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# The gut-brain axis affecting TLR4 in Parkinson's diseases

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## Abstract:

Parkinson's disease (PD) is a chronic neurodegenerative illness, which is increasing in developing countries and creating a burden on these economies. Multiple motor and non-motor symptoms have been connected to PD, and patients are diagnosed using clinical observations. Unfortunately, clinical symptoms are found in the late stages of the disease, when preventing is no longer an option. The gastrointestinal system, and more specifically the gut microbiota have an important role in the bidirectional communication taking place between the gut and brain. As a result, the gut microbiota dysbiosis and its effect on the brain-gut axis are among important factors to be considered in PD pathology. In addition, the role of Toll-like receptors (TLRs) in recognizing pathogenic molecules and creating immune responses can affect PD pathogenesis. In this review, we have tried to better understand the effect of TLR4 on the gut microbiota and their dysbiosis, creating a bidirectional feedback loop. Even though the effect of blocking TLR4 signaling on PD is still not well known, it affects PD, and brings hope of using microbial based medications to control this chronic neurodegenerative disease.

**Keywords:**Parkinson's disease; Toll-like receptors; TLR4; gut dysbiosis; non-motor symptoms; gastrointestinal dysfunction; alpha-synuclein

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## Introduction

Parkinson's disease (PD) is a progressive chronic neurodegenerative disorder [1]. The condition's clinical symptoms were first described in 1817 [2], and it has been estimated that by 2030 patients with PD disease will grow to somewhere between 8.7 and 9.3 million, shifting the burden of disease to developing countries in the east [3].

Multiple symptoms characterized as motor dysfunctions and non-motor ones are seen in PD patients [4]. Resting tremor, bradykinesia, and rigidity are three well-known motor characteristics considered when diagnosing PD. Postural reflex problems and postural instability usually take place later in the process, and are not primary diagnostic characteristics[5, 6]. These clinical symptoms usually occur during later stages of the disease. By the time a patient was diagnosed, PD can no longer be prevented, and doctors only try to help with existing and new symptoms[7]. Non-motor symptoms (NMSs) are also prevalent and can be seen in approximately 60% of patients. Unfortunately, compared to motor symptoms they are underreported. NMSs can create greater discomfort than motor symptoms and negatively affect the patient's quality of life [8].

Several possible causes, including genes and environmental factors, have been considered for this disease, and it is categorized as a complex and multifactorial condition resulting from their interactions [1, 9]. For instance, variations of genes found in Crohn's disease patients can be seen in PD patients [7]. Also, constipation increases the risk and physical exercise decreases it. However, it is believed these could be signs of PD preclinical development rather than being risk factors [9]. A number of toxins, especially chemicals and pesticides used in agriculture, have also shown links with PD, and in individuals with genetically altered function increased the risk associated with the toxins [2]. Healthy lifestyle choices and anti-inflammatory treatments used to reduce autoimmune conditions have been seen to help lower PD risk [1].

PD is usually characterized by a loss of dopaminergic neurons mainly in the substantia nigra (SN) region [10]. This feature is not specific to this disease and can be found in other neurodegenerative parkinsonian conditions as well. The difference lies in the pattern of degeneration. The ventral tegmental area (VTA) rarely changes in PD, while the ventrolateral SN shows a great loss [11].

TLRs are considered the front line in the immune system's defense against pathogens [12]. As a subfamily of pattern recognition receptors (PRRs), TLRs sense microorganisms' presence [13]. They are seen as the intestine and microbial signals' first point of contact, binding to pathogenic substances and leading to gut inflammation [1, 3,14].The gastrointestinal (GI) system has also been recognized for its involvement in PD. The gut microbiome (GM) regulates a relationship between the brain and GI system called the gut-brain axis by using immunological mechanisms, endocrine and neural signaling [3, 6, 13,15]. GM products including short-chain fatty acids (SCFA) are known to affect the central nervous system (CNS) [5]. Changes in this complex system can lead to GI dysfunctions associated with PD. Among the non-motor dysfunctions, GI ones are common, and can start long before motor dysfunctions, making the microbiome-gut-brain axis a possible less understood player in the cross-talk taking place between the gut and brain [1, 6, 13, 16].

In this review, we will look at the role of the GM in PD pathogenesis. Also, we will take into consideration the role of TLRs and their link to PD.

