CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
DOCTORAL SCHOOL
FIELD OF MEDICINE

DOCTORAL THESIS

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THE ROLE OF MICROBIOTA IN THE MECHANISMS OF AGGREGATION AND PROPAGATION OF ALPHA-SYNUCLEIN IN EXPERIMENTAL MODELS OF PARKINSON'S DISEASE

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Table of Contents of the PhD Thesis

I. General Part

II. Personal Contributions

Introduction, Hypotheses and Objectives

Parkinson's disease is the second most common neurodegenerative disorder, with a rising incidence and no available neuroprotective treatment, primarily due to the still incomplete understanding of its pathophysiology. A deeper insight into the mechanisms that initiate and drive the neurodegenerative process is essential for developing preventive and therapeutic strategies.

Until the early 2000s, it was believed that the pathological features of Parkinson's disease,namely α-synuclein aggregates and neuronal apoptosis,emerged simultaneously and progressively within neuroanatomical structures, affecting both dopaminergic neurons in the substantia nigra pars compacta and non-dopaminergic neurons. However, Heiko Braak and subsequent studies demonstrated that the pathological changes initially occur in peripheral regions, particularly in the nasal cavity and gastrointestinal tract, and progressively ascend in a centripetal, inter-neuronal manner. This spread resembles the progression of prion diseases, sequentially involving the medulla, pons, midbrain, basal ganglia, and cortical areas. As a result, many studies focusing on the initiation of α-synuclein aggregation now investigate interactions occurring within the gut.

A key player in these intestinal interactions is the gut microbiota. Its roles in pathology are closely tied to the amplification or initiation of autoimmune responses, promotion of local and systemic inflammation, and increased intestinal barrier permeability. Previous in vitro, in vivo, and even clinical studies have shown that alterations in gut microbiota composition are correlated with the onset and progression of Parkinson's disease. From a pathophysiological perspective, this potential role of the gut microbiota in initiating and propagating Parkinson's disease is scientifically grounded.

Intestinal bacteria locally form complex structures known as biofilms,which are communities of bacteria embedded in an extracellular matrix produced by the bacteria themselves. These biofilms serve as protective shelters against local antibacterial cells or compounds. A major structural component of these biofilms is protein aggregates. The proteins forming these aggregates are called functional amyloids and are capable of inducing not only their own aggregation but also cross-seeding aggregation of other aggregation-prone proteins. Examples of functional amyloids produced by gut microbiota include curli protein and phenol-soluble modulin α (PSMα).

Another class of molecules produced by gut microbiota includes bacterial amphiphiles, which contain both hydrophilic and hydrophobic components. These amphiphilic molecules can

form micelles that sequester specific proteins, potentially catalyzing aggregation if the sequestered proteins are prone to this process. Examples include lipopolysaccharide (LPS) and rhamnolipid.

Based on this information, evaluating the effects of microbial compounds derived from gut microbiota on the intestinal epithelium and enteric nervous system, as well as the interaction between gut epithelium exposed to these compounds and the nervous system, are essential objectives in studies exploring Parkinson's disease pathogenesis.

The central hypothesis of this doctoral thesis is that these microbial compounds derived from the gut microbiota exert pathological effects on intestinal system cells that trigger αsynuclein aggregation and subsequent inter-neuronal propagation.

Accordingly, this PhD thesis comprises three in vitro studies evaluating the effects of microbial compounds derived from the gut microbiota—namely lipopolysaccharide (LPS), rhamnolipid, the CsgA subunit of curli protein, and phenol-soluble modulin α1 (PSMα1)—on dopaminergic neurons (Study 1) and intestinal epithelial cells (Study 2). Additionally, the effects of these microbial compounds were assessed in terms of interaction between intestinal epithelial cells and dopaminergic neurons, simulating the interplay between the intestinal epithelium and the enteric nervous system (Study 3).

For assessing the effects of microbial compounds on dopaminergic neurons, the SHSY5Y human neuroblastoma cell line was used. These cells were differentiated into dopaminergic-like neurons using a specific protocol whose success was evaluated.

To assess the effects on the intestinal epithelium, the colorectal adenocarcinoma cell line Caco2 was used in vitro.

For evaluating the effects of microbial compounds on the interaction between the intestinal epithelium and the enteric nervous system, a co-culture system was used in vitro, involving Caco-2 cells pre-treated with microbial compounds and dopaminergic-differentiated SH-SY5Y neurons.

The assessment of microbial compound effects included evaluation of cell viability and cytotoxicity (via MTS viability assays and LDH cytotoxicity assays), analysis of cell adhesion (through surface impedance measurements using the xCELLigence platform), and analysis of intracellular α-synuclein levels (via α-synuclein gene expression measured by qRT-PCR and protein quantification using Western Blot).

The experimental research was conducted at the Laboratory of Cell Biology, Neuroscience, and Experimental Myology, within the "Victor Babeș" National Institute of Pathology.

Data are presented as mean ± standard error of the mean (SEM) and were statistically analyzed using GraphPad Prism v9.5.

