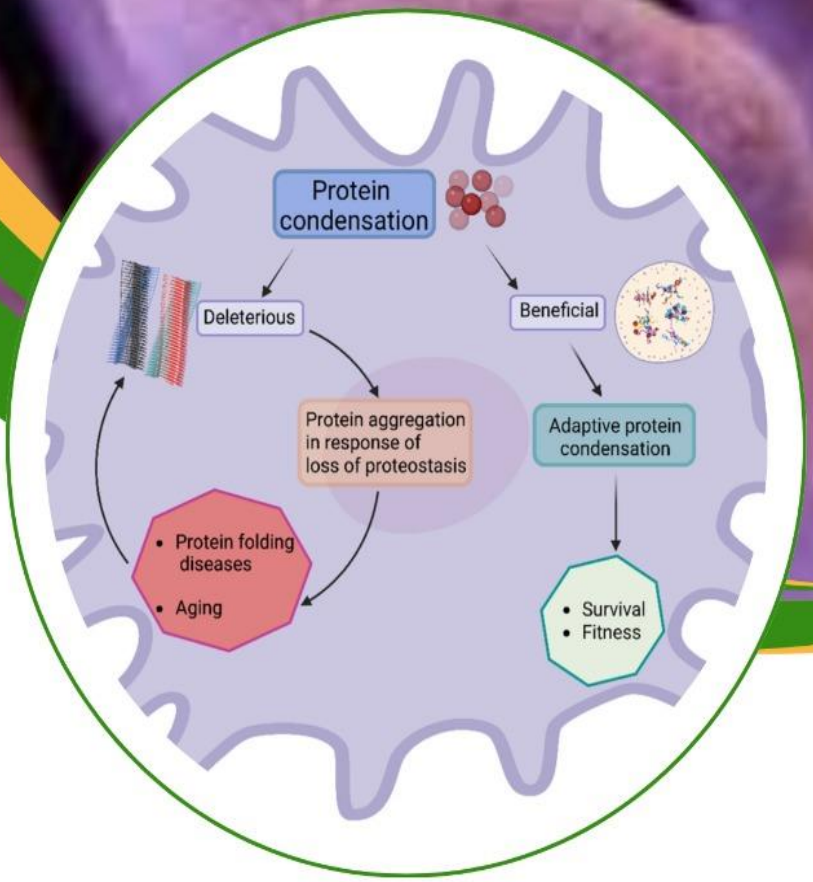

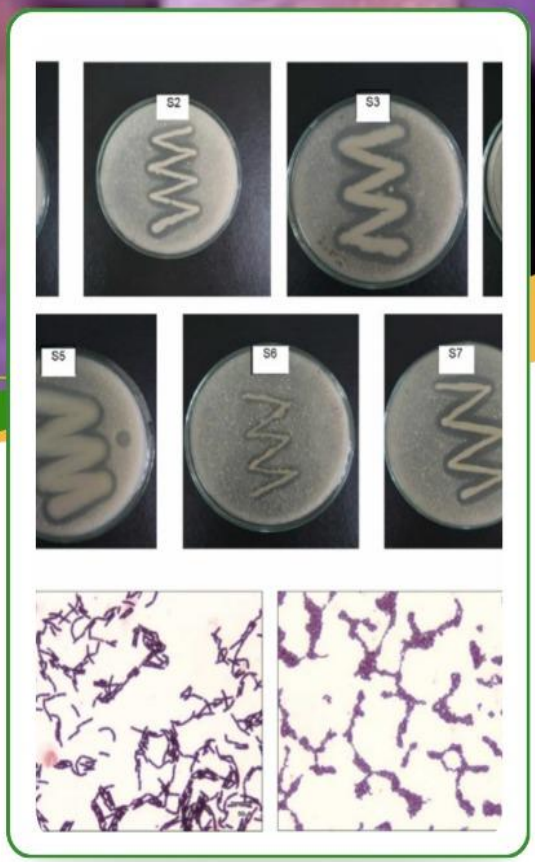

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Investigating the Role of Proteolytic Bacteria in Degrading Misfolded Proteins Associated with Neurodegenerative disease