**Pathophysiology and mechanism of Parkinson's disease**

There have always been different views concerning the influence of the environment and genes in PD [2, 17]. For instance, according to Goldman et al. who studied twins, when one twin had been diagnosed before reaching the age of 50, there was a greater consistency in PD among monozygotic twins compared to dizygotic ones. In cases diagnosed after this age the possibility was similar between both types of twins. These findings are in agreement with an environmental effect for the normal age of disease onset, and a more genetic basis for young-onset [18]. Sometimes the effect of one factor has been reduced in favor of the other. Currently, a combination of both factors is considered in creating the pathophysiologic process of PD [2, 17].

Exposure to certain metals or chemicals in the environment might increase the possibility of PD. This exposure can be occupational or resulting from living close to fields treated with pesticides [17]. Personal habits such as using gloves while working with some toxic chemicals and washing hands afterwards have been shown to reduce the risk [19]. Also, genetic factors could affect an individual's risk after exposure to toxic chemicals [17].

10% of genetic mutations are familial PD [20]. As a result, they are not found in most PD patients [17]. At least 17 genetic mutations have been found in familial PD investigations. Some are autosomal recessive gene mutations, and others are autosomal dominant ones [20, 21]. Some mutations cause rare familial forms of PD. Rarer cases cause diseases with pathological and clinical characteristics of PD, which are more like parkinsonism than idiopathic PD [20, 21]. Genetic mutations have greatly helped understand the underlying mechanisms of PD. Alpha-synuclein mutations are one of the most important genetic mutations in understanding the underlying mechanisms of PD. Although rare, their existence has led to discovering the major role alpha-synuclein has in Eosinophilic cytoplasmic neuronal inclusions, otherwise called Lewy bodies (LBs), and Lewyneutrites[17, 20]. A mutation in the gene that encodes for alpha-synuclein (SNCA) creates alpha-synucleins that are more likely to misfold and aggregate compared to the wild-type. Another mutation in this gene has been shown to affect its quantity leading to duplications of alpha-synuclein [22]. Both characteristics can be seen in PD.

Until now several possible sites have been considered as the organ where PD pathogenesis could begin. For instance, the appendix has more alpha-synuclein than other GI tract sites and is supplied with nerves from the vagus nerve. Also, tonsils participate in protecting the gut from bacteria and participate in mucosal immunity. Unlike the appendix, they are not directly supplied with vagus nerves. In such cases, appendectomy and tonsillectomy have been studied for possible prevention or reduction of PD symptoms. Neither has shown significant results [23].

The damage from gradual loss of dopaminergic neurons in the SN and dopamine deficiency causes some of the main motor characteristics and GI symptoms
observed in PD [2, 24]. In the motor loop, it innervates the motor portions, and the resulting deficiency leads to an impairment in the balance between inhibition and excitation phases. In these circumstances, pathways are shifted towards inhibition, which in turn cause a decrease in movement. Caligiore et al (2016) believes in the importance of alterations in cerebellar circuits and changes in the basal ganglia's interactions with the cerebellum in PD pathophysiology [2]. Dysfunction in the GI system is also associated with lesions in different areas of the autonomic nervous system [24].

**dysfunction in PD**

GI dysfunction is one of PD non-motor complications. In normal circumstances, several factors affect the GI function, the most important being muscle cells contraction and sphincters coordinated function [6, 15].

Individuals with GI dysfunctions are considered at a higher risk of PD [25]. For instance, over-expression of the CARD15 gene's nucleotide polymorphisms connected to Crohn's disease can be seen in PD patients. In addition, the drugs used for treating PD can lead to GI dysfunction in patients, and are considered as side effects [24]. Most PD patients experience GI dysfunctions in the form of hyper-salivation from difficulty swallowing, dysphagia, nausea, delayed gastric emptying, changes in bowel habits and constipation [13, 24, 25]. Enteric neurodegeneration is known to lead to bowel dysfunction [15]. These problems might not be life threatening, yet they do affect drug pharmacodynamics and a person's quality of life. Their frequency depends on the severity of PD and length of time an individual has been ill [25]. The GI tract and enteric nervous system (ENS) appear to have a role in PD pathogenesis [13, 26]. The ENS is one of the first places where lesions occur before the CNS. These lesions are thought to be the reason for digestive symptoms. They also affect dopaminergic neurons [24]. Gut dysfunction can cause changes in neurotransmission pathways [13, 26].