For Western Blot and qRT-PCR experiments, data were expressed as fold change relative to control and normalized to the control group (control value = 1). Statistical analysis was performed on the normalized values.

The normality of data distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. For parametric data, one-way ANOVA and Dunnett's multiple comparison tests were used. For non-parametric data, the Kruskal-Wallis and Dunn's multiple comparison tests were applied.

Statistical significance is indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Study 1 Summary – Effect of Microbial Compounds Derived from Gut Microbiota on Dopaminergic Neurons

Given the presence of dopaminergic neurons within the enteric nervous system and the ability of gut microbiota metabolites to disrupt the intestinal barrier, enter the bloodstream, impair the blood-brain barrier, and interact with dopaminergic neurons in the substantia nigra, a crucial step in understanding the role of gut microbiota in Parkinson's disease pathogenesis is to evaluate the impact of microbial compounds on dopaminergic neurons.

In this in vitro study, we treated dopaminergic-differentiated human SH-SY5Y cells, according to a validated differentiation protocol (Figure 1), with gut microbiota-derived molecules -LPS, rhamnolipid, curli CsgA, and PSMα1- and evaluated cell viability, surface impedance, growth curves, and α-synuclein levels.

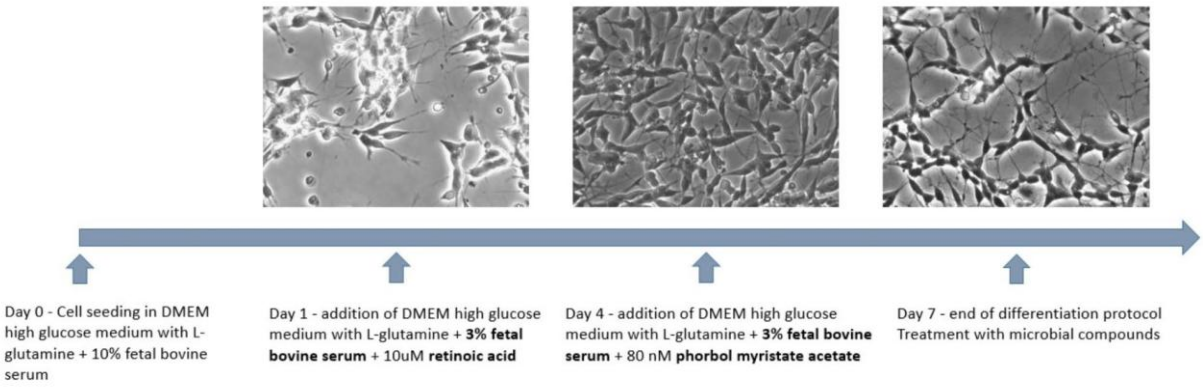

Figure 1. Graphical representation of the SH-SY5Y dopaminergic differentiation protocol with phasecontrast microscopy images corresponding to each stage (40x objective).

While many in vitro neuronal models use the human neuroblastoma cell line SH-SY5Y in its proliferative, undifferentiated state, in this study we employed dopaminergically differentiated SH-SY5Y cells, as they display a higher degree of similarity to human dopaminergic neurons. Even a simple observation of the morphological differences between undifferentiated and differentiated cells reveals that the latter constitute a much more accurate in vitro model.

Regarding the measurement of α-synuclein levels, although SH-SY5Y is a simple in vitro neuronal model, a significant advantage of this study is the use of a cell line that has not been genetically modified to overexpress α-synuclein or to express a mutant protein. Thus, any observed increases in α-synuclein levels occur within a native, basal expression context and provide valuable insights into the early pathogenesis of Parkinson's disease (PD).

To better understand the role of gut microbiota in the pathogenesis of PD, it is imperative to analyze how exposure to microbial compounds affects dopaminergic neurons. On one hand, the enteric nervous system contains dopaminergic neurons that are exposed to microbial compounds when the intestinal barrier is compromised. On the other hand, these microbial compounds can enter the bloodstream, disrupt the blood–brain barrier, and interact with dopaminergic neurons in the substantia nigra.

The discovery that the initial pathological changes in PD occur in the gastrointestinal tract has brought increased attention to the role of gut microbiota in this neurodegenerative disease. Therefore, to elucidate the precise role of microorganisms in the initiation of PD, it is essential to actively evaluate the microbial molecules that may induce the overexpression or

aggregation of α-synuclein. Bacterial amphiphiles such as rhamnolipid and LPS, as well as functional amyloids like curli and PSMα1, are microbial products that have garnered researchers' attention in PD studies.

