RESEARCH PAPER

Postbiotic-Mediated Neuroprotection by *Lentilactobacillus parabuchneri* and Fermented Germinated Brown Rice Through Multifunctional Anti-Neurodegenerative Mechanisms

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ABSTRACT

Oxidative stress, neuroinflammation, cholinergic deficit, and aberrant neuromelanin accumulation converge as principal drivers of Alzheimer's disease (AD) and Parkinson's disease (PD), yet safe and multi-target therapeutic strategies remain elusive. Lactic acid bacteria (LAB) and their postbiotic cell-free supernatants (CFS) have emerged as promising neuroprotective candidates via the gut–brain axis. This study evaluated the anti neuro degenerative potential of CFS from five LAB strains — Levilactobacillus brevis, Lactobacillus gasseri, Lentilactobacillus parabuchneri, Latilactobacillus sakei, and Lacticaseibacillus rhamnosus — and LAB-fermented germinated brown rice (GBR) through probiotic characterisation (NaCl, pH, bile tolerance), antioxidant (DPPH, H_2O_2 scavenging, reducing power), anti-inflammatory (albumin denaturation, haemolysis inhibition), acetylcholinesterase (AChE) and tyrosinase inhibition assays, and zebrafish (Danio rerio) embryo toxicity assessment. Lentilactobacillus parabuchneri exhibited the strongest gastrointestinal tolerance and consistently led across bioactivity assays, demonstrating 78.22% DPPH scavenging (vs. ascorbic acid 80.40%), 59.26% albumin denaturation inhibition (vs. aspirin 83.75%), 48.93% AChE inhibition, and 39.10% tyrosinase inhibition. Fermented GBR outperformed all CFS preparations in enzyme inhibition, achieving 64.79% AChE and 65.28% tyrosinase inhibitory activity — significantly exceeding unfermented GBR (13.04% and 23.53%, respectively) — underscoring LAB fermentation as a critical bioconversion step. Zebrafish embryo assays confirmed the developmental safety of both preparations, with no morphological abnormalities and enhanced larval growth observed relative to controls. These findings position Lentilactobacillus parabuchneri CFS and LAB-fermented GBR as safe, multi-target postbiotic candidates for functional food-based neuroprotective strategies against AD and PD.

Keywords: Lactic acid bacteria, cell-free supernatant, neuroprotection, fermented brown rice and zebrafish embryo toxicity.

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INTRODUCTION

Neurodegenerative diseases constitute a broad spectrum of progressive, debilitating disorders characterised by the selective loss of neuronal structure and function in the brain and spinal cord. Among these, Alzheimer's disease (AD) and Parkinson's disease (PD) represent the most prevalent and socioeconomically burdensome conditions, affecting millions of individuals worldwide and placing enormous strain on healthcare systems globally (Sivamaruthi et al., 2024). With rising life expectancy across most nations, the incidence of neurological disorders is predicted to increase substantially; dementia currently affects approximately 50 million individuals, with projections indicating this number may reach 130 million by 2050 (Mutalib et al., 2023). These conditions share converging pathological hallmarks including chronic neuroinflammation, oxidative stress, mitochondrial dysfunction, aberrant protein aggregation, and progressive cognitive decline, underscoring the urgent need for novel preventive and therapeutic strategies (Lana et al., 2024).

A rapidly emerging paradigm in neurological research concerns the gut–brain axis (GBA), a complex bidirectional communication network linking the gastrointestinal tract and the central nervous system (CNS) through neural, endocrine, immune, and metabolic signalling pathways (Sivamaruthi et al., 2024). Mounting evidence suggests that the gut microbiota plays a pivotal role in modulating the GBA, influencing neuroinflammatory tone, neurotransmitter synthesis, and cognitive function (Lana et al., 2024). Dysbiosis perturbation of the gut microbial community has been increasingly implicated as a susceptibility factor in the pathogenesis and progression of multiple neurodegenerative disorders, including AD, PD, and multiple sclerosis (Lana et al., 2024). In particular, gut microbiota-derived short-chain fatty acids, endogenous signals, and neuroactive compounds have been shown to modulate neuronal function, plasticity, and behaviour (Yin & Horzmann, 2024, Lakshmikanthan et al., 2026).

Probiotics, defined by the World Health Organization as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host," have attracted substantial scientific interest as therapeutic modulators of the gut–brain axis (Tyagi et al., 2021). Among probiotics, lactic acid bacteria (LAB) stand out for their well-established safety profile and diverse bioactive properties (Tyagi et al., 2021). LAB exhibit neuroprotective properties through various mechanisms of action, and LAB treatment alone or in probiotic formulations has demonstrated significant neuroprotective activities across in vitro, in vivo, and clinical studies (Mutalib et al., 2023). In animals and humans, LAB probiotic supplementation has improved memory and cognitive performance mainly through antioxidant and anti-inflammatory pathways (Mutalib et al., 2023). The potential role of probiotics in neurotherapy discovery against neurodegenerative diseases such as Alzheimer's and Parkinson's has thus been prominently highlighted in recent years (Varesi et al., 2023, Ayyankalai et al., 2025).

Beyond live probiotic cells, postbiotic preparations including cell-free supernatants (CFS) derived from LAB fermentation have emerged as a promising and safer alternative for delivering bioactive metabolites with neuroprotective potential. Probiotic CFS preparations have been shown to modulate inflammatory and oxidative responses in neuronal and microglial cell lines in vitro, exerting antioxidant effects through the nuclear factor erythroid 2–related factor 2 (NRF2) signalling pathway and upregulating superoxide dismutase 1 (SOD1), an enzyme with critical roles in neuroprotection (De Marco et al., 2018; Lattanzi et al., 2024). Cell-free supernatants of LAB strains have also been shown to reduce neurological deficits following ischaemic injury, downregulate pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, and attenuate oxidative stress markers in neuronal tissue (Samer et al., 2022). These findings collectively support the therapeutic relevance of LAB-derived CFS as postbiotic agents targeting neurodegeneration.

A key target in the search for anti-neurodegenerative agents is the acetylcholinesterase (AChE) enzyme, which mediates the hydrolysis of the neurotransmitter acetylcholine (ACh) in the synapse. In AD brains, overexpression of AChE results in markedly reduced ACh levels, contributing to profound cholinergic deficits and cognitive deterioration (Mutalib et al., 2023). AChE inhibitors represent the most clinically utilised pharmacological strategy against AD, and recent investigations into LAB metabolites have identified their potential to inhibit AChE activity and improve synaptic cholinergic function. Notably, oral administration of *Lactobacillus gasseri* MG4247 and *Lacticaseibacillus rhamnosus* MG4644 has been shown to significantly ameliorate cognitive impairment in amyloid beta (Aβ)-induced mouse models by protecting the antioxidant system, attenuating neuroinflammation, and enhancing synaptic function through the TLR4/Akt pathway (Choi et al., 2025). Similarly, *Lactobacillus acidophilus* LA4 and *Lacticaseibacillus paracasei* F5 reduced AChE activity, suppressed pro-inflammatory cytokines, and enhanced antioxidant enzymes in Alzheimer's disease models (Liu et al., 2026).

Parkinson's disease pathology is closely associated with the accumulation of neuromelanin (NM) in dopaminergic neurons of the substantia nigra. Neuromelanin is formed as a cellular by-product during dopamine synthesis, with tyrosinase implicated as a key enzyme catalysing the oxidation of dopamine-to-dopamine quinone an essential precursor for neuromelanin synthesis (Nagatsu et al., 2022). Elevated tyrosinase activity promotes neuromelanin accumulation, which above a critical threshold drives dopaminergic neuronal death and PD phenotypes (Carballo-Carbajal et al., 2019). The inhibition of tyrosinase activity therefore represents a rational strategy to attenuate neuro melanogenesis and reduce the associated oxidative burden in PD. Significantly, LAB fermentation supernatants have been reported to exhibit potent tyrosinase inhibitory activity, with the fermentation supernatant of the LAB strain

MNJ-9 achieving 68.9% inhibition of tyrosinase and 93.1% DPPH radical scavenging capacity, indicating dual anti-pigmentation and antioxidant benefits (Zhang et al., 2023).

Chronic neuroinflammation is a hallmark of virtually all major neurodegenerative diseases. Activated microglia and astrocytes release pro-inflammatory cytokines and reactive oxygen species (ROS) that perpetuate a neurotoxic cycle of oxidative damage and neuronal death. LAB-derived bioactive compounds have consistently shown capacity to modulate neuroinflammatory cascades, suppressing NF-κB signalling, downregulating TNF-α, IL-1β, and IL-6, and enhancing the activities of endogenous antioxidant enzymes including SOD and glutathione peroxidase (Choi et al., 2025). The modulation of these pathways by LAB CFS places them among the most promising candidates for in vitro anti neurodegenerative drug discovery pipelines.

Fermented foods represent another compelling source of neuroprotective bio actives. Fermented brown rice has attracted growing interest as a functional food matrix enriched in bioactive compounds following LAB fermentation. *Limosilactobacillus reuteri*-fermented brown rice has been characterised as a potent source of multifunctional bioactive peptides with antioxidant, anti-inflammatory, and neuroprotective properties, producing elevated levels of gamma-aminobutyric acid (GABA), an important brain neurotransmitter, alongside compounds such as quercetin, ferulic acid, cinnamic acid, and ascorbic acid (Tyagi et al., 2021). Metabolomic analyses of fermented brown rice have revealed an abundance of stress-reducing and antioxidant compounds with potential relevance to neuroprotection, including modulation of the gut-brain axis through the Keap1-NRF2 pathway, cytokine suppression, and neurotransmitter regulation (Tyagi et al., 2021; Tyagi et al., 2025, Muthu et al., 2025).