**Gut microbiota changes in PD**

The GI tract has so many neuronal cells it is considered the second brain. These cells interact with the microbiota, and any products it might have [26]. The gut microbiota with different microorganisms has been compared to a complete organ in the body [13]. The brain and GM use different nerves to create the gut-microbiome-brain axis (GMBA) – a bidirectional relationship between the ENS and CNS [27]. GMBA is known to influence neurotrophic factors and neurochemistry [1, 16]. The vagus nerve is an important part of the parasympathetic nervous system. It creates a connection between microbiota metabolites it identifies and responses it creates in the CNS [5]. Also, it is thought to have an anti-inflammatory role and control the intestine's immune activation [23].
Microbiota in the GI tract is unique for each person, but similar in all healthy adults. Medications, disease, diet or anything capable of changing the microbiota, which depends on the length of time the microbiota has undergone disruption and the extent of the problem, can affect its ability to recover its original proportions [13, 28]. PD patients have different gut microorganisms at different stages of the disease. For instance, *Clostridium coccoides*levels increase in the first stages of PD, while *Lactobacillus gasseri* grows in the advanced stages [16]. Altogether, concentrations of gut microbiota are lower in these patients. Researchers have not reached a common ground on changes in PD patient's microbiome [27]. They are aware that PD is connected to an increase in *Helicobacter pylori* infections and the possibility of ulcer disease [24]. Changes in gut microbiota and inflammatory reactions can begin PD pathogenesis or aggravate it [16]. The enteric microbiota is also affected by geography and aging. This can have repercussions for an individual's later stages of life [1, 13, 24], since the gut microbiota diversity decreases, gut permeability increases and gut movement is reduced with age. These differences could help better understand the effect of gut microbiota from different environments and countries in PD [24]. At the same time, it can make it difficult to understand which microorganisms have a consistent role in PD [1, 13]. PD patients are also at greater risk of motility dysfunction in the small intestine, which can make them susceptible to small intestinal bacterial overgrowth (SIBO). SIBO might cause small intestinal permeability and affect motor dysfunction [24].

**Alpha-synuclein aggregation in the GI**

The GI has the greatest level of interaction through a large surface with environmental factors. Toxins from the environment can create bacterial products in areas interacting with the neuronal network inducing oxidative stress. The importance of environmental toxins causing PD has led to the possibility of the GI becoming a candidate for oxidative stress forming phosphorylated alpha-synuclein aggregates [26]. In addition, there is a possible connection between microbial dysfunction and CNS related diseases like PD, which could be the result of the host and microorganisms adapting to each other [16]. Infections affecting the GI tract can also induce the expression of alpha-synuclein, possibly causing a defense mechanism [13].
In normal conditions, alpha-synuclein exists in the CNS and is associated with neurotransmission regulation. However, it can become phosphorylated and become involved in PD [2]. The intestinal submucosa could be where alpha synucleins start to deposit in neurons [15]. Changes in the GI barrier allow byproducts from microorganisms to move and cause inflammation, oxidative stress and mucosal permeability [16]. As an early sign of the disease, alpha-synuclein deposition could be the result of pathogens or environmental toxins and leaky gut syndrome – removing the barrier to microorganisms and their products. However, it can be triggered by enteric microbiota as well [5, 19]. Oxidative stress and neuro inflammation, which is triggered by gut microorganisms, can cause aggregation and misfolding, removing the need for any form of external contribution. Misfolded alpha-synuclein can then increase oxidative stress and inflammation by activating microglial cells neurodegeneration, beginning in the CNS and moving to the substantia nigra pars compacta (SNc) [5, 6, 29].