Rhamnolipid is a bacterial amphiphilic molecule produced by *Pseudomonas aeruginosa*, which can colonize both the gastrointestinal tract and the nasal cavity. There is a lack of research into the effects of rhamnolipid on PD pathogenesis, and direct studies evaluating rhamnolipid levels in PD are limited. The potential relevance of a link between rhamnolipid and PD is amplified by the increasing use of rhamnolipid as a biological alternative to chemical surfactants in household cleaners and cosmetics. This study demonstrates a concentration-dependent effect on neuronal viability, cytotoxicity, cell adhesion, and morphology. Furthermore, it shows a concentration-dependent increase in α-synuclein in dopaminergic neurons, surpassing the overexpression induced by rotenone (Figures 2 and 3). LPS, the endotoxin of Gram-negative bacteria, is a potent pro-inflammatory molecule that activates Toll-like receptor 4 (TLR-4) and is also an amphiphilic compound that promotes bacterial biofilm formation. This study yielded interesting results following neuronal treatment with LPS, such as a lack of change in cell viability, cytotoxicity, and adhesion compared to the control group, regardless of concentration. However, an increase in α-synuclein expression and intracellular levels was observed (Figures 2 and 3).

Regarding treatment with functional amyloids-curli CsgA and PSMα1-at the selected concentration range, we observed slight changes in neuronal viability and cytotoxicity at the highest concentration of both compounds (32 μg/mL). We also noted a trend toward decreased adhesion in cells treated with curli CsgA. Beyond these effects, no other changes were observed, and there was no increase in α-synuclein mRNA or intracellular α-synuclein levels. These findings do not exclude the potential role of functional amyloids in inducing intraneuronal αsynuclein aggregation, which was not evaluated in this study.

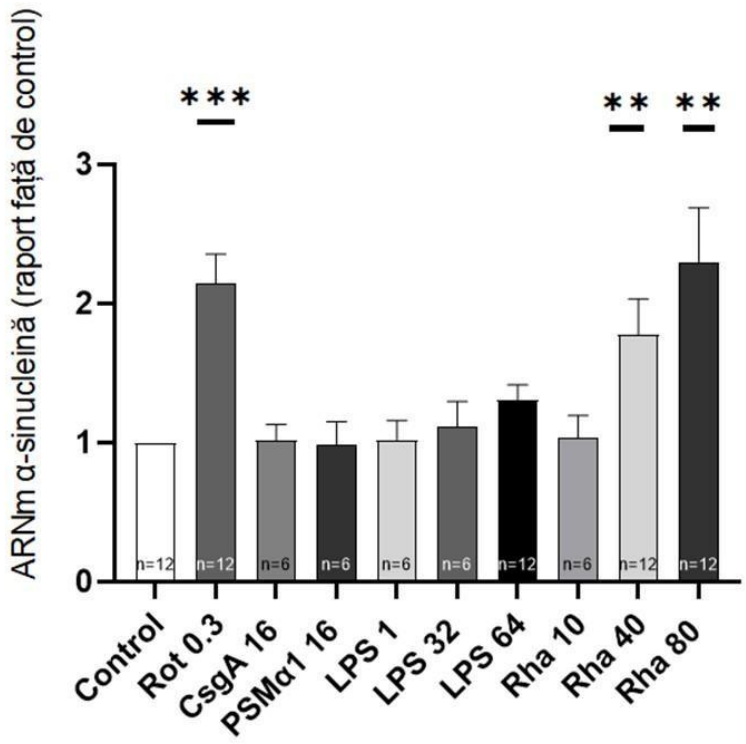

Figure 2. *qRT-PCR for α-synuclein mRNA performed 48 hours after treatment with bacterial compounds (curli CsgA 16 µg/mL, PSMα1 16 µg/mL, LPS 1 µg/mL, 32 µg/mL, and 64 µg/mL, rhamnolipid 10 µg/mL, 40 µg/mL, and 80 µg/mL) on dopaminergically differentiated SH-SY5Y cells. Rotenone 0.3 µM was used as a positive control. Data are presented as fold increase relative to the control, which was normalized to 1. α-synuclein levels were normalized to GAPDH. Data are expressed as mean ± SEM from three independent experiments, each performed at least in duplicate. **p < 0.01, ***p < 0.001.*