In the context of safety and translational toxicology assessment, the zebrafish (*Danio rerio*) embryo model has emerged as a highly valuable vertebrate platform for evaluating both the toxicity and neuroprotective potential of bioactive compounds. Zebrafish embryos offer unique advantages including optical transparency, rapid external development, genetic similarity to mammals, and conservation of key neurotransmitter systems dopaminergic, serotonergic, GABAergic, and cholinergic that are directly implicated in neurodegenerative disease pathways (Frontiers, 2025). The embryonic and larval stages of zebrafish provide real-time insights into neuronal development and disease progression without invasive procedures, and the model is well-suited for high-throughput toxicity screening of candidate neuroprotective agents (Yin & Horzmann, 2024).

The present study investigates the anti neuro degenerative potential of CFS derived from five selected LAB strains *Levilactobacillus brevis*, *Lactobacillus gasseri*, *Lectilactobacillus parabuchneri*, *Latilactobacillus sakei*, and *Lacticaseibacillus rhamnosus* — as well as LAB-fermented brown rice, through a comprehensive battery of in vitro assays. These include antioxidant activity assays, anti-inflammatory assessments, inhibition of tyrosinase (as a proxy for neuromelanin suppression relevant to PD), and inhibition of AChE (relevant to AD cholinergic pathology). In addition, zebrafish embryo toxicity analysis is employed to evaluate the safety profile of the test preparations. The selection of these five LAB strains is informed by their known safety, GRAS status, and emerging evidence of their bioactive metabolite profiles in the context of neuroprotection (Choi et al., 2025; Mutalib et al., 2023). This multi-target in vitro approach aims to provide a mechanistic basis for the potential use of LAB-derived postbiotics and fermented brown rice as functional food-based strategies for the prevention or management of neurodegenerative conditions.

Despite growing evidence supporting the neuroprotective potential of probiotics, limited information is available regarding the comparative anti neurodegenerative activities of probiotic cell-free supernatants and fermented brown rice products derived from different LAB strains. Therefore, the present study aimed to evaluate the neuroprotective potential of five probiotic lactic acid bacteria, namely *Levilactobacillus brevis*, *Lactobacillus gasseri*, *Lentilactobacillus parabuchneri*, *Latilactobacillus sakei*, and *Lacticaseibacillus rhamnosus*. The cell-free supernatants and fermented brown rice preparations were investigated for their antioxidant, anti-inflammatory, anti-tyrosinase, and acetylcholinesterase inhibitory activities. Furthermore, their safety and biocompatibility were assessed using the zebrafish (*Danio rerio*) embryo toxicity model. The findings of this study may provide valuable insights into the development of probiotic-derived functional foods and postbiotic formulations for neuroprotection and the prevention of neurodegenerative diseases.

MATERIALS AND METHODS

Source and Maintenance of Lactic Acid Bacteria Strains

The probiotic lactic acid bacteria employed in this study included *Lactobacillus sakei* (MTCC 12327), *Levilactobacillus brevis* (MTCC 12485), *Lentilactobacillus parabuchneri* (MTCC 13315), *Lactobacillus gasseri* (MTCC 12485), and *Lacticaseibacillus rhamnosus* (MTCC 1423). These strains were obtained from the Microbial Type Culture Collection (MTCC) and Gene Bank, India. The cultures were routinely propagated and maintained in de Man, Rogosa and Sharpe (MRS) broth and on MRS agar (HiMedia Laboratories, Mumbai, India) under anaerobic incubation at 37 °C. For long-term storage, the bacterial isolates were preserved in MRS broth containing 30% (v/v) glycerol and maintained at −86 °C. Before being utilized in experimental procedures, each strain was subcultured three consecutive times in freshly prepared MRS broth to ensure optimal viability, metabolic activity, and culture purity (Ma et al., 2019).

Characterization of Probiotic Lactic Acid Bacteria

The probiotic lactic acid bacteria used in this study, namely *Lactobacillus sakei*, *Levilactobacillus brevis*, *Lentilactobacillus parabuchneri*, *Lactobacillus gasseri*, and *Lacticaseibacillus rhamnosus*, were characterized for their tolerance to different environmental stress conditions. Prior to stress tolerance and functional analyses, each bacterial culture was grown in MRS broth at 37 °C for 24 h, and the cell density was adjusted to approximately 1.5 × 10^8 CFU/mL. To assess acid tolerance, the isolates were inoculated into MRS broth adjusted to different pH levels ranging from 3.0 to 9.0. Salt tolerance was evaluated by culturing the isolates in MRS broth supplemented with varying concentrations of NaCl (2–10%). Bile salt tolerance was determined by growing the cultures in MRS broth containing different concentrations of bile salts (0.3–2.0%, w/v). All cultures were incubated at 37 °C for 24 h under anaerobic conditions. Following incubation, bacterial growth was measured spectrophotometrically at 600 nm (OD600). The ability of each strain to survive and grow under acidic, saline, and bile salt conditions was used to determine its probiotic tolerance characteristics (Sasikumar et al., 2026).

In Vitro Anti-Inflammatory Activity

The anti-inflammatory activity of the cell-free supernatants (CFS) obtained from *Lactobacillus sakei*, *Levilactobacillus brevis*, *Lentilactobacillus parabuchneri*, *Lactobacillus gasseri*, and *Lacticaseibacillus rhamnosus* was evaluated using in vitro anti-inflammatory assays according to the method described by Ayman Geddawy et al. (2023). Briefly, bacterial cultures were centrifuged at 8,000 rpm for 10 min at 4 °C, and the resulting supernatants were collected and filtered through a 0.22 µm membrane filter to obtain sterile CFS. The CFS was tested at a concentration of 1mg/mL in all anti-inflammatory assays, with the final reaction volume adjusted to 1 mL. The anti-inflammatory potential was assessed through the inhibition of protein denaturation using the albumin denaturation assay and membrane stabilization activity using the heat-induced haemolysis assay. In the albumin denaturation assay, the ability of the CFS to prevent heat-induced denaturation of bovine serum albumin was determined, and absorbance was recorded at 660 nm. For the heat-induced haemolysis assay, freshly prepared red blood cell (RBC) suspension was used to evaluate membrane stabilization activity, and the absorbance was measured at 560 nm following incubation. The percentage inhibition of protein denaturation and haemolysis was calculated to determine the anti-inflammatory efficacy of each probiotic strain (Sasikumar et al., 2026).

The percentage inhibition in both assays was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

In Vitro Antioxidant Activity

The antioxidant activity of the cell-free supernatants (CFS) obtained from *Lactobacillus sakei*, *Levilactobacillus brevis*, *Lentilactobacillus parabuchneri*, *Lactobacillus gasseri*, and *Lacticaseibacillus rhamnosus* was evaluated using DPPH radical scavenging assay, hydrogen peroxide scavenging assay, and reducing power assay following standard methods (Sasikumar et al., 2026). Bacterial cultures were centrifuged, and the collected CFS was used directly for all assays. The CFS was tested at a concentration of 1mg/mL, and the final reaction volume was adjusted to 1 mL. The DPPH assay was used to determine free radical scavenging activity, while the hydrogen peroxide scavenging assay evaluated the ability of the samples to neutralize hydrogen peroxide. The reducing power assay was performed to assess the electron-donating capacity of the probiotic metabolites. The absorbance values were measured spectrophotometrically, and the antioxidant activity was expressed as percentage scavenging activity or reducing power relative to the control (Sasikumar et al., 2026).

The antioxidant activity was expressed as percentage inhibition and calculated using the following formula:

$$\text{Antioxidant (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Acetylcholinesterase (AChE) Inhibition Assay

The acetylcholinesterase (AChE) inhibitory activity of the cell-free supernatants (CFS) of *Lentilactobacillus parabuchneri*, Germinated Brown rice Extract and Fermented Germinated brown rice was evaluated using Ellman's colorimetric method. Briefly, the reaction mixture consisted of 20 µL of DTNB [5,5′-dithio-bis-(2-nitrobenzoic acid)] solution, 140 µL of 0.1 M sodium phosphate buffer (pH 7.0), and 20 µL of acetylcholinesterase enzyme solution (5 U/mL) prepared in 20 mM Tris-HCl buffer (pH 7.5). 1mg/ml concentration of were added separately to the reaction mixture and incubated under suitable conditions. The absorbance was measured at 412 nm using a UV–Visible spectrophotometer (Ayman Geddawy et al., 2023). The percentage inhibition of AChE activity was calculated using the following equation:

$$\text{AChE Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Tyrosinase Inhibition Assay

The tyrosinase inhibitory activity of the cell-free supernatants (CFS) of *Lentilactobacillus parabuchneri*, Germinated Brown rice Extract and Fermented Germinated brown rice was determined using a modified method described by Ayman Geddawy et al., 2023. Briefly, 1mg/ml concentrations of sample were mixed with 80 µL of 0.1 M sodium phosphate buffer (pH 7.0) and 40 µL of tyrosinase enzyme solution (46 U/mL). Subsequently, 40 µL of L-DOPA substrate solution was added to initiate the reaction. The reaction mixtures were incubated at 25 °C for 30 min, and the absorbance was measured at 475 nm using a UV–Visible spectrophotometer.

The percentage inhibition of tyrosinase activity was calculated using the following equation:

$$\text{Tyrosinase Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Zebra fish embryos/larval toxicity study

The zebra fish embryo toxicity study followed the OECD criteria 236 (OECD, 2024) and used healthy fertilized eggs, embryos and larvae of zebra fish. Twenty embryos at identical developmental stages were randomly chosen and placed in each of six well-plates (17.7 mL/each well), each holding provided a 4 mL water layer for the embryos. They were treated with MIC of CQDs, and the untreated group was kept as a control. The study used a photoperiod of light followed by darkness, 12 h and 12 h, respectively, at constant room temperature. Further it is washed with water and can be observed through fluorescent Microscope.