As previously mentioned, a loss of dopaminergic neurons mainly in the SN region is a sign of PD [2, 10]. LBs aggregation is also considered a clinical sign of PD, and cannot be found in other cases with parkinsonian signs. Neurodegeneration usually takes place when LBs exist. Misfolded alpha-synuclein aggregates are an important part of LBs – a pathological sign of PD [2, 19,30]. Alpha-synuclein toxicity is mostly the result of these insoluble aggregates [25]. Until 2003, it was believed to be limited to the brain but has been found to exist in the GI tract for years before other PD symptoms (7, 14). It can be seen along the esophagus till the rectum in gut ENS mucosal and submucosal plexuses and olfactory bulbs [5, 19]. It is seen in untreated patients ENS from the early stages of PD [25] and spreads as the disease progresses [31]. However, it is not always pathological and depends on the amount expressed, as it is also connected with aging and can be found when individuals grow older [25].It is possible that pathology can spread in a similar way as prions, moving from neuron to neuron. It can move from the gut to brain, beginning from the CNS and moving towards the ENS or the other way round. The alpha-synuclein movements can happen at the same time as from brain to gut [32]. Alpha-synucleinhas been shown to spread from rats' gastrointestinal tract to their brain[22]. Also, it has been observed that alpha-synuclein can use the vagal nerve in mice to move from the ENS to the CNS [14]. The proximal and anterior vagus nerves receive fibers from the left and right cervical vagus nerves when entering the abdominal cavity[7]. Therefore, alpha-synuclein can spread from the periphery to midbrain. This brings up the possibility of PD beginning outside the CNS. The manner in which the deposition moves up to the brain can also support the movement of alpha synucleins through the GI tracts' submucosal layer and reaching the CNS using the vagus nerve[14]. The reverse direction of alpha-synuclein movement using the vagus nerve has been confirmed. Studies in small groups of volunteers showed removing 1-2 cm of the vagus nerve (vagotomy) decreased PD, but has not proven to significantly protect from it [7, 33]. Due to many possible factors that could have affected the circumstances (e.g. type of PD and vagotomy procedure), it needs more research on a larger group. The ability of alpha-synuclein pathology to start at peripheral or central nerve terminals makes it difficult to understand PD pathogenesis[7] Also, alpha synucleinopathy spreading between the interconnected areas of these nerve systems is controversial in clinical research [35].

**Role of Tlr4 and its absence in PD**

The communication taking place with the help of microorganisms' neurohormones, neuromodulators and neurotransmitters creates a network. They might be able to use TLRs to regulate neurotransmitter levels, or directly use neuroactive molecules. As previously mentioned, TLRs are a subfamily of PRRs that sense microorganisms' presence [33]. PRRs are part of the innate immunity and activate microglia, causing them to begin an inflammatory immune response. Genes connected to TLRs might have a role in familial PD as well. They have an important role in inflammation as they increase the release of pro-inflammatory proteins [16].

TLRs are type I integral transmembrane glycoproteins made of three domains: a C-terminal domain which faces the cytoplasm, an N-terminal domain on the outside of the membrane (ectodomain), and a single helix transmembrane domain. The single helix is situated between the other domains, and spans the transmembrane connecting the two sides. TLRs determine ligands on the cell surface. Once a ligand such as MAMPs/PAMPs (Microbial associated molecular pattern or Pathogen associated molecular pattern) attaches to the ectodomain, it induces dimerization. Subsequently, the C-terminal domain begins a series of interactions leading to downstream signaling. The signaling cascade that takes place depends on the TLR, the downstream adaptor molecule used for signaling and the kind of ligand that initiates the process [3, 4, 31].

TLRs are the first point of contact between the intestine and microbial signals. They are mediators between gene-environment interactions and the resulting gut inflammation. TLRs are considered the reason behind the enteric neurons and gut microorganisms' interactions [1, 14]. The manner in which they are spread along the nervous system shows their role in the neural cells homeostasis and development. It also shows the neural cells role in immune responses [8]. In the intestine, TLR expression changes, and different amounts of micro-organism products from the gut could pass through when they are blocked. Suppression increases the risk of infections [29]. TLR4 (shock proteins) are one of several TLRs bound to plasma membrane that interact with the bacterial membranes and cell walls molecules [8]. It regulates neuro inflammation and has a small level of expression in healthy intestines. When TLR4 changes, it causes microflora dysbiosis that has been indicated in PD patients [14, 30, 33]. However, gut inflammation and dysbiosis does not always lead to PD [1]. Dysbiosis affects the intestine barrier, activates the immune response and causes systemic inflammation [26]. In the long run, dysbiosis leads to gut permeability, which in

turn causes colitis and leaky gut [14,30]. Microflora dysbiosis also changes the normal gut-brain connection, affecting all functions related to this connection [25].

