiotics may increase glucose metabolism via reducing insulin resistance in PD patients when compared with placebo.

In particular, 12-week treatment with *Lactobacillus acidophilus, Lactobacillus Fermentum, Lactobacillus reuteri,* and *Bifidobacterium bifidum* reduced triglycerides and very low-density lipoprotein (VLDL)-cholesterol plasma levels in PD patients when compared with placebo. However, the probiotics treatment did not change total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol and MDA plasma levels (Tamtaji et al., 2019). Additionally, probiotics supplementation reduced insulin resistance and insulin plasma levels, and increased insulin sensitivity, when compared with

placebo (Tamtaji et al., 2019). Similarly, following 12-week treatment with the same mixture of probiotics to PD patients, PMBCs isolated post-treatment were found to have upregulated the expression of PPAR-γ gene when compared with PBMCs isolated from the placebo group; however, there were no changes in the expression of low-density lipoprotein receptor (LDLR) (Borzabadi et al., 2018).

3.2.2 Inflammation and oxidative stress

Three clinical studies investigated the effect of probiotics on inflammation and oxidative stress (Borzabadi et al., 2018; Tamtaji et al., 2019; Tan et al., 2020) (Table 2), 2 of which were previously introduced in section 3.2.1 (Borzabadi et al., 2018; Tamtaji et al., 2019). Overall, they found that probiotics may decrease peripheral inflammation in PD patients when compared with placebo.

Interestingly, 12-week treatment with a mixture of probiotics belonging to the *Firmicutes* or *Actinobacteria* phylum reduced peripheral levels of high-sensitivity C-reactive protein (CRP) and increased plasma levels of the antioxidant glutathione in PD patients, when compared with placebo (Tamtaji et al., 2019). However, in another study, 12-week treatment with the same mixture of probiotics did not change plasma levels of glutathione, when compared with placebo (Borzabadi et al., 2018). In the latter study, however, probiotics supplementation downregulated the gene expression of IL-1, IL-8 and TNF-α in PBMCs isolated from PD patients post-probiotic treatment, when compared with placebo (Borzabadi et al., 2018). Additionally, 4-week treatment with *Lactobacillus acidophilus*, *Lactobacillus reuteri* and *Bifidobacterium bifidum* did not affect levels of faecal calprotectin, a marker of intestinal inflammation (Tan et al., 2020).

In conclusion, evidence suggests that probiotics can reduce peripheral systemic inflammation (expression of CRP, IL-1, IL-8 and TNF-α levels), whereas no effect was

observed on peripheral intestinal inflammation (faecal calprotectine levels). However, findings remain inconclusive concerning the effect of probiotics on oxidative stress in PD patients.

3.2.3 Neurodegeneration

No clinical studies evaluated the effects of probiotics on peripheral or cerebrospinal markers of neurodegeneration in PD patients.

3.2.4 Motor function

Only 1 study, previously introduced in sections 3.2.1 and 3.2.2, investigated the effect of probiotics on motor function (Tamtaji et al., 2019). The study showed that 12-week treatment with a mixture of probiotics from the *Firmicutes* or *Actinobacteria* phylum improved overall PD symptomatology, including tremor, bradykinesia, rigidity, and gait dysfunction, as measured by the Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS).

3.2.5 Non-motor function

Four clinical studies investigated the effects of probiotics on non-motor function in PD (Cassani et al., 2011; Georgescu et al., 2016; Tamtaji et al., 2019; Tan et al., 2020) (Table 2). Two of these studies were previously introduced in sections 3.2.1 (Tamtaji et al., 2019), 3.2.2 (Tamtaji et al., 2019; Tan et al., 2020), and 3.2.4 (Tamtaji et al., 2019). Overall, results showed that probiotics may decrease constipation in patients with PD when compared with placebo.