RESULTS

Characterization of Probiotic Lactic Acid Bacteria

NaCl Tolerance

The salt tolerance of *Lactobacillus sakei*, *Levilactobacillus brevis*, *Lentilactobacillus parabuchneri*, *Lactobacillus gasseri*, and *Lacticaseibacillus rhamnosus* was evaluated by measuring bacterial growth (OD600) in MRS broth supplemented with increasing concentrations of NaCl (2–10%) (Fig1A). A concentration-dependent decline in growth was observed for all strains, indicating that elevated salinity imposed osmotic stress and reduced bacterial proliferation. Among the tested isolates, *Lentilactobacillus parabuchneri* exhibited the highest salt tolerance, maintaining the greatest growth throughout the assay, with OD600 values decreasing from 0.3594 at 2% NaCl to 0.1138 at 10% NaCl. *Levilactobacillus brevis* also demonstrated strong tolerance, retaining substantial growth at higher salt concentrations (OD600 = 0.1117 at 10% NaCl). In contrast, *Lactobacillus gasseri* was the most sensitive strain, showing a marked reduction in growth from 0.2164 at 2% NaCl to 0.0205 at 10% NaCl. *Lactobacillus sakei* and *Lacticaseibacillus rhamnosus* displayed moderate tolerance, with OD600 values declining from 0.1385 to 0.0440 and from 0.1397 to 0.0541, respectively.

pH tolerance

The pH tolerance of *Lactobacillus sakei*, *Levilactobacillus brevis*, *Lentilactobacillus parabuchneri*, *Lactobacillus gasseri*, and *Lacticaseibacillus rhamnosus* was evaluated by measuring bacterial growth (OD600) in MRS broth adjusted to pH 3–7 (Fig1B). All strains exhibited a progressive increase in growth with increasing pH, indicating improved adaptation under near-neutral conditions. Among the tested isolates, *Levilactobacillus brevis* showed the highest growth at pH 7 (OD600 = 2.0863), followed by *Lacticaseibacillus rhamnosus* (1.9864), *Lentilactobacillus parabuchneri* (1.8247), *Lactobacillus sakei* (1.7725), and *Lactobacillus gasseri* (1.7158). At the highly acidic condition of pH 3, all strains maintained measurable growth, with *Lacticaseibacillus rhamnosus* exhibiting the highest OD600 value (0.7148).

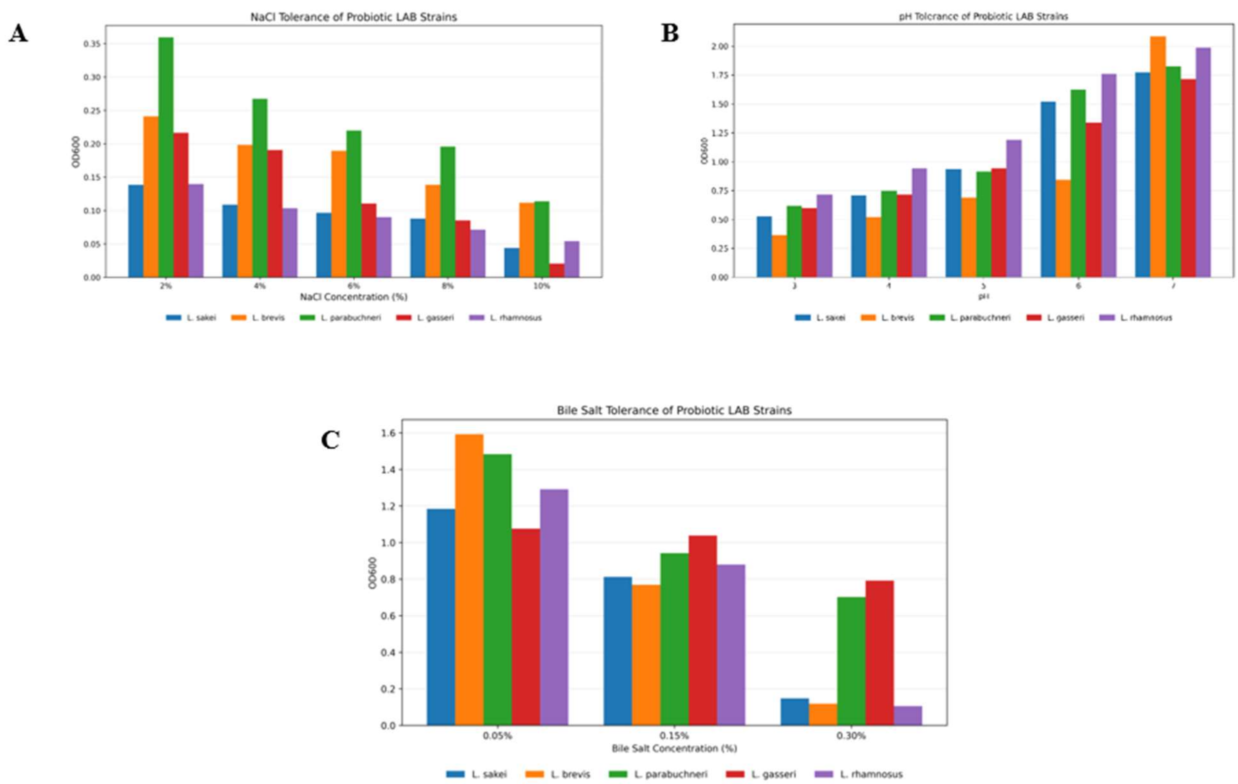

Figure 1: Characterization of Probiotic Lactic Acid Bacteria. A) NaCl Tolerance, B) pH Tolerance, C) Bile Salt Tolerance

Bile tolerance

The bile salt tolerance of *Lactobacillus sakei*, *Levilactobacillus brevis*, *Lentilactobacillus parabuchneri*, *Lactobacillus gasseri*, and *Lacticaseibacillus rhamnosus* was assessed by measuring bacterial growth (OD600) in MRS broth supplemented with increasing concentrations of bile salts (0.05–0.30%) (Fig1C). All strains exhibited a concentration-dependent reduction in growth, indicating that elevated bile salt levels exerted stress on bacterial cells. Among the tested isolates, *Levilactobacillus brevis* demonstrated the highest growth at 0.05% bile salt (OD600 = 1.5927), while *Lentilactobacillus parabuchneri* maintained superior tolerance at higher bile salt concentrations, recording OD600 values of 0.9418 and 0.7024 at 0.15% and 0.30% bile salts, respectively. In contrast, *Lacticaseibacillus rhamnosus* and *Levilactobacillus brevis* showed the greatest reduction in growth at 0.30% bile salt, with OD600 values of 0.1053 and 0.1186, respectively. *Lactobacillus sakei* and *Lactobacillus gasseri* exhibited moderate bile tolerance, maintaining OD600 values of 0.1482 and 0.7917, respectively, at the highest bile salt concentration.

Anti-inflammatory activity

Inhibition of Albumin Denaturation Assay

The anti-inflammatory activity of the probiotic cell-free supernatants was evaluated using the albumin denaturation assay, which measures the ability of samples to inhibit protein denaturation (Fig2A). Aspirin, used as the positive control, exhibited the highest inhibition of albumin denaturation (83.75%). Among the tested probiotic strains, *Lentilactobacillus parabuchneri* demonstrated the strongest anti-inflammatory activity with 59.26% inhibition, followed by *Lactobacillus gasseri* (48.19%), *Lacticaseibacillus rhamnosus* (41.92%), *Levilactobacillus brevis* (39.21%), and *Lactobacillus sakei* (23.02%).

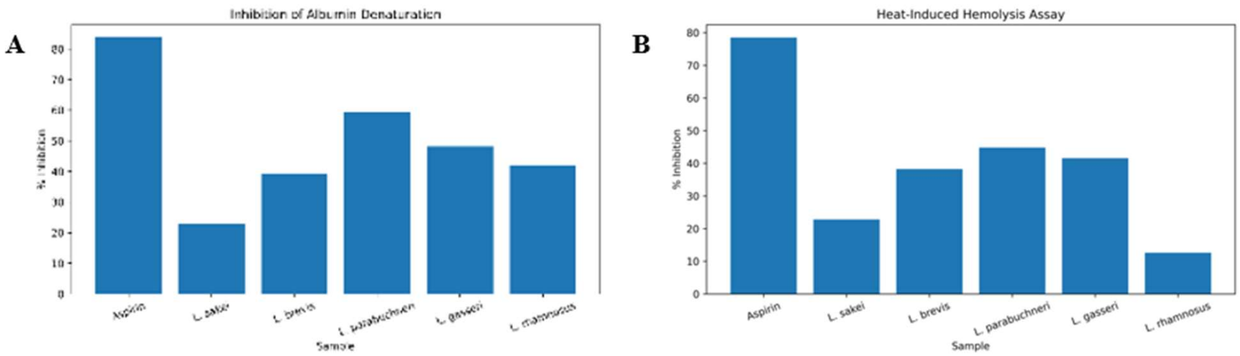

Figure 2: Anti-Inflammatory. A) Inhibition Albumin Denaturation, B) Heat Induced Haemolysis

Heat Induced Haemolysis assay

The anti-inflammatory activity of the probiotic cell-free supernatants was further evaluated using the heat-induced haemolysis assay, which assesses the ability of samples to stabilize erythrocyte membranes against thermal stress (Fig2B). Aspirin, used as the positive control, exhibited the highest inhibition of haemolysis (78.52%). Among the probiotic strains, *Lentilactobacillus parabuchneri* showed the greatest membrane stabilization activity with 44.82% inhibition, followed by *Lactobacillus gasseri* (41.58%), *Levilactobacillus brevis* (38.27%), *Lactobacillus sakei* (22.82%), and *Lacticaseibacillus rhamnosus* (12.61%).