The inflammatory nature of dysbiosis brings up the possibility of using the TLR4 signaling pathway to trigger the colon's mucosal immune activation. This has also been hypothesized by Perez et al. who believed it could cause neurodegeneration and neuro inflammation in PD patients. In PD patients the intestinal mucosa shows a growth of TLR4 + cells. This increase is accompanied by a reduction of natural colonic bacteria that produce SCFA [28]. Fecal SCFAs, which can pass the blood-brain barrier and affect the CNS, have been seen to differ between healthy individuals and PD patients [9, 27, 33]. SCFAs are usually seen in the colon, and can regulate towards increased or reduced levels of inflammation. Their epigenetic mechanism or antimicrobial receptors' effects are believed to have anti-inflammatory properties [9, 26].

Lipopolysaccharides (LPS) are known to trigger TLR4 [33]. When barriers are removed microorganisms and bacterial products such as LPS can transfer into circulation creating possible contact with cell surface PRRs, including TLR4 that binds to LPS and other molecules, activating nuclear factor kappa B (NF-κB) triggering neurodegeneration by generating pro-inflammatory protein production [1, 16, 30]. In the liver TLR4 activation also suppresses phosphoenolpyruvate carboxy kinase (PEPCK) [34]. Alpha-synuclein uses the blood-brain barrier for its bidirectional movement. LPS induced inflammation disrupts this barrier and can increase its movement towards the brain [35].

Alpha-synuclein might use TLRs to act like Damage-associated molecular patterns on microglia, increasing their expression and causing neuro inflammation Fig 1. [16]. TLR4 activated by alpha-synucleins in microglial cells increase Tumor necrosis factor-α (TNF-α, triggering signaling pathways including the PI3K/AKT/GSK3β and helping activate proteins that deliver signals to downstream pathways, including NF-κB. The NF-κB can then lead to release pro-inflammatory cytokines release, and ultimately result in the elevated expression of inflammatory genes damaging neurological function [1, 8, 35]. TLR4 antagonists have been able to prevent this series of inflammatory events caused by alpha-synucleins [1]. TLR4-knockout has been shown to decrease motor impairments. It significantly decreases brain neuroinflammation, believed to have a role in PD neuroinflammation, neurodegeneration, gut permeability and gut-derived inflammation. TLR4 loss causes a case of dysbiotic pro-inflammatory microbiota. This new microbiota creates a TLR4/TNF-α signaling leading to a TLR4 immune activation in PD patients' brain and colon [1, 30].