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ABSTRACT

Neurodegenerative diseases (NDs) such as Alzheimer's, Parkinson's, and Huntington's disease are characterized by the accumulation of misfolded proteins, leading to neuronal dysfunction. While proteolytic enzymes regulate protein homeostasis, emerging evidence suggests that proteolytic bacteria influence disease progression by either clearing toxic aggregates or exacerbating neurotoxicity through the gut-brain axis. This review explores bacterial proteases in neurodegeneration, emphasizing their ability to traverse the blood-brain barrier (BBB) and their therapeutic potential. We discuss experimental and clinical evidence on their dual role as both neuroprotective agents and contributors to pathology. Understanding these interactions could open avenues for novel diagnostic and therapeutic strategies, harnessing bacterial proteases to mitigate neurodegeneration and improve brain health. Furthermore, elucidating the mechanisms of bacterial protease activity may provide insights into broader microbial influences on neurodegenerative disorders. Advancing this knowledge could drive innovative microbiome-based interventions for neuroprotection and disease management.

Keywords: Neurodegenerative diseases, Misfolded protein degradation, Proteolytic bacteria, Gut-brain axis, Bacterial proteases

1. Introduction

Neurodegenerative diseases represent a class of chronic, progressive disorders characterized by the degeneration of the nervous system, particularly the loss of neurons in key areas of the brain responsible for cognitive, motor, and emotional functions. Alzheimer's Disease (AD), Parkinson's Disease (PD), and Huntington's Disease (HD) are some of the most common and debilitating neurodegenerative conditions [1]. According to recent estimates, over 50 million people worldwide are living with dementia, and that number is projected to triple by 2050 due to an aging global population [2]. The prevalence of these diseases has spurred significant research into their molecular and cellular mechanisms to identify effective therapeutic strategies.

One of the central pathological features of neurodegenerative diseases is the accumulation of misfolded proteins. In AD, the accumulation of amyloid-beta peptides and hyper phosphorylated tau protein form toxic plaques and tangles, which disrupt cellular homeostasis, impair synaptic function, and trigger neuroinflammation. In PD, the aggregation of alpha-synuclein leads to the formation of Lewy bodies, which similarly contribute to neuronal dysfunction and death. In HD, the expansion of CAG repeats in the huntingtin gene results in the accumulation of mutant huntingtin protein, which forms insoluble aggregates that interfere with normal neuronal function. The deposition of these aggregated proteins in the brain has become one of the hallmark features of neurodegeneration, and they are thought to contribute directly to neuronal death and disease progression [3,4]

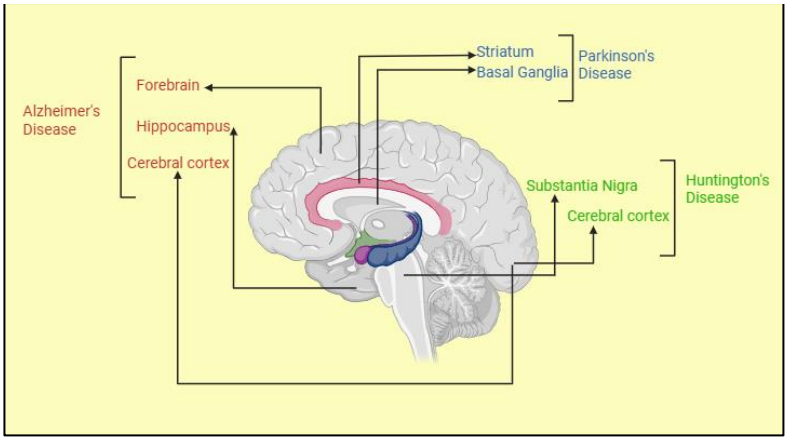

Figure 1: Sagittal section of the human brain depicting major areas affected in selected neurodegenerative diseases. The basal ganglia, including the striatum, are shown in the context of Parkinson's disease, along with the substantia nigra, a key site of dopaminergic neuron loss in this condition. The hippocampus and cerebral cortex, regions critical for memory and cognition, are indicated in relation to Alzheimer's disease. The cerebral cortex is also highlighted in Huntington's disease, a disorder characterized by motor and cognitive decline.

Protein misfolding and aggregation play a key role in neurodegenerative diseases and offer potential therapeutic targets [3]. Normally, proteins fold into specific structures for function, but mutations, oxidative stress, or environmental factors can disrupt this process, leading to toxic aggregates. These misfolded proteins resist degradation by cellular proteostasis mechanisms, triggering inflammation and oxidative damage that worsen neuronal injury [5]. This cycle highlights the need for strategies to prevent aggregation or enhance the clearance of misfolded proteins.