Antioxidant Activity

DPPH Scavenging Activity

The antioxidant activity of the probiotic cell-free supernatants was evaluated using the DPPH radical scavenging assay, which measures the ability of samples to neutralize free radicals through hydrogen or electron donation (Fig3A). Ascorbic acid, used as the positive control, exhibited the highest scavenging activity (80.40%). Among the tested probiotic strains, *Lentilactobacillus parabuchneri* demonstrated the strongest antioxidant activity with 78.22% DPPH radical scavenging, closely approaching the activity of the positive control. This was followed by *Levilactobacillus brevis* (43.50%), *Lacticaseibacillus rhamnosus* (37.38%), *Lactobacillus sakei* (21.21%), and *Lactobacillus gasseri* (21.19%).

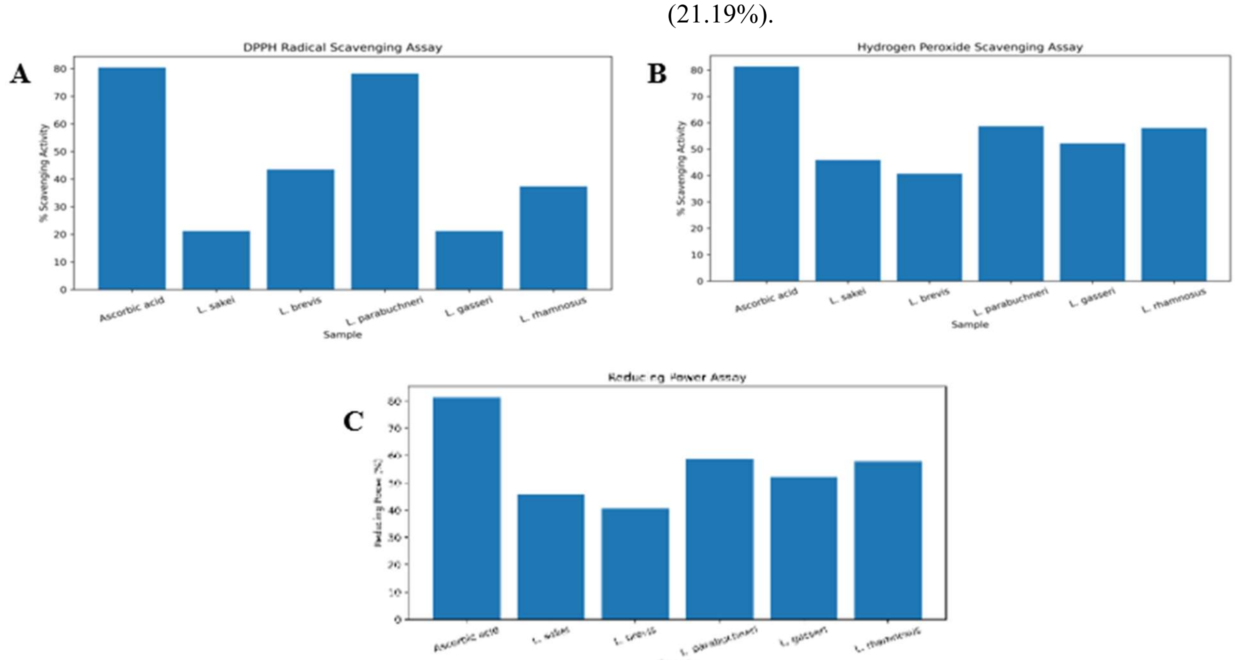

Figure 3: Antioxidant Activity. A) DPPH, B) Hydrogen Peroxide Scavenging Assay, C) Reducing Power

Hydrogen peroxide Scavenging Assay

The antioxidant activity of the probiotic cell-free supernatants was further assessed using the hydrogen peroxide scavenging assay, which evaluates the ability of samples to neutralize hydrogen peroxide and thereby reduce the formation of reactive oxygen species (Fig3B).

Ascorbic acid, used as the positive control, exhibited the highest scavenging activity (81.34%). Among the probiotic strains, *Lentilactobacillus parabuchneri* demonstrated the strongest hydrogen peroxide scavenging activity (58.69%), closely followed by *Lacticaseibacillus rhamnosus* (58.02%) and *Lactobacillus gasseri* (52.18%). Moderate scavenging activity was observed for *Lactobacillus sakei* (45.88%) and *Levilactobacillus brevis* (40.71%).

Reducing Power Assay

The reducing power of the probiotic cell-free supernatants was evaluated to determine their electron-donating capacity, an important indicator of antioxidant potential (Fig3C). Ascorbic acid, used as the positive control, exhibited the highest reducing power (81.34%). Among the tested probiotic strains, *Lentilactobacillus parabuchneri* demonstrated the greatest reducing activity (58.69%), followed closely by *Lacticaseibacillus rhamnosus* (58.02%) and *Lactobacillus gasseri* (52.18%). Moderate reducing power was observed for *Lactobacillus sakei* (45.88%) and *Levilactobacillus brevis* (40.71%).

Acetylcholinesterase (AChE) Inhibition Assay

The acetylcholinesterase (AChE) inhibitory activity of the test samples was evaluated to determine their potential neuroprotective effects through the prevention of acetylcholine degradation (Fig4A). Galantamine, used as the positive control, exhibited the highest AChE inhibition (92.35%). Among the tested samples, fermented germinated brown rice (GBR) showed the strongest inhibitory activity with 64.79% inhibition, followed by *Lentilactobacillus parabuchneri* cell-free supernatant with 48.93% inhibition. In contrast, the unfermented GBR extract exhibited relatively low AChE inhibition (13.04%).

Tyrosinase Inhibition Assay

The tyrosinase inhibitory activity of the test samples was evaluated to assess their potential role in reducing melanin and neuromelanin formation, which are associated with oxidative stress and neurodegenerative disorders (Fig4B). Kojic acid, used as the positive control, exhibited the highest inhibition of tyrosinase activity (78.42%). Among the tested samples, fermented germinated brown rice (GBR) demonstrated the strongest inhibitory activity with 65.28% inhibition, followed by *Lentilactobacillus parabuchneri* cell-free supernatant (39.10%). The unfermented GBR extract showed comparatively lower inhibition (23.53%).

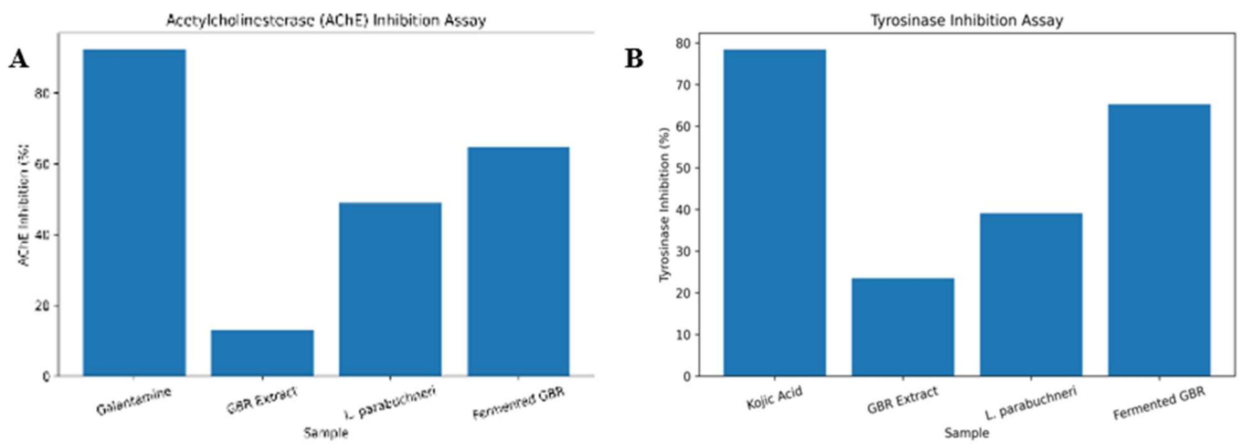

Figure 4: Anti neuro degenerative. A) AchE Inhibition Assay, B) Tyrosinase inhibition Assay

Zebra fish embryos/larval toxicity study

The toxicity and developmental effects of *Lentilactobacillus parabuchneri* cell-free supernatant (CFS) and fermented germinated brown rice (GBR) were evaluated using zebrafish (*Danio rerio*) embryos at different developmental stages (Fig5). Microscopic observations revealed normal embryonic development from the blastula stage through hatching and larval stages in both treatment groups when compared with the untreated control. No significant morphological abnormalities, including pericardial edema, yolk sac edema, spinal curvature, developmental delay, or mortality, were observed throughout the experimental period. Interestingly, embryos treated with *Lentilactobacillus parabuchneri* CFS and fermented GBR exhibited slightly enhanced growth and developmental progression compared to the control group, as evidenced by improved body elongation, normal yolk sac absorption, and healthy larval morphology. Fluorescence imaging further confirmed the absence of apparent toxicity or apoptotic damage in the treated larvae.