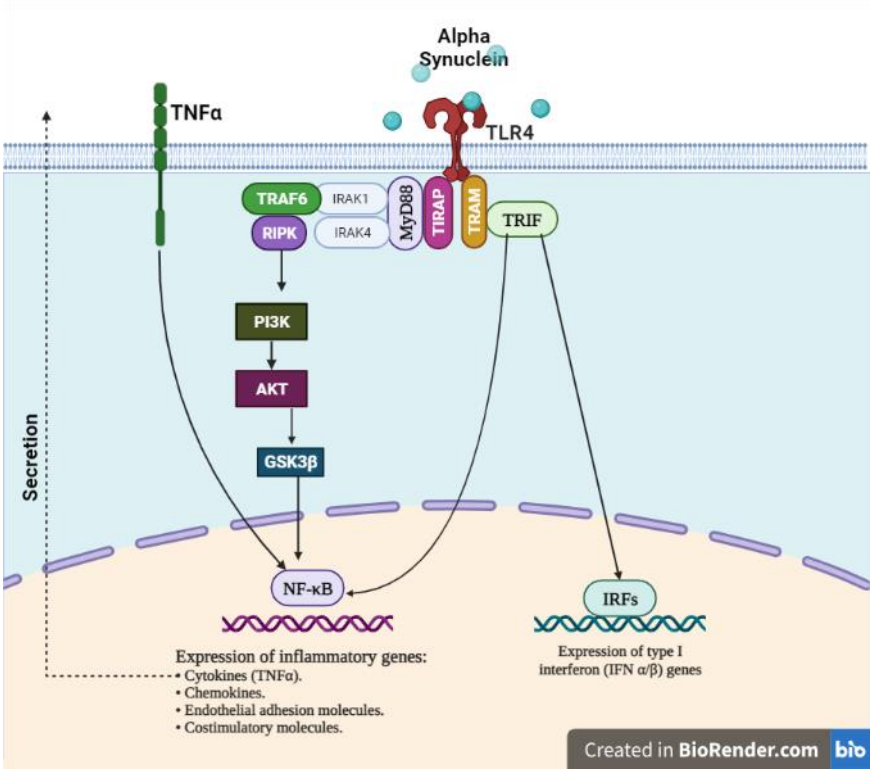


**Figure1.** A general view of the TLR4 inflammation pathway after activation with alpha-synuclein.

## Constipation and Microbiota Treatments

Constipation and bloating are very common in patients. Caputi et al. considered constipation as an early biomarker of PD seen long before motor symptoms [8, 18]. However, Forsyth et al. did not have any complaints from newly diagnosed patients, and Perez et al. didn't find clinically significant symptoms in their study [18, 30].

Slower colon movement has been observed in patients without symptomatic constipation, and in patients who have or have not been treated[8]. Constipation has been known to occur long before motor symptoms begin, sometimes it can start decades earlier [35]. It can be the result of losing central dopaminergic neurons and colonic ones. In advanced stages of the disease infusing duo dopa within the jejunum has been shown to help non-motor symptoms including constipation [10].

PD-related constipation could affect the composition of the microbiota [26]. There have been several studies on changes in PD patients' different fecal microorganisms compared to healthy individuals which can be seen in PD characteristics [14]. Type three secretion systems are found in conditions that could help with inflammation caused by bacterial products. Their increase in PD patients' fecal samples supports the possibility of intestinal dysbiosis increasing neuroinflammation [26]. *Enterobacteriaceae* levels increase in PD patients' fecal samples compared to healthy individuals, while *Prevotellaceae* are less than normal. Reduced *Prevotellaceae* commensals that take part in the gut's mucosal layer mucin synthesis and SCFA production are connected to intestinal permeability resulting in bacterial antigens transfer, dysbiosis and alpha synucleinopathy [14, 28, 33,35]. Keshavarzian et al. also found a decrease in *Prevotellaceae*, but did not see a significant reduction [26]. Decrease in *Prevotellaceae* adds to bacterial endotoxins and antigens exposure, which is caused by increased intestinal permeability. This could cause overexpression of alpha-synuclein or misfolding in the colon [28]. *Roseburia*, *Blautia* and *Coprococcus* which produce SCFA and are connected to anti-inflammatory characteristics decrease in patients' fecal samples, leading to a leaky gut [26].

Fecal Microbiota Transplantation (FMT) used in several diseases has been shown to improve the gut's microbial dysbiosis. This improvement also creates a form of neuroprotection in PD, inhibiting the TLR4/TBK1/NF-κB/TNF-α signaling pathway resulting in a reduction of p-P13K, p-AKT, TLR4, NF-κB and TNF-α expression mediated by alpha-synuclein. Thus, reversing gut microbial dysbiosis, reducing the number of dopaminergic neurons lost in the brain and improving motor function by inhibiting glial cell activation and fecal SCFAs [1]. FMT has been shown to have positive results in mice, but the amount of research on their effect in humans with PD is extremely limited[13,28]. Antibiotics have also been shown to help improve motor symptoms, but need more well-designed research. Results from these studies could then help better understand the effect of FMT [28].

Also, probiotic supplements have been shown to restore the gut and other tissue homeostasis by making the GI environment more favorable for the host. They depend on the vagal activation to affect CNS functionality [28, 35]. Studies on the possible benefits of probiotics in PD are still limited in the preclinical and clinical stages (Table 1).