Three studies showed that 12-week treatment with probiotics, including *Lactobacillus casei Shirota* only (Cassani et al., 2011) or *Lactobacillus acidophilus* among multiple strains (Georgescu et al., 2016; Tamtaji et al., 2019) improved constipation, including frequency of bowel movements, stool consistency, abdominal bloating and pain, as well as the sensation of

incomplete evacuation (Cassani et al., 2011; Georgescu et al., 2016; Tan et al., 2020). Additionally, 12-week treatment with a mixture of probiotics, containing *Lactobacillus acidophilus* among other strains, improved overall PD symptomatology, including cognitive impairments, anxiety, and depression, as measured by the MDS-UPDRS (Tamtaji et al., 2019).

In conclusion, evidence suggests that probiotics treatment can reduce constipation-related symptoms in PD, as well as improve overall PD symptomatology, including cognitive alterations and affective symptoms.

4 Discussion

This is the first review to systematically investigate the effects of probiotics on energy metabolism, peripheral and central inflammation, oxidative stress, neurodegeneration and motor and non-motor function, in preclinical and clinical studies of PD. Findings from preclinical studies suggest that treatment with probiotics can increase glucose metabolism (increased secretion of GLP-1), reduce peripheral and central inflammation (reduced peripheral and central levels of IL-6 and TNF-α), reduce peripheral and central oxidative stress (reduced peripheral superoxide anion levels and increased central antioxidant glutathione levels), decrease neurodegeneration (increased numbers of TH+ dopaminergic neurons and levels of the neuroprotective factor BDNF), and increase motor (increased motor agility) and non-motor function (decreased memory deficits). Similarly, findings from clinical studies seems to suggest that probiotics can increase glucose metabolism (reduced insulin resistance), reduce peripheral inflammation (reduced peripheral gene expression of TNF-α and CRP levels), and increase motor and non-motor function (reduced overall PD symptomatology and constipation). However, findings on oxidative stress were inconclusive and no clinical studies, thus far, have investigated the effects of probiotics on neurodegeneration in PD. Overall, this

review suggests a beneficial role for probiotics across a variety of cellular and molecular mechanisms, as well as symptoms characterising the PD pathology.

Firstly, results from our review demonstrate that probiotics can re-establish normal lipid glucose metabolism, which is commonly dysregulated in PD (Alecu and Bennett, 2019; Dunn et al., 2014) (Figure 2). Interestingly, in both clinical and preclinical studies probiotics treatment were able to activate the GLP-1 metabolic pathway (Borzabadi et al., 2018; Castelli et al., 2020; Sun et al., 2020; Tamtaji et al., 2019), which leads to increased cellular expression of PPARγ, a transcription factor involved in fatty acid storage and glucose metabolism (d'Angelo et al., 2019). Interestingly, PPARγ expression was increased by a variety of probiotic strains, including *Lactobacillus* and *Bifidobacterium* strains, in both human and animal studies (Borzabadi et al., 2018; Castelli et al., 2020). Furthermore, GLP-1 activation contributes to a reduction in insulin resistance (Tamtaji et al., 2019), which is of relevance for PD as loss of insulin signalling may contribute to the development of the pathological features of this neurodegenerative disease (Athauda and Foltynie, 2016). In line with this, the GLP-1 receptor agonist exenatide, a commonly prescribed medication for type 2 diabetes mellitus (Syed and McCormack, 2015), is now considered to be one of the most promising therapeutic agents for PD (Athauda et al., 2017). Overall, this seems to suggest a putative role for probiotics in the regulation of the GLP-1 metabolic pathway in the context of PD. In addition, it is worth noting that none of the identified studies investigated the effect of probiotics on the kynurenine pathway metabolism which represents an interesting direction for future research, given the presence of alterations of this metabolic pathway in PD and the ability of probiotics to modulate it (Purton et al., 2021; Venkatesan et al., 2020).

