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Impact of mushroom nutrition on gut microbiota modulation and association with neuroprotective capacity

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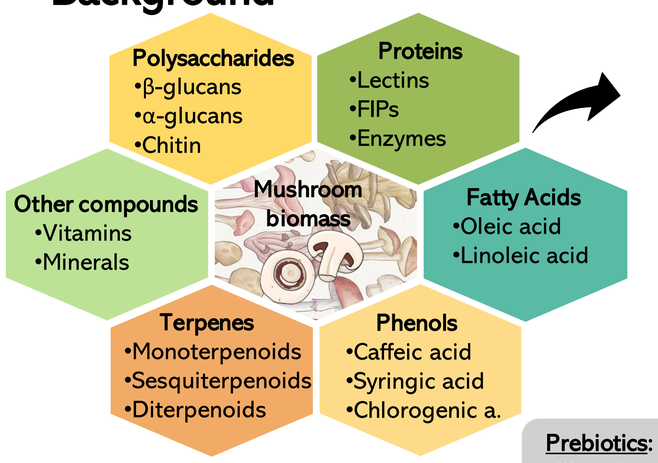
Abstract

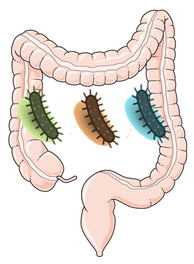
Alzheimer disease (AD) is a neurodegenerative disorder characterized by a progressive loss of memory and cognitive functions. To date, an effective treatment is not established, but it has been suggested that AD is associated with a decreased level of some microbial-derived products, such as short-chain fatty acids (SCFAs), gamma aminobutyric acid (GABA), and serotonin. Thus, gut microbiota modulation has been proposed as a potential tool for AD prevention and control. Given the importance of diet in this modulation, this PhD program explores the modulatory effect of mushroom biomass nutrition due to their prebiotic, antioxidant, anti-inflammatory, and neuroprotective properties. Regarding this, the direct neuroprotective potential of medicinal and edible mushroom species, as well as their modulatory effect on the production of gut microbiota-related neuroactive metabolites, will be accessed. Their impact through the gut-brain axis on inflammatory brain cells and in AD pathologies will be unrolled to propose novel strategies for preventing or ameliorating this neurological condition.

Background

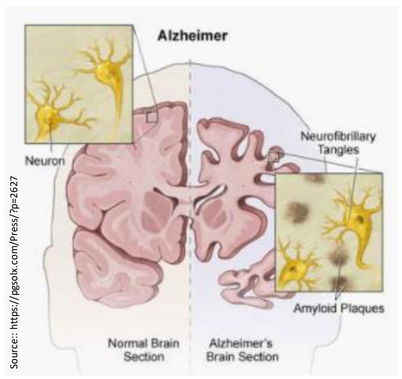


Prebiotics: "a substrate that is selectively utilised by host microorganisms conferring a health benefit" (Gibson *et al.*, 2017)

- In the colon, **gut microbiota** can **metabolize polysaccharides**, producing several metabolites such as **SCFAs**, **GABA** and **serotonin**, that have several **health benefits**.
- Due to the importance of these metabolites, **gut microbiota dysbiosis** has been directly connected with the **development of several disorders**, including **neurodegenerative diseases**.



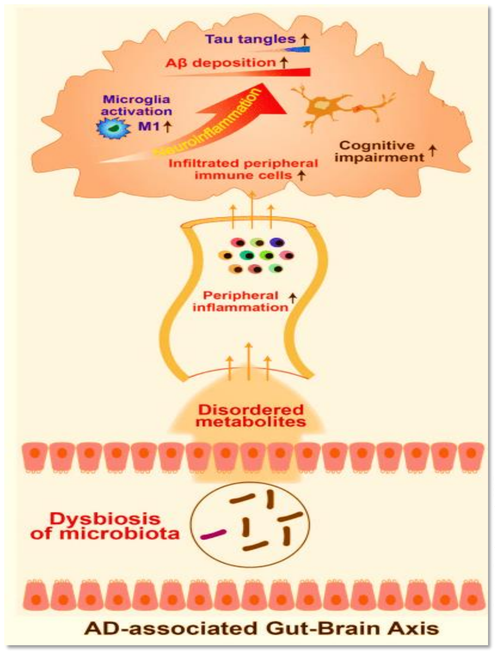
Gut-brain axis



AD has been linked to **reducing** SCFAs, GABA, serotonin, and other **gut microbiota metabolites**.