**Table 1.** Studies on the possible effects of probiotics in PD [3, 11, 17, 24].

| Organism | Probiotic | Results |
|---|---|---|
| Peripheral blood mononuclear cells (PBMCs) isolated from PD patients | *Lactobacillus salivarius* LS01 DSM 22775, *L. plantarum* LP01 LMG P-21021, *L. acidophilus* LA02 DSM 21717, *L. rhamnosus* LR06 DSM 21981, *Bifidobacteriumanimalis* subsp. lactis BS01 LMG P-21384, and *B. breve* BR03 DSM 16604 | The production of pro-inflammatory cytokines was down regulated.<br>The production of anti-inflammatory cytokines was promoted. |
| PD patient | 4-strain probiotic suspension (Symprove) | The relative proportions of the main bacterial phyla in the microbiotas of PD patients differed from those of healthy subjects, with levels of *Firmicutes* raised and levels of *Bacteroidetes* reduced. |
| 6-hydroxydopamine (6-OHDA) mouse model | A mixture of nine bacterial strains: *Streptococcus thermophilus*, *B. longum*, *Bifidobacterium breve*, *Bifidobacteriuminfantis*, *L. acidophilus*, *L. plantarum*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, and *Lactobacillus brevis*, formulation SLAB51 | Conferred neuroprotection with reduced nigral dopaminergic neuronal loss. |
| MPTP and rotenone toxin-induced PD mouse models | Probiotic cocktail containing *L. rhamnosus GG*, *Bifidobacteriumanimalislactis*, and *Lactobacillusacidophilus* | Promoted the production of butyrate |
| The genetic MitoPark PD mouse model | Six common probiotic strains (*Bifidobacteriumbifidum*, *Bifidobacteriumlongum*, *Lactobacillus rhamnosus*, *L. rhamnosus GG*, *Lactobacillus plantarum* LP28, and *Lactococcus* | Detected better motor performance (gait, balance, and coordination) in the treated animals.<br>Reduced degeneration of nigral dopaminergic neurons, suggesting a neuroprotective effect of the probiotic mixture. |

**Table 1 (continued).** Studies on the possible effects of probiotics in PD [3, 11, 17, 24].

| Organism | Probiotic | Results |
|---|---|---|
| MPTP-treated PD mice | *Lactococcuslactis* subsp. *cremori* | Exhibited reduced locomotor impairment, increased neurons positive for tyrosine hydroxylase (the enzyme producing dopamine and other catecholamines), and reduced inflammation.<br>Suppressed *Enterobacteriaceae* (that may act as an intestinal pathogen) and enhanced the number of probiotic *Lactobacillus* and *Akkermansia* species. |
| alpha-synucleinexpressing worm (*Caenorhabditiselegans*) model | *Bacillus subtilis* PXN21 | Reduced alpha-synucleinaccumulation in the host. |
| *Caenorhabditiselegans* model of synucleinopathy | *Bacillus subtilis* | Inhibited alpha-synuclein aggregation<br>Cleared pre-formed aggregates |
| *[1]NMS rats | VSL#3( *lactobacilli (Lactobacillus casei, L. plantarum, L. acidophilus and L. delbrueckii subsp. bulgaricus), three strains of bifidobacteria (Bifidobacteriumlongum, B. breve and B. infantis) and Streptococcus salivariussubsp*) | Normalization of the inflammation-related genes pattern.<br>Counter-regulates genes involved in the inflammatory cascade, and genes that encode for factors that regulate the innate and adaptive immune response, as TLRs, NFκB and MAPKs, thus inhibiting inflammatory processes. |
| Multi-hit LPS plus paraquat model of PD mice | VLS#3 , *[2]DDS-1 | DDS-1 Altered gut microbiota<br>Probiotic VSL#3 did not influence any PD-like outcomes and appeared to have minimal consequences overall. |

1.Neonatal maternal separation model (NMS)
2. *Lactobacillus acidophilus* DDS-1

## Conclusion

The manifestation of GI symptoms before motor symptoms can be an indicator of PD beginning in the gut and moving to the brain in later stages. The gut-brain axis is bidirectional and there seems to be a bidirectional relationship creating a feedback loop. In this loop increased gut permeability can result from neuroinflammation and cause neuroinflammation by allowing environmental toxins and microorganisms entry, forming phosphorylated alpha-synuclein aggregates.

There also seems to be a link between the gut microbiota and what happens in the brain. Previous reviews show that although it has not been confirmed whether changes in gut microbiota cause PD or the other way round, changes in gut microbiota might cause oxidative stress and increase inflammation in the brain. GM influences the brain and behavior, using the neural, endocrine and immune systems to alter the brain's neurochemistry [14]. Contact between bacterial substances such as LPS with TLR4 might not be directly connected to PD, but they can show possible underlying problems. As part of the innate immune system, TLR4 activated by pro-inflammatory factors can affect the gut microbiota, altering the immune system and causing systemic inflammation [30, 33]. Although it is not yet known whether blocking TLR4 signaling will help decrease PD, this knowledge could open new possibilities to use microbial-based medications such as probiotics and FMT. However, a better understanding of the role of gut induced inflammation role and its effect on PD's initiation and the causal mechanism is also necessary.

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