Additionally, findings from our review suggest that probiotics can also reduce peripheral inflammation, both in preclinical and clinical studies of PD (Figure 2). In preclinical

studies, supplementation with probiotics decreased the protein levels of the inflammatory cytokines, including IL-6 and TNF-α, both in the brain and in the periphery of PD models (Liao et al., 2020; Magistrelli et al., 2019; Perez Visñuk et al., 2020), and, of note, this effect was independent of probiotic strain, PD model and treatment duration. Interestingly, these findings were partly replicated in human studies, where gene expression of similar cytokines, IL-1, IL-8 and TNF-α, was also reduced in the periphery (Borzabadi et al., 2018).

In contrast, findings from this review show inconsistencies in the effect of probiotics on oxidative stress, especially when focusing on clinical data (Figure 2). Part of this may be due to differences in study methodology, including differences in the concentrations of probiotics used across the study cohorts. For example, one clinical study administered high concentrations of a *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus reuteri* and *Bifidobacterium bifidum* probiotics mixture (8×10^9 CFU/day) and did not find significant changes in plasma levels of glutathione (Borzabadi et al., 2018), whereas another study administered a much lower concentration of the same probiotic mixture (2×10^9 CFU/day) and found an increase in plasma levels of glutathione (Tamtaji et al., 2019). Considering that these two studies were similar in duration, sample size, study design, as well as probiotic mixture, having a high or low probiotic concentration could play a relevant role in the effects of these bacteria on oxidative stress in patients with PD, although additional studies are required to confirm this observation.

Our review also suggests that probiotics exert potential neuroprotective properties in preclinical studies of PD (Figure 2). This was observed across a variety of studies, irrespective of the model, treatment duration or probiotic strain employed (Alipour Nosrani et al., 2020; Castelli et al., 2020; Goya et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019; Sun et al., 2020). The increase in the levels of dopaminergic neurons in the substantia nigra demonstrates the ability of these bacteria

to exert a central neuroprotective action. Indeed, this may be the result of a concomitant inhibition of pro-inflammatory markers, including the production of pro-inflammatory cytokines (Magistrelli et al., 2019; Perez Visñuk et al., 2020) and oxidant molecules (Liao et al., 2020; Magistrelli et al., 2019), and activation of neurotrophic factors, including BDNF and NGF (Silva et al., 2020; Yang et al., 2020), which exert neurogenic properties and contribute to the maintenance of brain plasticity.

The majority of the preclinical studies reviewed here also suggests that probiotics treatment can prevent the reduction in motor agility (Figure 2), despite the probiotic strain, the animal model of PD or duration of treatment employed (Alipour Nosrani et al., 2020; Castelli et al., 2020; Goya et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019; Sun et al., 2020). The beneficial effect of probiotics on motor function can be explained by the prevented loss of dopaminergic neurons in the substantia nigra observed after the bacterial supplementation (Alipour Nosrani et al., 2020; Castelli et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019). In humans, only one study has tested the effects of probiotics on motor function upon treatment with a mixture of bacteria belonging to *Firmicutes* or *Actinobacteria* phylum and showed a significant improvement in overall PD symptomatology, including tremor, bradykinesia, rigidity, and gait dysfunction (Tamtaji et al., 2019). While this approach is promising, it needs to be replicated in larger placebo-controlled trials, specifically looking at the motor aspects of PD.

Concerning non-motor symptoms, findings suggest that treatment with probiotics may prevent cognitive-related deficits in a PD model in rodents (Alipour Nosrani et al., 2020; Xie and Prasad, 2020) (Figure 2). These preclinical results are consistent with findings from one clinical study, where probiotics supplementation led to an improvement in overall PD symptomatology, including cognitive impairments (Tamtaji et al., 2019). Several mechanisms

of action can be implicated in the beneficial effects of probiotics on cognitive impairments in PD, specifically enhanced levels of neurotrophic factors (Castelli et al., 2020; Liao et al., 2020; Srivastav et al., 2019), reduced loss of dopaminergic neurons (Alipour Nosrani et al., 2020; Dwyer et al., 2021; Goya et al., 2020; Hsieh et al., 2020; Liao et al., 2020; Marsova et al., 2020; Perez Visñuk et al., 2020; Srivastav et al., 2019; Sun et al., 2020), as well as improved glucose tolerance and insulin sensitivity (Borzabadi et al., 2018; Castelli et al., 2020; Sun et al., 2020; Tamtaji et al., 2019), all of which are key mechanisms involved in the pathophysiology of cognitive dysfunction in PD (Aarsland et al., 2017).