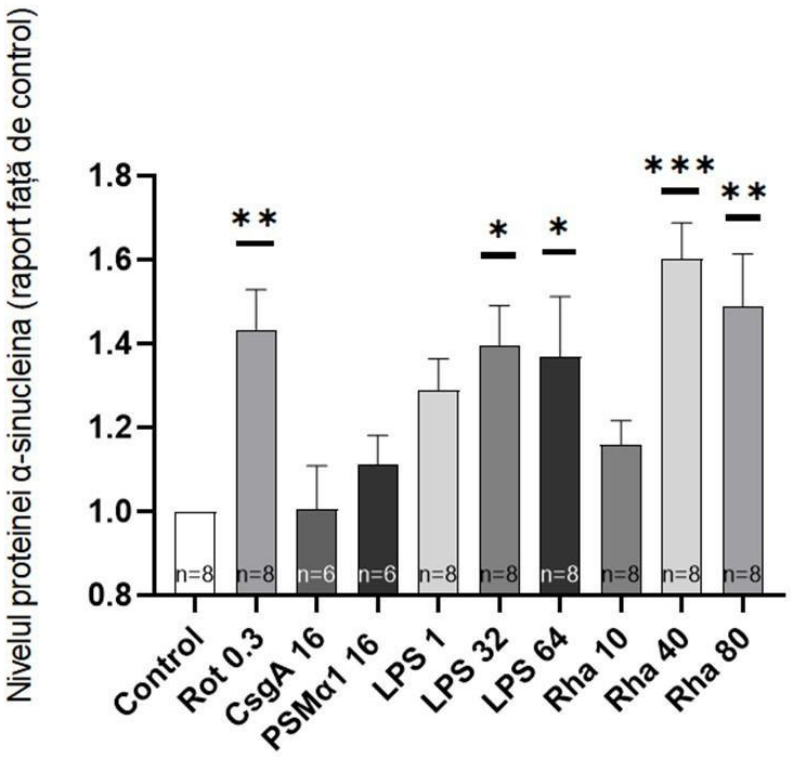

Figure 3. *Western blot analysis for α-synuclein performed 48 hours after treatment with bacterial compounds (curli CsgA 16 μg/mL, PSMα1 16 μg/mL, LPS 1 μg/mL, 32 μg/mL, and 64 μg/mL, rhamnolipid 10 μg/mL, 40 μg/mL, and 80 μg/mL) on dopaminergically differentiated SH-SY5Y cells. Rotenone 0.3 μM was used as a positive control. Intracellular α-synuclein protein levels were normalized to GAPDH. Data are presented as fold increase relative to the control, which was normalized to 1. Results are expressed as mean ± SEM from four independent experiments performed in duplicate (for curli CsgA 16 μg/mL and PSMα1 16 μg/mL – three independent experiments performed in duplicate). *p < 0.05, **p < 0.01, ***p < 0.001.*

Although we did not assess intracellular α-synuclein aggregation, it is well known from genetic forms of PD, such as SNCA duplication or triplication, that elevated intracellular levels of α-synuclein undeniably favor the initiation of aggregation—the onset of this process being only a matter of time (Singleton et al., 2003b; Chartier-Harlin et al., 2004b; Iannielli et al., 2022).

Our in vitro study provides significant insights into the potential mechanisms by which microbiota-derived molecules, particularly rhamnolipid and LPS, may contribute to PD pathogenesis. Although it does not definitively resolve the complexity surrounding the initiation of PD pathology in the gastrointestinal tract, it highlights the critical role these microbial compounds may play. The α-synuclein overexpression observed following exposure to bacterial amphiphiles introduces a novel mechanism through which gut microbiota may contribute to

PD-related pathological changes, expanding the current concept of α-synuclein aggregation being directly induced by these compounds.

Study 2 Summary – The Effect of Microbial Compounds Derived from Gut Microbiota on Intestinal Epithelial Cells

The Caco-2 cell line, derived from a human colorectal adenocarcinoma, is frequently used in experimental models that assess intestinal epithelium. When maintained as a monolayer on inserts for at least 21 days, these cells become polarized and form tight junctions, establishing both basolateral contact ,through the porous membrane of the inserts, and apical contact. As such, this cell line is commonly used in a differentiated state in *in vitro* models testing the effects of various compounds on the intestinal barrier. At the same time, it serves as a useful model for evaluating the impact of different compounds on epithelial cell function without requiring differentiation.

Following the evaluation of the effects of microbiota-derived compounds on dopaminergic neurons (Study 1), the next step was to assess the effects of these compounds on intestinal epithelial cells, which are in continuous interaction with gut microbiota. Intestinal epithelial cells may represent the first domino to fall, triggering α-synuclein aggregation and the initiation of PD. In addition to treatment with microbial compounds, the effect of rotenone on Caco-2 cells was also evaluated. Excluding permeability studies involving differentiated monolayers, the literature lacks investigations into the effect of rotenone on Caco-2 cells with respect to viability, cytotoxicity, and especially α-synuclein expression. Microbial compounds -particularly the bacterial endotoxin LPS- have an inflammatory role at the intestinal barrier, leading to increased permeability. This facilitates the translocation of bacteria and microbial compounds into local tissue and potentially into the bloodstream. At the same time, these microbial compounds have been shown to exert local effects on intestinal wall cells. In enteroendocrine cells, exposure to LPS induces an increase in α-synuclein levels and alters intracellular α-synuclein.