- **Gut microbiota modulation**: a promising tool
- **Diet**: a powerful driver of gut microbiota modulation
- **Mushrooms**: prebiotic capacity and preliminary studies suggested neuroprotective properties

However, the mechanisms are not totally understood and an association between the prebiotic effect and neuroprotective properties was not established.



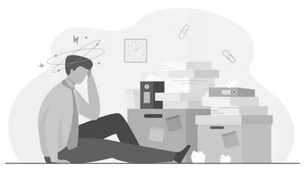
Objectives

Coriolus versicolor (CV) | *Pleurotus ostreatus* (PO) | *Hericium erinaceus* (He)

Impact of mushroom consumption on gut microbiota and establishment of its role in Alzheimer's disease to propose an effective strategy to prevent or ameliorate this disease

- Analyze the influence of gastrointestinal tract on bioactive compounds present in the mushroom biomass and understand their impact on the gut microbial population dynamics
- Explore beneficial and detrimental neuroactive metabolites present in absorbed digestive-derived and fermentative-derived fractions and assess their bioavailability
- Evaluate the anti-inflammatory potential of digested non-absorbed fractions and the impact upon probiotic microbial adhesion in the gut cells
- Identify and quantify the neuroactive compounds that pass through BBB and recognize their anti-inflammatory and antioxidant impact on the microglia inflammatory brain cells, astrocytes and neurons

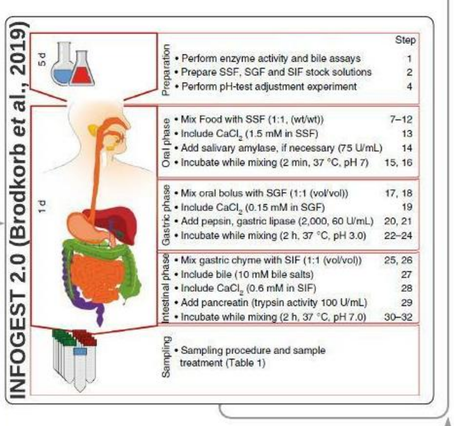
Experimental plan and expected results



T1: Literature review and experimental protocols design → Two review papers and one book chapter Experimental protocols design

T2: Biochemical characterization and gastrointestinal impact — *Coriolus versicolor*, *Hericium erinacius*, *Pleurotus ostreatus* Mushroom biomass → Biochemical characterization

HPLC, GC-MS, LC-MS and FPLC: Polysaccharides, and sugars, phenolic compounds, lipids, proteins, peptides and free amino acids.



Probiotic growth stimulation. Capacity of **pathogenic growth inhibition**. Biochemical characterization.

Expected results: Assess the stability of bioactive compounds present in mushroom biomass under GIT conditions and their bioacessibility.

In vitro simulation of the digestive process → Non-absorbed fraction | Absorbed fraction

T3: Modulation of gut microbiota through *in vitro* fermentation → Fermentation with single bacterial strains

Fermentation by human feces gut microbiota

Microbial population dynamics: RT-PCR. Biochemical characterization.

Expected results: Validate gut microbiota modulation and gut pathogens inhibition capacity. Comprehend the gut microbial population dynamics and quantify the main gut microbial-derived metabolites.

T4: *In vitro* impact on gut model cells — Co-culture of intestinal cell lines Caco-2/HT29-MTX → Permeability analysis: Transwell inserts

Layer integrity: transepithelial eletrical resistance. **Cell viability and cytotoxity:** MTT and LDH assays. Quantification of metabolites permeation.

Modulation of inflammation Bacterial adhesion assays

Anti-inflammatory and pro-inflammatory markers detection: ELISA and RT-PCR (e.g. IL-8, TNF-alpha) Impact upon the **adhesion of probiotic bacteria** to the intestinal epithelium.

Expected results: Assess the bioavailability of neuroactive compounds present in digestive-derived and fermentative-derived fractions. Understand the anti-inflammatory potential and bacteria adhesion promotion.

T5: *in vitro* impact on brain model cells → Permeability tests: Transwell inserts

hCMEC/D3: blood-brain barrier. Quantification of neuroactive metabolites permeation. MTT and LDH assays.

Neuroprotective potential evaluation

Primary microglia cortical cultures: cytokines production (IL-6, IL-1 and TNF-alpha) by ELISA. **CHME3:** FRET to quantify inflammatory biomarkers **Astrocyte and neuron cell lines:** conditionated medium, immunocytochemistry test

Expected results: Recognize the microglia anti-inflammatory and oxidative responses and respective impact on astrocytes and neurons in the presence of BBB fractions. Select the most promising mushroom species.

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