In addition to cognitive assessments, another non-motor outcome assessed in the clinical studies was constipation (Figure 2), where findings consistently showed that probiotics can improve constipation-related features, such as abdominal bloating, pain and stool consistency (Cassani et al., 2011; Georgescu et al., 2016; Tan et al., 2020). This is not surprising, considering the ability of probiotics to modify the intestinal luminal environment, ultimately regulating intestinal motility and secretion (Dimidi et al., 2017). Indeed, probiotics have been recently recommended by the Movement Disorders Society Evidence-Based Medicine Committee as a clinically useful therapeutic option for the treatment of constipation in PD (Barichella et al., 2016; Seppi et al., 2019). However, while a relative amount of evidence has been generated so far on the efficacy of probiotics in improving constipation (Dimidi et al., 2014), the exact underlying mechanisms involved in these beneficial effects are still to be identified and therefore require additional investigations. Furthermore, considering the ability of probiotics to improve abdominal pain and alter peripheral and central mechanisms involved in pain modulation in PD (Guo et al., 2019), the latter might represent a novel future research area to explore from both preclinical and clinical perspectives.

Limitations of this systematic review include the paucity of data available in the clinical setting, which reflects the novelty of the topic. Nevertheless, we believe our review is

meaningful as it provides a systematic summary of the currently available evidence and highlights research gaps to be considered for future translational research. In addition, we acknowledge the mixed quality of the sources of reference material and, thus, further hight-quality studies with rigorous methodology are needed. Other limitations include the use of different strains of probiotics and different outcome measures across the preclinical and clinical studies, which makes it challenging to draw a firm conclusion on the relationship between individual strains of bacteria and specific effects. The latter, together with the well-known intrinsic challenges in translating research findings from animal model to humans, can partially explain some of the inconsistencies found among preclinical and clinical studies. It is also worth noting that although some evidence seems to suggest increased levels of *Lactobacillaceae* (usually considered to be probiotic strains) in faecal samples from patients with PDwhen compared to healthy controls, findings are still equivocal rather than conclusive. Should the increased levels of *Lactobacillaceae* be confirmed as a biosignature of PD, the underlying mechanisms would need to be elucidated and it may be argued that this might represent a compensatory mechanism reactive to a proinflammatory intestinal environment and provide the rational for probiotics to become an effective anti-inflammatory therapeutic option.

In conclusion, this is the first systematic review that highlights the potential beneficial effect of probiotics on metabolism, inflammation, neurogenesis and clinical aspects of PD across both preclinical and clinical studies. Despite the increasing evidence outlined in our review, which supports the beneficial effects of probiotic interventions in PD, further investigations are needed, especially in clinical contexts. Future research should aim at validating preclinical findings in larger, longitudinal and more controlled clinical studies, to identify the best therapeutic strategy (type of probiotic strain, concentration, treatment duration) for patients with PD.