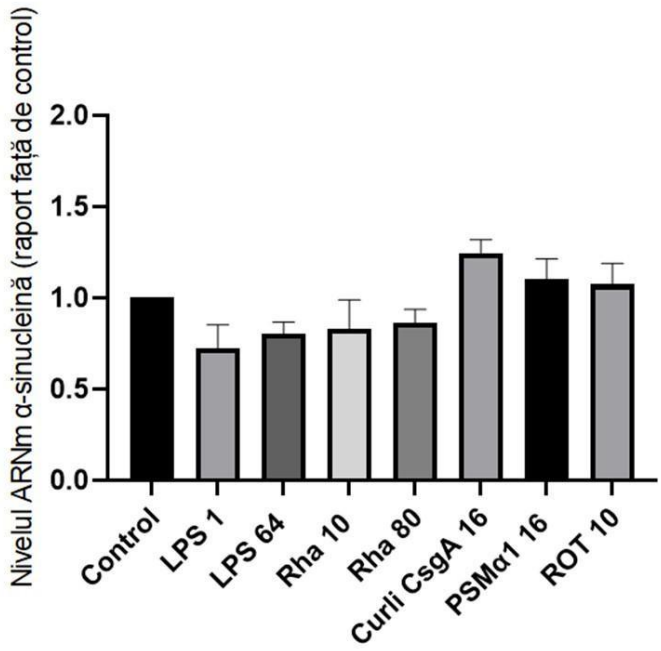

Figure 4. *qRT-PCR for α-synuclein mRNA performed 48 hours after treatment of Caco-2 intestinal epithelial cells with rotenone (10 µM) and bacterial compounds (curli CsgA 16 µg/ml, PSMα1 16 µg/ml, LPS 1 µg/ml and 64 µg/ml, rhamnolipid 10 µg/ml and 80 µg/ml). Data are presented as fold change relative to the control, which was normalized to 1. α-synuclein levels were normalized to GAPDH. Data are expressed as mean ± SEM from two independent experiments performed in duplicate or quadruplicate.*

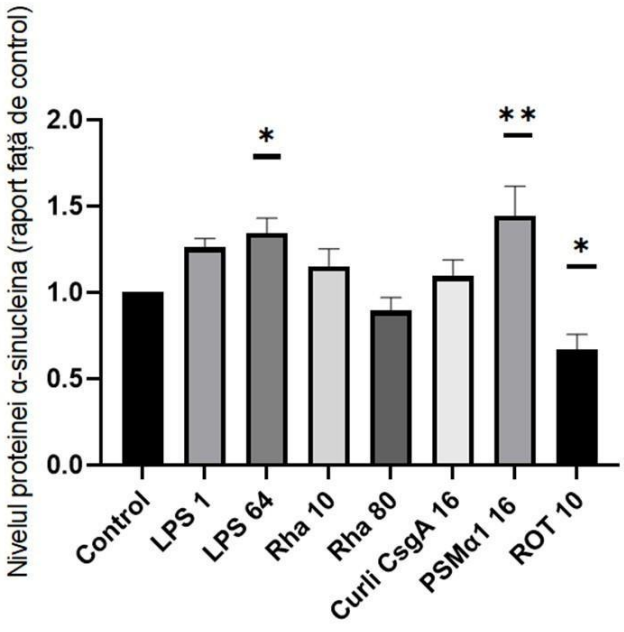

Figure 5. *Western Blot for α-synuclein performed 48 hours after treatment of Caco-2 intestinal epithelial cells with rotenone (10 µM) and bacterial compounds (curli CsgA 16 µg/ml, PSMα1 16 µg/ml, LPS 1 µg/ml and 64 µg/ml, rhamnolipid 10 µg/ml and 80 µg/ml). Intracellular α-synuclein protein levels were normalized to GAPDH. Data are presented as fold change relative to the control, which was normalized to 1. Results are expressed as mean ± SEM from four independent experiments performed in duplicate. *p < 0.05, **p < 0.01*

Rhamnolipid had no significant effect on intracellular α-synuclein levels in Caco-2 cells (Figures 4 and 5). Cell viability decreased in a dose-dependent manner, but without associated cytotoxicity, which only appeared at the highest concentration of 160 µg/ml. Therefore, we can conclude that rhamnolipid does not have a significant effect on intestinal epithelial cells. This effect contrasts with that observed in dopaminergic neurons, where rhamnolipid led to a marked increase in α-synuclein levels (Study 1).

Similar to the effect observed in Study 1 on dopaminergic neurons, **LPS** did not alter the viability or cytotoxicity of intestinal epithelial cells. However, it had a very interesting effect on α-synuclein levels (Figures 4 and 5). LPS caused a slight decrease in α-synuclein mRNA expression, by 20–28% compared to control, accompanied by an increase of up to 35% in intracellular α-synuclein protein levels. This discrepancy between decreased expression and increased protein levels requires further investigation to determine the cause. One current hypothesis, considering the pathophysiological mechanisms of α-synuclein aggregation discussed in the introduction, is that LPS impairs protein degradation systems, leading secondarily to intracellular α-synuclein accumulation and, as a feedback response, a reduction in its expression. Studies evaluating the activity of the two main protein degradation systems, ubiquitin-proteasome and autophagy-lysosome, could support this hypothesis.

PSMα1 had a similar effect to LPS, increasing intracellular α-synuclein levels while maintaining α-synuclein expression within normal limits (Figures 4 and 5). This also suggests an impairment of protein degradation, though not followed by decreased expression. PSMα1 was not cytotoxic and showed only a slight decrease in cell viability at the highest tested concentration of 32 µg/ml.

In contrast, **curli CsgA** showed a different effect compared to LPS and PSMα1, leading to an increase in both α-synuclein expression (by 25%) and intracellular protein levels (by 10%) (Figures 4 and 5). This increase could be significant in chronic or repeated exposure under physiological conditions. Curli caused a slight progressive reduction in viability with increasing concentration, without significant cytotoxicity except at the highest concentration (32 µg/ml), where a minor cytotoxic effect was observed.