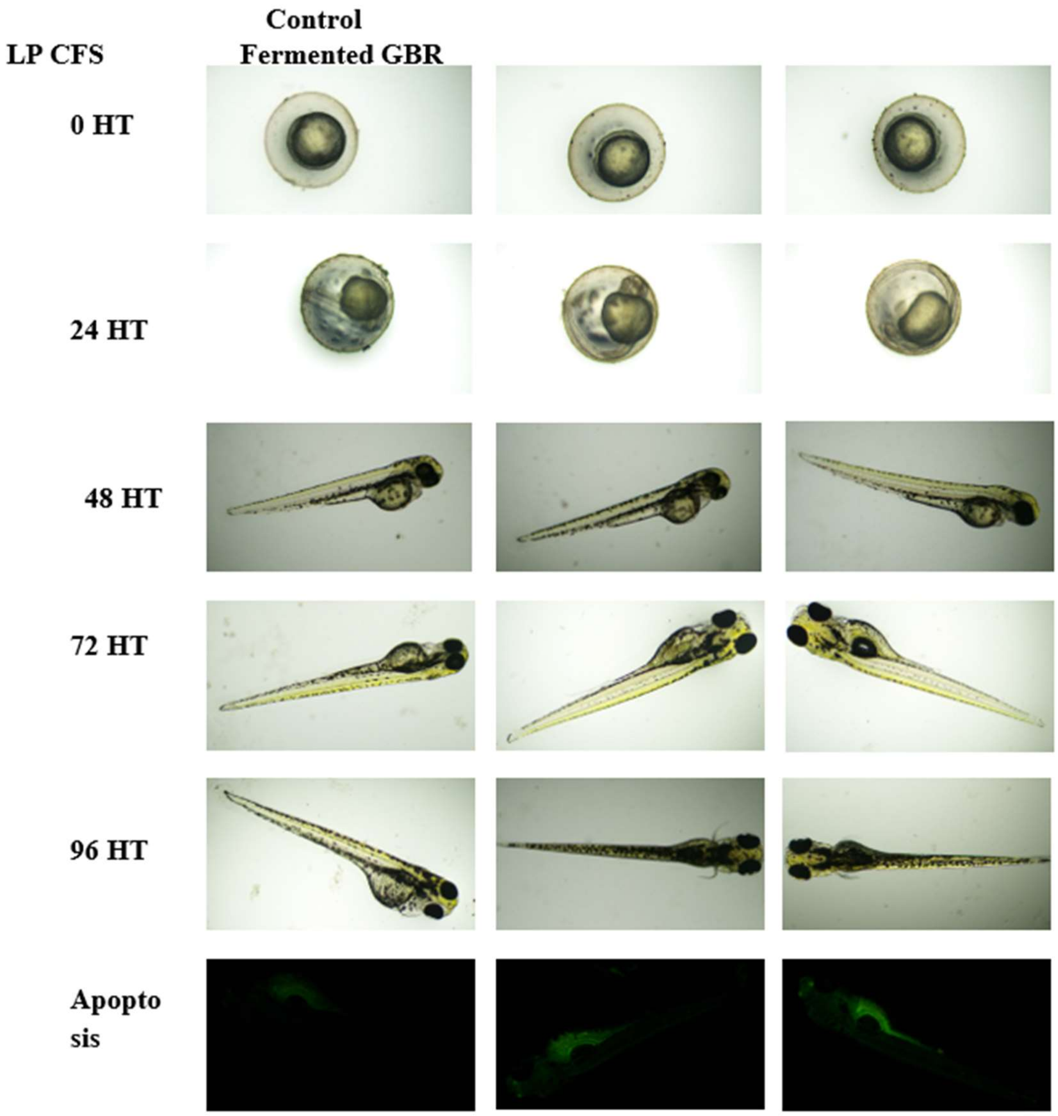

Figure 5: Zebra fish embryos/larval toxicity study

DISCUSSION

The present study systematically evaluated the probiotic characterisation and anti neuro degenerative potential of CFS derived from five lactic acid bacteria strains *Lentilactobacillus parabuchneri*, *Levilactobacillus brevis*, *Lactobacillus gasseri*, *Latilactobacillus sakei*, and *Lacticaseibacillus rhamnosus* alongside LAB-fermented germinated brown rice (GBR), through a battery of in vitro mechanistic assays encompassing antioxidant activity, anti-inflammatory activity, AChE inhibition, tyrosinase inhibition, and zebrafish embryo developmental toxicity. The collective findings demonstrate that these preparations possess significant multi-target anti neuro degenerative potential, with *Lentilactobacillus parabuchneri* CFS and fermented GBR consistently emerging as the most bioactive preparations across many assays. These results are discussed considering existing literature, with emphasis on the mechanistic basis of the observed activities and their translational relevance to AD and PD pathology.

The probiotic fitness of the five LAB strains was first assessed through evaluation of their tolerance to acid, bile salt, and osmotic stress the primary physiological barriers encountered during gastrointestinal transit. All five strains exhibited concentration-dependent reductions in growth under increasing NaCl, decreasing pH, and increasing bile salt concentrations, consistent with the general Osmo sensitivity and membrane-stress responses characteristic of LAB (Frontiers in Microbiology, 2023). However, the degree of tolerance varied significantly among strains, reflecting differences in membrane fatty acid composition, efflux pump activity, and stress-response gene expression. Among all isolates, *Lentilactobacillus parabuchneri* demonstrated the highest tolerance to both

NaCl (OD600 = 0.1138 at 10%) and bile salts at higher concentrations (OD600 = 0.7024 at 0.30%), suggesting that this strain possesses robust Osmo protective mechanisms, possibly involving the accumulation of compatible solutes such as betaine or proline, and bile salt hydrolase (BSH) activity that enables deconjugation of bile acids a well-recognised mechanism of bile resistance among LAB (Chen et al., 2022). This is consistent with findings by Liu et al. (2022), who demonstrated significant inter-strain variability in bile salt delay times among *Lactobacillus* isolates, with strains displaying longer delay times exhibiting superior bile acid tolerance linked to bile salt hydrolase activity. In contrast, *Lactobacillus gasseri* was the most sensitive strain under osmotic stress (OD600 = 0.0205 at 10% NaCl), while *Lacticaseibacillus rhamnosus* and *Lactobacillus sakei* displayed moderate tolerance profiles. Under acidic conditions, *Levilactobacillus brevis* achieved the highest growth at pH 7, while *Lacticaseibacillus rhamnosus* demonstrated the best survival at the most acidic condition (pH 3), which is consistent with the extensively documented acid tolerance of *L. rhamnosus* strains, attributed to F0F1-ATPase-mediated proton extrusion and constitutive expression of glutamate decarboxylase systems (Mutalib et al., 2023). This differential tolerance profiling is important from a functional standpoint, as strains capable of surviving gastrointestinal transit in adequate numbers are more likely to deliver viable and metabolically active cells to the colon, thereby sustaining bioactive CFS production in situ (Selvaraj & Gurumurthy, 2023).

Neuroinflammation is a primary and self-perpetuating driver of neuronal loss in both AD and PD, mediated by activated microglia and astrocytes secreting TNF-α, IL-1β, IL-6, and reactive oxygen species that collectively constitute a neurotoxic environment (Choi et al., 2025). The anti-inflammatory potential of the CFS preparations was evaluated through two complementary in vitro models: inhibition of albumin denaturation and inhibition of heat-induced haemolysis. Protein denaturation is a hallmark of inflammatory and arthritic pathologies, as structural alterations in serum albumin expose cryptic epitopes that elicit autoimmune responses and amplify pro-inflammatory signalling via NF-κB activation (Madhuranga & Samarakoon, 2023). The capacity of a bioactive preparation to inhibit albumin denaturation thus reflects its ability to stabilise extracellular protein structure under inflammatory conditions — a mechanism that is particularly relevant to neurodegenerative disease, where systemic inflammation and blood-brain barrier disruption expose neuronal proteins to denaturation-induced immunogenicity. *Lentilactobacillus parabuchneri* CFS demonstrated the highest inhibition of albumin denaturation (59.26%) among the five strains tested, approaching but not reaching the activity of aspirin (83.75%). This level of inhibition is notable and consistent with previous reports on flavonoid-rich and phenolic bioactive preparations exhibiting anti-denaturation activities; for instance, membrane-stabilising plant extracts rich in phenolics have achieved 69.89% inhibition at comparable concentrations (Peltophorum pterocarpum study, 2020), suggesting that the anti-inflammatory efficacy observed in *Lentilactobacillus parabuchneri* CFS may similarly reflect its phenolic and bioactive metabolite content. This is supported by findings from Bianchi et al. (2025), who demonstrated that CFS from *Lacticaseibacillus rhamnosus* exerted marked anti-inflammatory effects on LPS-stimulated peripheral blood mononuclear cells (PBMC), with the low molecular weight fraction (≤ 3 kDa) bearing the principal immunomodulatory activity, suggesting that small organic acids and bioactive peptides in LAB CFS are primary mediators of the anti-inflammatory response. The heat-induced haemolysis assay further corroborated these findings, with *Lentilactobacillus parabuchneri* demonstrating the greatest erythrocyte membrane stabilisation (44.82%), followed by *Lactobacillus gasseri* (41.58%). This erythrocyte membrane-stabilising activity is mechanistically interpreted as an indicator of lysosomal membrane integrity protection the stabilisation of lysosomal membranes prevents the release of lysosomal enzymes that otherwise amplify inflammatory cascades (Madhuranga & Samarakoon, 2023). The lower inhibitory activity of *Lacticaseibacillus rhamnosus* (12.61%) in the haemolysis assay, despite moderate albumin denaturation inhibition (41.92%), suggests that this strain's anti-inflammatory metabolites may be more effective against denaturation-related mechanisms than haemolytic stress pathways. These findings collectively reinforce the proposition that LAB-derived CFS modulates neuroinflammation through multiple overlapping mechanisms, encompassing protein stabilisation, membrane protection, and pro-inflammatory cytokine downregulation (De Marco et al., 2018).