Proteolytic bacteria offer a novel approach to combating protein aggregation [2]. Certain strains produce enzymes that degrade misfolded proteins like amyloid-beta, alpha-synuclein, and huntingtin [1]. Studies show gut microbiome proteases can break down amyloid-beta and reduce its toxicity [6]. Through the gut-brain axis, microbiota-derived proteases may directly interact with misfolded proteins, presenting a promising therapeutic strategy [7]

Furthermore, recent evidence has highlighted the potential of bacterial proteases to modulate neuroinflammation, a key feature of neurodegenerative diseases. By degrading amyloid plaques or other aggregated proteins, these enzymes may reduce the inflammatory response triggered by the accumulation of toxic protein species, thus providing a dual therapeutic effect—targeting both the misfolded proteins and the neuroinflammatory pathways. The use of proteolytic bacteria could also serve as a complementary treatment alongside other emerging therapies, such as small molecules or gene therapies, to enhance protein clearance mechanisms and slow disease progression [5]

Although promising, the therapeutic application of proteolytic bacteria in neurodegenerative diseases remains in its infancy. Further research is needed to identify the most effective bacterial strains, characterize the specific proteases involved, and explore the mechanisms through which these bacteria interact with the central nervous system [6]. In this article, I will be exploring the potential of proteases in neurodegenerative diseases, analysing their enzymatic properties, and assessing their relevance to neurodegenerative disease research.

2. Neurodegenerative diseases

Neurodegenerative diseases are a group of disorders characterized by progressive degeneration of the nervous system, leading to cognitive, motor, and functional impairments. These diseases primarily affect older populations, although they can occur in younger individuals, and they result from the gradual loss of neurons and synaptic connections in specific regions of the brain. Alzheimer’s disease (AD), Parkinson’s disease (PD), and

Huntington's disease (HD) are among the most well-known neurodegenerative disorders, each with distinct pathophysiological mechanisms, yet sharing the common feature of protein misfolding and aggregation.

2.1 Alzheimer's Disease (AD)

AD is the most prevalent form of dementia, affecting millions of individuals worldwide. It is primarily characterized by progressive memory loss, cognitive decline, and changes in behaviour. AD is associated with the accumulation of two key pathological hallmarks: amyloid-beta plaques and tau tangles. Amyloid-beta peptides, which arise from the cleavage of the amyloid precursor protein (APP), aggregate to form plaques that disrupt neuronal communication and trigger inflammation [9]. Tau, a microtubule-associated protein, becomes hyperphosphorylated in AD, leading to the formation of neurofibrillary tangles inside neurons, which impair cellular function and contribute to neuronal death. These aggregates interfere with cellular homeostasis, synaptic plasticity, and neuronal signaling, contributing to the cognitive and functional decline seen in AD patients [10]. Genetic mutations in APP, presenilin-1, and presenilin-2, as well as the APOE4 allele, are known to increase the risk of AD, though environmental factors such as oxidative stress and inflammation also play a significant role in disease development and progression [11].

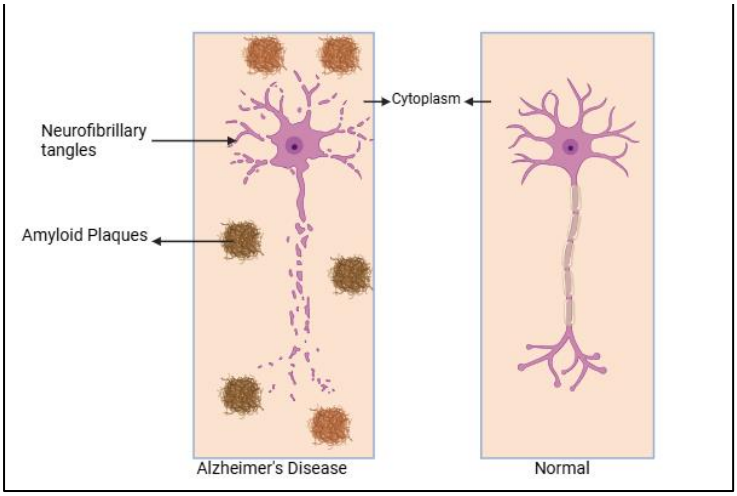

Figure 2: Structural comparison of a normal neuron and an Alzheimer's disease-affected neuron. The normal neuron displays a healthy structure with a well-defined cytoplasm. In contrast, the Alzheimer's disease-affected neuron shows the presence of neurofibrillary tangles within the cell body and amyloid plaques accumulating outside the cell, both of which disrupt neuronal function. The image was created using BioRender.com.