Rotenone also showed an interesting effect, as it did not alter α-synuclein mRNA expression but did cause a reduction of up to 33% in intracellular α-synuclein protein levels (Figures 4 and 5). This effect also warrants further investigation, since rotenone is known to selectively affect dopaminergic neurons by inducing mitochondrial dysfunction, conformational

changes in α-synuclein, and increased α-synuclein expression. However, its effect on intestinal epithelial cells in terms of α-synuclein has not yet been analyzed.

In conclusion, these observed effects suggest that intestinal epithelial cells may play an important, previously overlooked role in the initiation of α-synuclein aggregation, and the mechanisms by which microbial compounds exert their influence on these cells appear to be multifactorial.

Study 3 Summary – The effect of microbial compounds in a co-culture system between intestinal epithelial cells and dopaminergic neurons

In this study, we sought to answer the question surrounding the initiation of α-synuclein aggregation in the gut and the role of the gut microbiota in this process: Is there a transmission of α-synuclein aggregates from other intestinal cells to neurons, or is there intercellular communication between neurons and other intestinal cells that, in response to stress from the intestinal lumen (caused by microbial compounds derived from the microbiota), triggers changes in neurons that initiate α-synuclein aggregation?

To explore this, we developed a **co-culture system** between Caco-2 intestinal epithelial cells and dopaminergic SH-SY5Y neuronal cells (Figure 6). Only the intestinal epithelial cells were pre-treated with microbial compounds, thereby simulating the physiological conditions in the gut where these cells are in constant contact with the microbiota and its metabolites. This system did not allow for direct contact between the two cell types - intercellular communication occurred only via soluble factors in the medium. This better mimics the physiological conditions of the intestinal tract, where epithelial cells do not directly contact neurons.

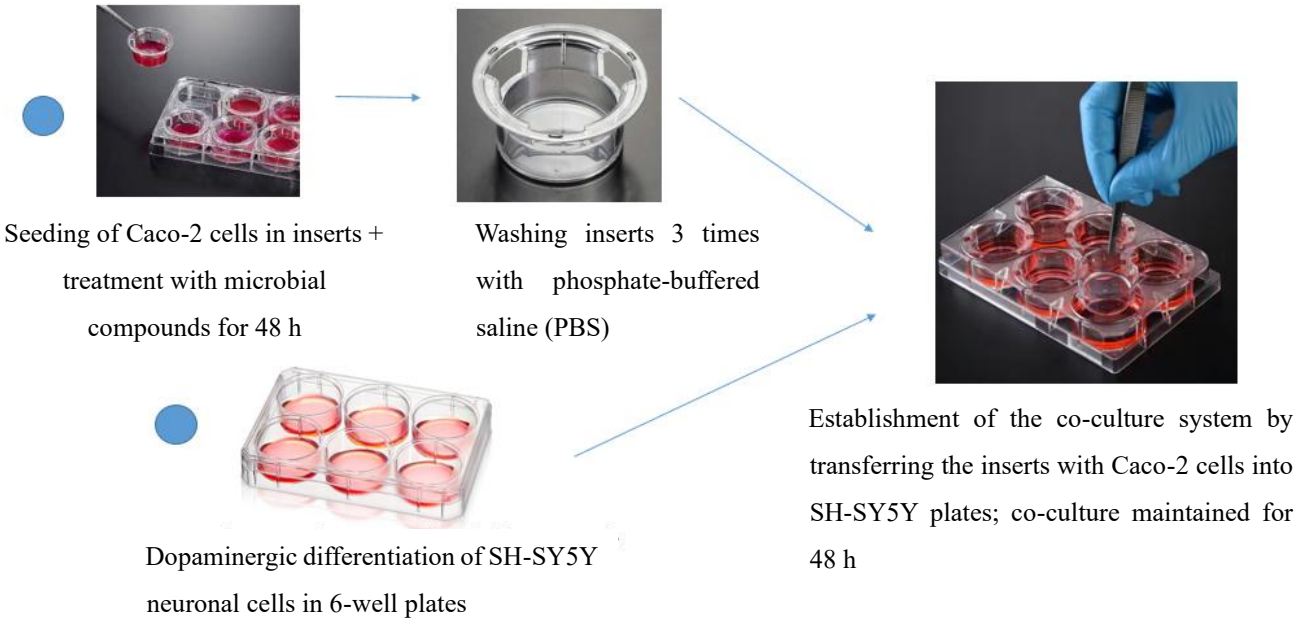

Figure 6. *Schematic representation of the co-culture protocol used in the experiments.*

The Caco-2 epithelial cells were seeded in inserts and subsequently treated with microbial compounds and rotenone, using the concentrations previously applied. After 48 hours of incubation, the inserts containing Caco-2 cells were thoroughly washed to remove any residual treatment medium and transferred into plates containing dopaminergically differentiated SH-SY5Y neuronal cells. The dopaminergic differentiation was performed in parallel with the Caco-2 treatment. Notably, the neurons were not directly exposed to microbial compounds or rotenone at any point. During the co-culture period, Caco-2 cells did not come into direct contact with SH-SY5Y neurons, as the insert remained suspended; however, both cell types shared the same medium, with the insert featuring 0.4 µm pores. After 48 hours of co-culture, the inserts containing Caco-2 cells were removed, and SH-SY5Y neurons were processed for α-synuclein level assessment via qRT-PCR and Western blot. The control group consisted of Caco-2 cells treated with the solvent of the compounds and subjected to the full co-culture protocol.