Oxidative stress resulting from excessive production of reactive oxygen species (ROS) is a central and early event in the pathogenesis of neurodegenerative diseases, triggering mitochondrial dysfunction, lipid peroxidation, protein oxidation, and DNA damage in neurons (Choi et al., 2025). The antioxidant activity of the LAB CFS was evaluated across three complementary assays DPPH radical scavenging, hydrogen peroxide scavenging, and ferric-reducing power to provide a comprehensive mechanistic portrait of the preparations' radical-neutralising capacity. *Lentilactobacillus parabuchneri* CFS demonstrated outstanding DPPH radical scavenging activity (78.22%), substantially higher than all other strains and approaching the activity of the ascorbic acid positive control (80.40%). This result is highly significant. The DPPH assay measures the capacity of electron or hydrogen donors to neutralise the stable DPPH radical through a hydrogen atom transfer (HAT) or single electron transfer (SET) mechanism; accordingly, this exceptionally high scavenging capacity in *Lentilactobacillus parabuchneri* CFS suggests that this strain's metabolic output is particularly enriched in hydrogen-donating bioactive compounds, potentially including short-chain organic acids, bioactive peptides, exopolysaccharides, or phenolic derivatives generated during fermentation (Łepecka et al., 2025). This is consistent with findings by Wu et al. (2025), who demonstrated that antioxidant activity in LAB CFS is primarily concentrated in the cell-free fermentation supernatant fraction rather than intact cells, and that the antioxidant capacity is strain-dependent and linked to the diversity of secreted metabolites. Notably, the pronounced DPPH scavenging activity of *Lentilactobacillus parabuchneri* CFS (78.22%) is comparable to the activity reported for exopolysaccharide fractions of *L. delbrueckii* and *L. plantarum* studied by Sirin & Aslim (2020), which similarly achieved high DPPH scavenging levels and demonstrated protective effects against Aβ1-42-induced apoptosis in neuronal SH-SY5Y cells a finding that raises the possibility that *Lentilactobacillus parabuchneri* CFS may possess comparable direct neuroprotective capacity in neuronal cell culture models. For hydrogen peroxide scavenging and reducing power, *Lentilactobacillus parabuchneri* maintained the highest activity (58.69%), followed by *Lacticaseibacillus rhamnosus* (58.02%) and *Lactobacillus gasseri* (52.18%). Hydrogen peroxide is a key mediator of intracellular oxidative stress through the Fenton and Haber-Weiss reactions, generating hydroxyl radicals that inflict substantial oxidative damage on neuronal membranes and macromolecules (Sirin & Aslim, 2020). The capacity of CFS to scavenge H2O2 is therefore of direct mechanistic relevance to neuroprotection. The convergence of *Lentilactobacillus parabuchneri*, *Lacticaseibacillus rhamnosus*, and *Lactobacillus gasseri* as the top performers across H2O2 scavenging and reducing power suggests that these strains produce overlapping pools of low-molecular-weight reductants and electron donors. This is further supported by reports that *L. rhamnosus* CCFM 1107 displayed high antioxidative capacity comparable to the reference strain *L. rhamnosus* GG ATCC 53103 across multiple antioxidant assays including DPPH, hydroxyl radical scavenging, and reducing power (Deng et al., 2015), validating the antioxidant potential of this well-characterised probiotic clade and situating the present findings within a coherent comparative framework.

The cholinergic hypothesis of AD posits that the progressive loss of cholinergic neurons in the basal forebrain and the consequent reduction in acetylcholine (ACh) availability at synapses underlie the cognitive and memory deficits that define the disease (Mutalib et al., 2023). Overexpression and dysregulation of AChE in AD brains accelerate ACh hydrolysis, exacerbating cholinergic deficits. Accordingly, AChE inhibition represents the most clinically validated pharmacological strategy in AD management, and the identification of natural AChE inhibitors from fermented food matrices and postbiotic preparations has emerged as a priority research direction. In the present study, fermented GBR demonstrated the strongest AChE inhibitory activity (64.79%), substantially exceeding the unfermented GBR extract (13.04%) a finding that underscores the critical role of LAB fermentation in releasing, modifying, and enriching bioactive compounds that target AChE. This fermentation-enhanced AChE inhibition is mechanistically consistent with the liberation of bioactive peptides, ferulic acid, and gamma-aminobutyric acid (GABA) during the fermentation process, compounds that have been shown to inhibit AChE through competitive or non-competitive binding at the active site or peripheral anionic site of the enzyme (Varesi et al., 2023). This is strikingly consistent with a directly comparable study by Oliveira et al. (2025), who demonstrated that fermented malted rice products exhibited AChE and tyrosinase inhibitory activities that were significantly elevated compared to unfermented controls, and that these neuroprotective properties were largely attributed to bioactive peptides generated during germination, fermentation, and simulated gastrointestinal digestion. Among the LAB CFS preparations, *Lentilactobacillus parabuchneri* CFS demonstrated notable AChE inhibitory activity (48.93%), suggesting that this strain secretes AChE-inhibitory metabolites during fermentation. This is consistent with findings from kefir-derived fractions studied by Malta et al., 2022, who demonstrated that the low molecular weight peptidic fraction (< 10 kDa) of kefir exhibited the highest AChE inhibitory capacity, and that this activity was closely associated with antioxidant capacity in the same fraction a pattern mirrored by the co-occurrence of high antioxidant and AChE inhibitory activities in *Lentilactobacillus parabuchneri* CFS in the current study. Furthermore, the neuroprotective potential of *L. gasseri* and *L. rhamnosus* against AD-related AChE dysregulation has been reported by Choi et al. (2025), who demonstrated that oral administration of *L. gasseri* MG4247 and *L. rhamnosus* MG4644 ameliorated cognitive impairment in Aβ-induced mice by restoring antioxidant enzyme activities and suppressing neuroinflammation, effects that would complement direct AChE inhibition to produce a synergistic neuroprotective outcome. The relatively low AChE inhibition observed for unfermented GBR (13.04%) further reinforces that fermentation is not merely a preservation method, but an active bioconversion process that dramatically amplifies the neuropharmacological value of the rice substrate by releasing bound phenolics, generating GABA, and producing peptide fragments with enzyme-inhibitory activity (Tyagi et al., 2023; Franck Polia et al., 2022).

Tyrosinase is a copper-containing oxidoreductase enzyme that catalyses the rate-limiting steps in melanin biosynthesis: the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone. In dopaminergic neurons of the substantia nigra, aberrant tyrosinase activity mediates the conversion of dopamine-to-dopamine quinone a precursor for neuromelanin (NM) synthesis (Nagatsu et al., 2022). While neuromelanin at physiological levels serves a neuroprotective role by chelating iron and toxic metals, its accumulation above a critical threshold drives oxidative damage, mitochondrial dysfunction, and dopaminergic neuronal death the hallmark of PD neuropathology (Carballo-Carbajal et al., 2019). The inhibition of tyrosinase activity therefore represents a rational mechanistic strategy for attenuating neuromelanogenesis and reducing the associated oxidative burden in PD. In the present study, fermented GBR demonstrated the highest tyrosinase inhibitory activity (65.28%), again far exceeding unfermented GBR (23.53%), while *Lentilactobacillus parabuchneri* CFS exhibited substantial inhibition (39.10%). The higher activity of fermented GBR compared to the individual LAB CFS preparations suggests that the combination of the rice substrate's endogenous phytochemicals including gamma-oryzanol, ferulic acid, and phenolic acids that are known copper-chelating agents with LAB fermentation-derived metabolites produces a synergistic tyrosinase inhibitory effect that exceeds that of either component alone (Tyagi et al., 2021). This is consistent with the mechanism described by (Song et al., 2022), who established that bioactive peptides derived from food proteins inhibit tyrosinase through three principal mechanisms: chelation of active site copper ions, competitive binding at the substrate recognition site, and downregulation of tyrosinase gene expression primarily through the cAMP/PKA/CREB pathway, with additional modulation via MEK/ERK/MITF and p38 MAPK/CREB/MITF pathways. The copper-chelating capacity of fermented GBR-derived metabolites may therefore underlie its potent tyrosinase inhibitory activity. The ability of LAB-fermented milk whey from *Lactobacillus helveticus* to suppress melanin production by inhibiting tyrosinase through downregulation of MITF transcription factor expression (Kato et al., 2020) provides further comparative evidence that LAB fermentation products possess direct tyrosinase-modulating capacity via transcriptional as well as enzymatic mechanisms. Taken together, the tyrosinase inhibitory activities observed for fermented GBR and *Lentilactobacillus parabuchneri* CFS place these preparations among the most promising natural anti-PD candidates in the fermented food landscape, particularly in the context of attenuating neuromelanin-driven oxidative neurotoxicity in dopaminergic neurons. The substantially higher tyrosinase inhibition reported for kojic acid (78.42%) as the positive control reflects its well-established mechanism as a competitive inhibitor of copper at the tyrosinase active site and situates the inhibitory activity of fermented GBR (65.28%) as approaching clinically relevant levels of enzyme suppression.