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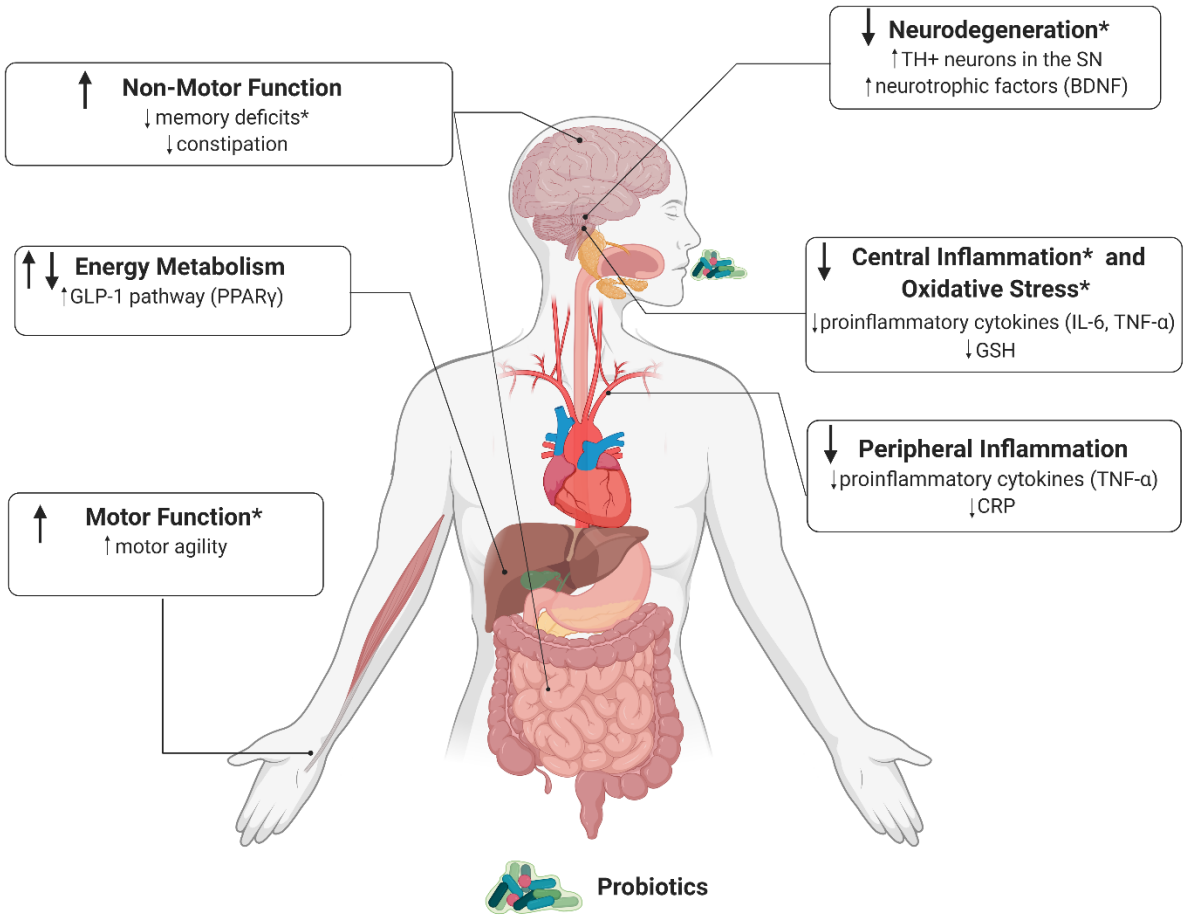

Figure 2. *Effects of probiotics in Parkinson's disease*

The figure shows the potential beneficial effects associated with probiotics supplementation in Parkinson's disease, which include modulation of energy metabolism, reduction of peripheral and central inflammation as well as oxidative stress, reduction of neurodegeneration, and increase motor and non-motor function. Effects demonstrated in preclinical studies only are marked by a star (*) and thus translational research is needed to confirm this basic scientific result. *Abbreviations:* BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; GFAP, glial fibrillary acid protein; GLP-1, glucagon-like peptide 1; GSH, glutathione; Iba1, ionized calcium-binding adaptor molecule 1; IL-6, interleukin 6; PPARγ, peroxisome

proliferator-activated receptor gamma; SN, substantia nigra; TH+, tyrosine hydroxylase positive; TNF-α, tumour necrosis factor-alpha.

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