Treatment with curli CsgA led to a slight decrease in α-synuclein expression, down to 22% compared to the control, alongside an increase in intracellular α-synuclein levels (Figures 7 and 8). This effect resembles the impact of LPS on Caco-2 cells observed in Study 2 and suggests a potential impairment in protein degradation mechanisms at the neuronal level, with

a compensatory downregulation of α-synuclein expression. Further studies are required to elucidate this mechanism.

Treatment with PSMα1 did not alter α-synuclein gene expression, yet the intracellular protein level increased significantly, reaching 43% above control levels. Similarly, LPS treatment resulted in an intracellular α-synuclein increase between 16% and 22%, without affecting gene expression (Figures 7 and 8). These similar effects currently support the hypothesis of a shared mechanism involving impaired protein degradation. Investigating the two main degradation pathways—ubiquitin-proteasome and autophagy-lysosome systems—may confirm this hypothesis.

Rotenone treatment significantly upregulated α-synuclein gene expression, with a 2.6fold increase compared to control; however, the corresponding rise in intracellular α-synuclein was only 13% (Figures 7 and 8). This discrepancy requires further investigation, as no clear biological explanation has yet been established—similar to observations from rotenone-treated Caco-2 cells.

In conclusion, the findings of this study highlight the potential role of epithelial–neuronal communication within the gut in initiating α-synuclein aggregation**.**

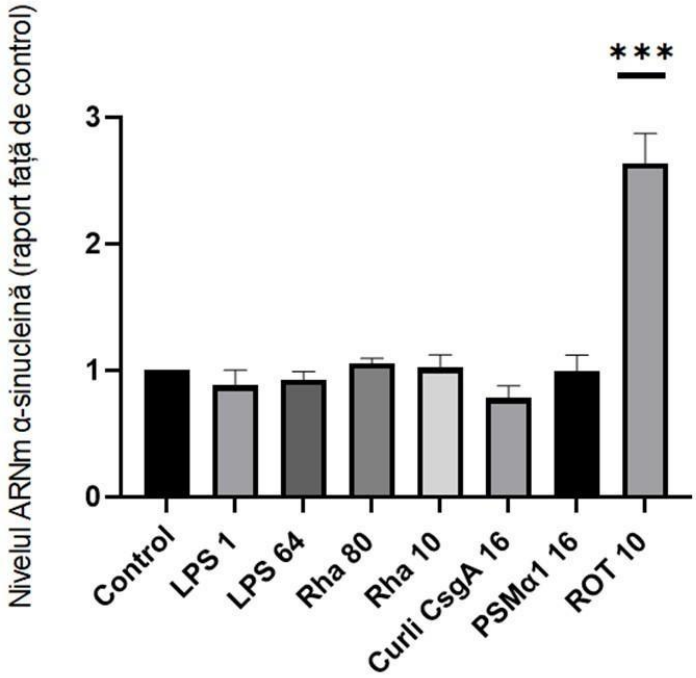

Figure 7. *qRT-PCR analysis of α-synuclein mRNA in SH-SY5Y neuronal cells after 48 hours of coculture with Caco-2 cells pre-treated for 48 hours with rotenone (10 µM) and bacterial compounds (curli CsgA 16 µg/ml, PSMα1 16 µg/ml, LPS 1 and 64 µg/ml, rhamnolipid 10 and 80 µg/ml). Data are presented as fold change relative to control, which was normalized to 1. α-synuclein levels were normalized to GAPDH. Results are expressed as mean ± SEM of at least two independent experiments performed in duplicate or quadruplicate. ***p < 0.001*