The translational utility of any candidate neuroprotective agent is contingent upon its safety profile, and the zebrafish (*Danio rerio*) embryo model is now firmly established as the premier vertebrate platform for early-stage in vivo toxicity screening of bioactive compounds. Acute toxicity studies dominate the zebrafish toxicology literature, followed by neurotoxicity assessments, reflecting the widespread adoption of high-throughput embryonic assays such as the Fish Embryo Acute Toxicity (FET) test. The zebrafish embryo is particularly well-suited for evaluating the developmental safety of food-derived bioactive preparations due to its optical transparency, rapid external development, conservation of vertebrate neurotransmitter systems, and the sensitivity of early developmental stages to any perturbation of cellular redox balance, membrane integrity, or signalling pathway homeostasis (Yin & Horzmann, 2024, Ayyadurai et al., 2026). In the present study, zebrafish embryos treated with *Lentilactobacillus parabuchneri* CFS and fermented GBR throughout blastula, hatching, and larval stages exhibited no significant morphological abnormalities specifically, the absence of pericardial oedema, yolk sac oedema, spinal curvature, developmental delay, or mortality confirms that neither preparation induced systemic or developmental toxicity at the concentrations tested. Common zebrafish embryonic toxicity indicators include pericardial edema, darkened yolk sac, body curvature, and retardation in dose-dependent manners, followed eventually by death the absence of all such endpoints in the present study provides strong evidence for the intrinsic safety of these LAB-derived preparations. Notably, embryos treated with *Lentilactobacillus parabuchneri* CFS and fermented GBR exhibited slightly enhanced growth and developmental progression relative to untreated controls, as evidenced by improved body elongation, normal yolk sac absorption, and healthy larval morphology confirmed by fluorescence imaging. This growth-promoting effect in zebrafish larvae is mechanistically plausible considering the antioxidant and anti-inflammatory activities demonstrated by these preparations: by reducing oxidative stress and suppressing pro-inflammatory signalling, the CFS preparations may create a more favourable cellular environment for embryonic cell proliferation, differentiation, and developmental progression. This is consistent with findings by Wu et al. (2024), who reported that *L. rhamnosus* supplementation improved survival, hatching, and growth rates in zebrafish larvae exposed to microcystin-LR toxicity, and that the probiotic-mediated protection was associated with reductions in ROS levels and enhancements in glutathione (GSH) content and catalase (CAT) activity effects that mirror the antioxidant mechanisms demonstrated by *Lentilactobacillus parabuchneri* CFS in the present study. Furthermore, Ene et al. (2025) demonstrated that post-exposure administration of *L. rhamnosus*, *Bifidobacterium longum*, and *Bifidobacterium animalis lactis* to zebrafish exposed to zinc chloride-induced AD-like neurotoxicity produced anxiolytic and partial neuroprotective effects, underscoring the capacity of LAB-derived preparations to modulate the gut-brain axis in zebrafish in a manner relevant to neurodegenerative disease models. The confirmation of developmental safety through zebrafish embryo bioassay therefore strengthens the translational case for these preparations as safe and efficacious postbiotic candidates for functional food and nutraceutical development targeting neurodegeneration.

Taken collectively, the results of the present study describe a multi-target neuroprotective profile for *Lentilactobacillus parabuchneri* CFS and fermented GBR that is mechanistically complementary and covers the principal pathological axes of both AD and PD. *Lentilactobacillus parabuchneri* CFS demonstrated the highest or near-highest activity in DPPH radical scavenging (78.22%), hydrogen peroxide scavenging (58.69%), reducing power (58.69%), albumin denaturation inhibition (59.26%), haemolysis inhibition (44.82%), AChE inhibition (48.93%), and tyrosinase inhibition (39.10%), while exhibiting the best probiotic tolerance across salt, acid, and bile environments. This convergent multi-target activity profile spanning antioxidant, anti-inflammatory, pro-cholinergic, and anti-neuro melanogenic mechanisms simultaneously is precisely the type of multi-modal bioactivity that is increasingly recognised as the most therapeutically relevant approach to neurodegenerative diseases, given their complex, multifactorial pathogenesis (Varesi et al., 2023; Mutalib et al., 2023). The superior multi-target activity of *Lentilactobacillus parabuchneri* CFS is particularly noteworthy given the relatively limited published literature on this specific species in the context of neuroprotection. While *L. parabuchneri* has historically been studied in the context of histamine production in fermented dairy (Gumustop & Ortakci, 2022), the present findings suggest that specific strains or conditions may yield bioactive CFS with remarkable anti neuro degenerative potential that warrants dedicated future investigation, including metabolomic profiling to identify the specific molecular entities responsible. Fermented GBR's consistent dominance in AChE inhibition (64.79%) and tyrosinase inhibition (65.28%) further positions it as a substrate-mediated amplifier of LAB bioactivity, in which the fermentation of a polyphenol- and gamma-oryzanol-rich matrix by LAB generates a uniquely rich pool of neuroprotective metabolites. The dramatic enhancement of bioactivity in fermented GBR over unfermented GBR in both enzyme inhibition assays is consistent with Oliveira et al. (2025), who reported that fermentation-enhanced neuroprotective properties of malted rice were attributable to the synergistic liberation of bioactive peptides and free phenolic acids by germination, fermentation, and digestion. These findings collectively provide a robust mechanistic and comparative foundation for the development of *Lentilactobacillus parabuchneri* CFS and LAB-fermented GBR as functional food-based postbiotic strategies for neuroprotection and anti neuro degenerative disease management.

Ethics Committee Approval

Not Applicable

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Conflict of interest

The authors have no conflicts of interest to declare.

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REFERENCES

1. Ayman Geddawy, Karukuvelraja Karukuvelraja, K.P. Shamna, & Muhammad Musthafa Poyil. (2023). Antioxidant, anti-inflammatory and anti-neurodegenerative activities of Jatropha integerrima Jacq. floral methanolic extract. Annals of Phytomedicine, 12(1). https://doi.org/10.54085/ap.2023.12.1.32.
2. Ayyankalai, N.K.; Sundararaj, P.; Baskar, M.G.; Duraisamy, N.; Muthu, S.; Lakshmikanthan, M.; Abdi, G. Green Production of Silver Nanoparticles from Cassia Occidentalis and Alternanthera Pungens and Evaluation of Their Nematicidal Activity against Meloidogyne Javanica. Sci. Rep. 2025, 15, 26228. https://doi.org/10.1038/s41598-025-12096-2.
3. Ayyadurai, T., Mohan, P. K., Annamalai, A., Kamaraj, C., & Waheeb, M. Q. (2026). Biogenic silver nanoparticles synthesis from Guilandina bonduc L. seed kernel extract with antibacterial, antioxidant and anticancer potential against ovarian Cancer cells. Results in Chemistry, 103246.
4. Bianchi, M., Kaya, E., Logiudice, V., Giuseppantonio Maisetta, Curtis, A., Kavanagh, K., Batoni, G., & Semih Esin. (2025). Biotherapeutic potential of different fractions of cell-free supernatants from Lacticaseibacillus rhamnosus against Pseudomonas

aeruginosa. Frontiers in Cellular and Infection Microbiology, 15. https://doi.org/10.3389/fcimb.2025.1608897

5. Carballo-Carbajal, I., Laguna, A., Romero-Giménez, J., Cuadros, T., Bové, J., Martinez-Vicente, M., Parent, A., Gonzalez-Sepulveda, M., Peñuelas, N., Torra, A., Rodríguez-Galván, B., Ballabio, A., Hasegawa, T., Bortolozzi, A., Gelpi, E., & Vila, M. (2019). Brain tyrosinase overexpression implicates age-dependent neuromelanin production in Parkinson's disease pathogenesis. Nature Communications, 10(1). https://doi.org/10.1038/s41467-019-08858-y
6. Chen, C., Yu, L., Tian, F., Zhao, J., & Zhai, Q. (2022). Identification of Novel Bile Salt-Tolerant Genes in Lactobacillus Using Comparative Genomics and Its Application in the Rapid Screening of Tolerant Strains. Microorganisms, 10(12), 2371. https://doi.org/10.3390/microorganisms10122371
7. Choi, H. J., Lee, H. L., Kim, I. Y., Ju, Y. H., Heo, Y. M., Na, H. R., Lee, J. Y., Choi, S.-I., & Heo, H. J. (2025). Oral Administration of Lactobacillus gasseri and Lacticaseibacillus rhamnosus Ameliorates Amyloid Beta (Aβ)-Induced Cognitive Impairment by Improving Synaptic Function Through Regulation of TLR4/Akt Pathway. Antioxidants, 14(2), 139–139. https://doi.org/10.3390/antiox14020139
8. De Marco, S., Sichetti, M., Muradyan, D., Piccioni, M., Traina, G., Pagiotti, R., & Pietrella, D. (2018). Probiotic Cell-Free Supernatants Exhibited Anti-Inflammatory and Antioxidant Activity on Human Gut Epithelial Cells and Macrophages Stimulated with LPS. Evidence-Based Complementary and Alternative Medicine, 2018, 1–12. https://doi.org/10.1155/2018/1756308
9. Di Chiano, M., Rocchetti, M. T., Spano, G., Russo, P., Allegretta, C., Milior, G., Gadaleta, R. M., Sallustio, F., Pontrelli, P., Gesualdo, L., Avolio, C., Fiocco, D., & Gallone, A. (2024). Lactobacilli Cell-Free Supernatants Modulate Inflammation and Oxidative Stress in Human Microglia via NRF2-SOD1 Signaling. Cellular and Molecular Neurobiology, 44(1), 60. https://doi.org/10.1007/s10571-024-01494-1
10. Ene, M., Savuca, A., Ciobica, A.-S., Jijie, R., Gurzu, I. L., Hritcu, L. D., Chelaru, I.-A., Plavan, G.-I., Nicoara, M. N., & Gurzu, B. (2025). The Neurobehavioral Impact of Zinc Chloride Exposure in Zebrafish: Evaluating Cognitive Deficits and Probiotic Modulation. Toxics, 13(3), 193. https://doi.org/10.3390/toxics13030193
11. Franck Polia, Pastor-Belda, M., Martínez-Blázquez, A., Marie-Noelle Horcajada, Tomás-Barberán, F. A., & Rocío García-Villalba. (2022). Technological and Biotechnological Processes To Enhance the Bioavailability of Dietary (Poly)phenols in Humans. Journal of Agricultural and Food Chemistry, 70(7), 2092–2107. https://doi.org/10.1021/acs.jafc.1c07198
12. Ismail Gumustop, & Fatih Ortakci. (2022). Comparative genomics of Leuconostoc lactis strains isolated from human gastrointestinal system and fermented foods microbiomes. BMC Genomic Data, 23(1). https://doi.org/10.1186/s12863-022-01074-6
13. Khushboo, Arun Karnwal, & Malik, T. (2023). Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1170725
14. Lakshmikanthan, M.; Muthu, S.; Krishnan, K.; Altemimi, A.B.; Abedelmaksoud, T.G. Poly(d,l-Lactide-Co-Glycolide) (PLGA) Nanoparticles in Oncological Therapeutics: Mechanisms of Targeted Delivery and Encapsulation Strategies for Chemother-apeutics, Gene Modulators, and Phytochemicals. Nanomedicine 2026, 74, 102935. https://doi.org/10.1016/J.NANO.2026.102935.
15. Lana, D., Magni, G., & Giovannini, M. G. (2024). Editorial: The role of the microbiota-gut-brain axis in the pathogenesis of neurodegenerative diseases. Frontiers in Neuroscience, 18. https://doi.org/10.3389/fnins.2024.1393840
16. Łepecka, A., & Kołożyn-Krajewska, D. (2025). Antioxidant Properties of Food-Derived Lactic Acid Bacteria: A Review. Microbial Biotechnology, 18(9). https://doi.org/10.1111/1751-7915.70229
17. Lima, C., Gusso, D., Disner, G. R., Pinto, F. J., Falcão, M. A. P., Rosa, J. G. S., & Lopes-Ferreira, M. (2026). The zebrafish in toxicology: a bibliometric analysis reveals current trends and future avenues for predictive safety assessment. Frontiers in Toxicology, 7, 1700031. https://doi.org/10.3389/ftox.2025.1700031
18. Liu, C., Han, F., Cong, L., Sun, T., Bilege Menghe, & Liu, W. (2021). Evaluation of tolerance to artificial gastroenteric juice and fermentation characteristics of Lactobacillus strains isolated from human. Food Science & Nutrition, 10(1), 227–238. https://doi.org/10.1002/fsn3.2662
19. Liu, J., Ren, G., Niu, S., Liu, Y., Zhao, Y., Sun, Z., Zhu, Q., Zhang, J., Mao, Y., Liu, Z., Guo, Q., & Liu, H. (2026). Probiotics Lactobacillus acidophilus LA4 and Lacticaseibacillus paracasei F5 Alleviate Cognitive Dysfunction in Alzheimer's Disease Models: A Dual-Screening Study in Drosophila and Mice. Foods, 15(3), 429. https://doi.org/10.3390/foods15030429
20. M. Onyszko, A. Markowska-Szczupak, Rakoczy, R., Paszkiewicz, O., Janusz, J., A. Gorgon-Kuza, K. Wenelska, & E. Mijowska. (2020). Few Layered Oxidized h-BN as Nanofiller of Cellulose-Based Paper with Superior Antibacterial Response and