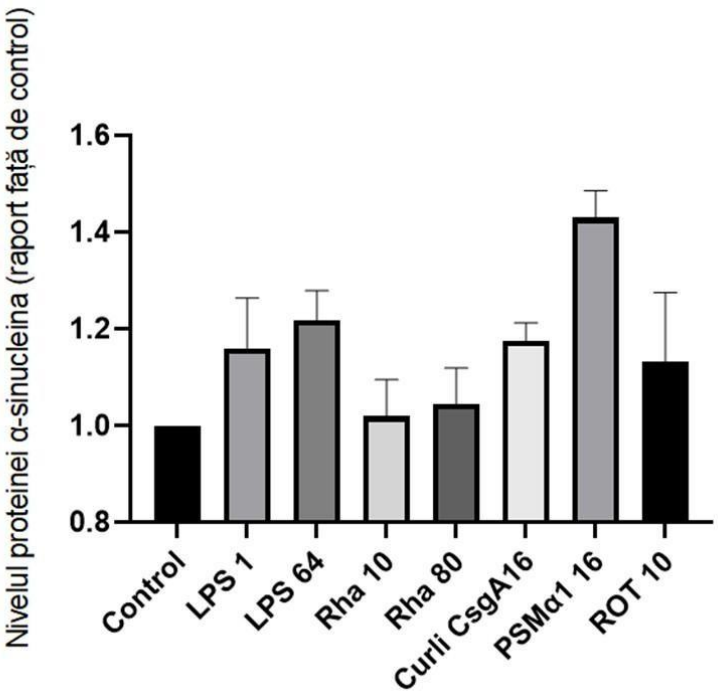

Figure 8. Western Blot for α-synuclein in differentiated dopaminergic SH-SY5Y neuronal cells after 48 hours of co-culture with intestinal epithelial Caco-2 cells previously treated for 48 hours with rotenone 10 μM and bacterial compounds (curli CsgA 16 μg/ml, PSMα1 16 μg/ml, LPS 1 μg/ml and 64 μg/ml, rhamnolipid 10 μg/ml and 80 μg/ml). Intracellular α-synuclein protein levels were normalized to GAPDH. Data are presented as fold increase relative to control, which was normalized to 1. Results represent the mean ± SEM of two independent experiments for curli CsgA and PSMα1, and four independent experiments for the other treatments, all performed in duplicate. Statistical analysis was conducted using one-way ANOVA followed by Dunnett's test.

Conclusions and Original Contributions

In the three studies presented, I consider that all proposed milestone objectives have been achieved. Below, I will briefly present the significant results obtained in this work, especially those relevant to the pathophysiology of Parkinson's disease (PD), namely the evaluation of α-synuclein levels.

1. All microbiota-derived molecules used in the experiments—rhamnolipid, LPS, curli CsgA, and PSMα1—contribute to the initiation of pathological mechanisms in PD by increasing intracellular α-synuclein levels in epithelial cells (represented in vitro by the Caco-2 cell line) and/or in dopaminergic neurons (represented in vitro by the differentiated SH-SY5Y cell line).
2. The co-culture system between intestinal epithelial Caco-2 cells and differentiated dopaminergic SH-SY5Y neuronal cells represents a suitable and reliable in vitro experimental model for studying the interaction between the intestinal epithelium and the enteric nervous system.
3. Rhamnolipid has a significant effect on increasing both the expression and intracellular amount of α-synuclein in differentiated dopaminergic SH-SY5Y neuronal cells, exceeding the increase caused by rotenone.
4. LPS increases both the expression and intracellular amount of α-synuclein in differentiated dopaminergic SH-SY5Y neuronal cells.
5. LPS increases the intracellular amount of α-synuclein (without increasing expression) in both intestinal epithelial Caco-2 cells and SH-SY5Y cells in co-culture with previously treated Caco-2 cells.
6. Curli CsgA increases α-synuclein expression in intestinal epithelial Caco-2 cells.
7. Curli CsgA increases intracellular α-synuclein levels (without increasing expression) in SH-SY5Y cells in co-culture with previously treated Caco-2 cells.
8. PSMα1 increases intracellular α-synuclein levels (without increasing expression) in intestinal epithelial Caco-2 cells and in SH-SY5Y cells in co-culture with previously treated Caco-2 cells.

The scientific hypothesis regarding the results showing increased intracellular αsynuclein levels without increased expression is that the treatment induces intracellular impairment of protein degradation systems, leading secondarily to α-synuclein accumulation.

Further studies are needed to evaluate this effect.

As previously mentioned, although intracellular α-synuclein aggregation was not assessed here, it is known from genetic forms of PD causing duplication (1.5-fold increase) or triplication (2-fold increase) of intracellular α-synuclein that elevated intracellular levels undoubtedly favor the initiation of aggregation, which is just a matter of time.

In conclusion, we have demonstrated that the microbiota may play a significant role in PD initiation at the intestinal level, as microbiota-derived molecules have the capacity to increase intracellular α-synuclein levels in intestinal epithelial or neuronal cells, promoting αsynuclein aggregation. Thus, we have fulfilled the main objective of this work. I believe this study can have a significant impact on research investigating the role of microbiota in PD initiation, representing a starting point for numerous future in vitro and in vivo studies.

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List of Publications

1. **Ioghen OC**, Gaina G, Lambrescu I, Manole E, Pop S, Niculescu TM, Mosoia O, Ceafalan LC, Popescu BO. Bacterial products initiation of alpha-synuclein pathology: an in vitro study.
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Chapter 8, pages 43–61; Chapter 9, pages 70–92

2. **Ioghen OC**, Ceafalan LC, Popescu BO. SH-SY5Y Cell Line In Vitro Models for Parkinson Disease Research-Old Practice for New Trends. Journal of Integrative Neuroscience.
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Chapter 6, pages 32–39

3. **Ioghen OC**, Ioghen MR, Popescu BO. Neurodegeneration: A Tale of Microbes and Neurons. Modern Medicine. 2025; 32(1); https://doi.org/10.31689/rmm.2025.32.1.7

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Chapter 4, pages 22-26, Chapterl 5, pages 27-31