Enhanced Mechanical/Thermal Performance. International Journal of Molecular Sciences, 21(15), 5396–5396. https://doi.org/10.3390/ijms21155396

21. Ma, C., Zhang, S., Lu, J., Zhang, C., Pang, X., & Lv, J. (2019). Screening for Cholesterol-Lowering Probiotics from Lactic Acid Bacteria Isolated from Corn Silage Based on Three Hypothesized Pathways. International Journal of Molecular Sciences, 20(9), 2073. https://doi.org/10.3390/ijms20092073
22. Malta, S. M., Batista, L. L., Silva, H. C. G., Franco, R. R., Silva, M. H., Rodrigues, T. S., Correia, L. I. V., Martins, M. M., Venturini, G., Espindola, F. S., da Silva, M. V., & Ueira-Vieira, C. (2022). Identification of bioactive peptides from a Brazilian kefir sample, and their anti-Alzheimer potential in Drosophila melanogaster. Scientific Reports, 12(1), 11065. https://doi.org/10.1038/s41598-022-15297-1
23. Med, N., Hdt, M., & Dnaw, S. (2023). Committed to Create Value for Researchers In vitro Anti-Inflammatory Egg Albumin Denaturation Assay: An Enhanced Approach In vitro Anti-Inflammatory Egg Albumin Denaturation Assay: An Enhanced Approach. Journal of Natural & Ayurvedic Medicine, 7(3). https://doi.org/10.23880/jonam-16000411.
24. Muthu, S.; Altemimi, A.B.; Lakshmikanthan, M.; Krishnan, K.; ALKaisy, Q.H.; Awlqadr, F.H.; Hesarinejad, M.A. Phycocolloids from Sargassum Microcystum: Immunomodulatory and Antioxidant Activities of Alginic Acid and Fucoidan. Food Hydrocoll. Health 2025, 7, 100209. https://doi.org/10.1016/J.FHFH.2025.100209.
25. Nagatsu, T., Nakashima, A., Watanabe, H., Ito, S., & Wakamatsu, K. (2022). Neuromelanin in Parkinson's Disease: Tyrosine Hydroxylase and Tyrosinase. International Journal of Molecular Sciences, 23(8), 4176. https://doi.org/10.3390/ijms23084176
26. Nurliana Abd Mutalib, Syed, A., Nor Atiqah Jusril, Nur Intan Hasbullah, Mohd, & Nor Hadiani Ismail. (2023). Lactic Acid Bacteria (LAB) and Neuroprotection, What Is New? An Up-To-Date Systematic Review. Pharmaceuticals, 16(5), 712–712. https://doi.org/10.3390/ph16050712
27. Ojha, S., Patil, N., Jain, M., Kole, C., & Kaushik, P. (2023). Probiotics for Neurodegenerative Diseases: A Systemic Review. Microorganisms, 11(4), 1083. https://doi.org/10.3390/microorganisms11041083
28. Samer, A., Toumi, R., Soufli, I., & Touil-Boukoffa, C. (2022). Cell-free probiotic supernatant (CFS) treatment alleviates indomethacin-induced enterocolopathy in BALB/c mice by down-modulating inflammatory response and oxidative stress: potential alternative targeted treatment. Inflammopharmacology, 30(5), 1685–1703. https://doi.org/10.1007/s10787-022-00996-y
29. Sasikumar, A., Shanmugam, R., Sengottuvelu, S., Saranya, N., & Karukuvelraja, R. (2026). GABA production from buckwheat derived probiotics (Cytobacillus oceanisediminis): a promising approach for neurological health enhancement. International Microbiology. https://doi.org/10.1007/s10123-025-00763-3
30. Sirin, S., & Aslim, B. (2020). Characterization of lactic acid bacteria derived exopolysaccharides for use as a defined neuroprotective agent against amyloid beta1–42-induced apoptosis in SH-SY5Y cells. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-65147-1
31. Sivamaruthi, B. S., Suganthy, N., & Pellegrini, C. (2024). Editorial: Reviews in neuropharmacology 2023: microbiota gut-brain axis, therapeutic insights for neurodegenerative diseases. Frontiers in Pharmacology, 15. https://doi.org/10.3389/fphar.2024.1499072
32. Song, Y., Chen, S., Li, L., Zeng, Y., & Hu, X. (2022). The Hypopigmentation Mechanism of Tyrosinase Inhibitory Peptides Derived from Food Proteins: An Overview. Molecules, 27(9), 2710. https://doi.org/10.3390/molecules27092710
33. Tyagi, A., Chelliah, R., Banan-Mwine Daliri, E., Sultan, G., Madar, I. H., Kim, N., Shabbir, U., & Oh, D.-H. (2023). Antioxidant activities of novel peptides from Limosilactobacillus reuteri fermented brown rice: A combined in vitro and in silico study. Food Chemistry, 404, 134747. https://doi.org/10.1016/j.foodchem.2022.134747
34. Tyagi, A., Shabbir, U., Chelliah, R., Daliri, E. B.-M., Chen, X., & Oh, D.-H. (2021). Limosilactobacillus reuteri Fermented Brown Rice: A Product with Enhanced Bioactive Compounds and Antioxidant Potential. Antioxidants, 10(7), 1077. https://doi.org/10.3390/antiox10071077
35. Tyagi, A., Yeon, S.-J., Daliri, E. B.-M., Chen, X., Chelliah, R., & Oh, D.-H. (2021). Untargeted Metabolomics of Korean Fermented Brown Rice Using UHPLC Q-TOF MS/MS Reveal an Abundance of Potential Dietary Antioxidative and Stress-Reducing Compounds. Antioxidants, 10(4), 626. https://doi.org/10.3390/antiox10040626
36. Wu, Q., Gong, A., Liu, X., Hou, J., Liu, H., Yang, Z., & Zhu, Y. (2024). Probiotics Alleviate Microcystin-LR-Induced Developmental Toxicity in Zebrafish Larvae. Toxics, 12(7), 527. https://doi.org/10.3390/toxics12070527
37. Xing, J., Wang, G., Zhang, Q., Liu, X., Gu, Z., Zhang, H., Chen, Y. Q., & Chen, W. (2015). Determining Antioxidant Activities of Lactobacilli Cell-Free Supernatants by Cellular Antioxidant Assay: A Comparison with Traditional Methods. PLOS ONE, 10(3), e0119058. https://doi.org/10.1371/journal.pone.0119058
38. Xuefeng, L. (2023). Screening and Compositional Analysis of Lactic Acid Bacteria with Tyrosinase

Inhibitory Activity. Shipin Kexue, 44(18), 193–199. https://agris.fao.org/search/en/providers/122436/records/675ac1dd0ce2cede71d0594b

39. Yin, J.-H., & Horzmann, K. A. (2024). Embryonic Zebrafish as a Model for Investigating the Interaction between Environmental Pollutants and Neurodegenerative Disorders. Biomedicines, 12(7), 1559–1559. https://doi.org/10.3390/biomedicines12071559