2.2 Parkinson's Disease (PD)

PD is a neurodegenerative disorder primarily affecting movement. It is characterized by the loss of dopaminergic neurons in the substantia nigra, a brain region responsible for controlling movement and coordination. The hallmark of PD is the aggregation of alpha-synuclein, a protein involved in synaptic function. In PD, alpha-synuclein misfolds and aggregates to form Lewy bodies, which disrupt neuronal function and lead to the degeneration of dopaminergic neurons. This loss of dopamine causes the motor symptoms characteristic of PD, including tremors, rigidity, bradykinesia (slowness of movement), and postural instability [12]. PD is also associated with non-motor symptoms, such as cognitive impairment, depression, and autonomic dysfunction. The exact cause of PD remains unclear, but genetic factors, such as mutations in the LRRK2 and SNCA genes, and environmental factors, such as exposure to toxins, have been implicated in the disease [13].

2.3 Huntington's Disease (HD)

HD is a genetic neurodegenerative disorder caused by an expansion of CAG repeats in the huntingtin gene. This expansion leads to the production of a mutant form of the huntingtin protein, which forms toxic aggregates that accumulate within neurons, primarily in the striatum and cortex. The presence of these aggregates disrupts cellular processes, such as protein degradation and mitochondrial function, and leads to neuronal dysfunction and death. HD is characterized by progressive motor impairment, including chorea (involuntary movements), as well as

cognitive and psychiatric symptoms [14]. Unlike AD and PD, HD is inherited in an autosomal dominant pattern, meaning that individuals with one copy of the mutated gene will eventually develop the disease, typically around 40 years [15]. The CAG repeat expansion in the huntingtin gene leads to the production of an abnormally long polyglutamine tract in the huntingtin protein, which is thought to be toxic to neurons [16].

3. Proteolytic Enzymes and Their Role in Protein Metabolism

Proteolytic enzymes are fundamental to maintaining cellular integrity and function by regulating protein turnover. These enzymes degrade misfolded, damaged, or unneeded proteins, preventing toxic accumulation and ensuring proper cellular function. The proteolytic process is tightly regulated and occurs through specialized pathways that coordinate protein breakdown, recycling, and clearance. Disruptions in proteolytic systems have been implicated in a range of diseases, including neurodegenerative disorders, where the accumulation of misfolded proteins is a hallmark of pathology [17]. This section explores the fundamental properties of proteolytic enzymes, their involvement in protein homeostasis, their role in neurodegenerative diseases, and their potential therapeutic applications.

2.1. Overview of Proteolytic Enzymes

Proteolytic enzymes, also known as proteases, are critical regulators of protein metabolism. They catalyse the breakdown of proteins into smaller peptides or amino acids, a process essential for cellular maintenance, energy production, and regulation of various biochemical pathways. These enzymes are involved in numerous biological functions, such as protein turnover, cellular signalling, and response to stress. They help to degrade damaged, misfolded, or surplus proteins, maintaining cellular homeostasis and ensuring proper protein function. Proteases are categorized based on their active sites, including serine, cysteine, metalloproteases, and aspartic proteases, each of which plays a distinct role in proteolysis [17].

2.2. The Role of Proteolytic Pathways in Protein Homeostasis

Proteolysis is primarily carried out by two systems: the ubiquitin-proteasome system (UPS) and the autophagy-lysosome system. The UPS targets short-lived or damaged proteins by tagging them with a molecule called ubiquitin. Once tagged, these proteins are recognized and degraded by the proteasome. In contrast, autophagy involves the engulfment of larger aggregates or entire organelles in vesicles, which are then transported to lysosomes for degradation. Both pathways are essential for maintaining cellular homeostasis by preventing the accumulation of toxic or defective proteins that could impair cellular functions. Disruptions in these proteolytic systems can lead to various diseases, particularly neurodegenerative disorders [18].

2.3. Proteolytic Enzymes in Neurodegenerative Diseases

In neurodegenerative diseases, the failure of proteolytic systems leads to the accumulation of misfolded or aggregated proteins, contributing to neuronal dysfunction and cell death. In Alzheimer's disease (AD), the accumulation of amyloid-beta plaques and tau tangles overwhelms the proteolytic capacity of neurons, impairing synaptic function and contributing to cognitive decline. In Parkinson's disease (PD), the aggregation of alpha-synuclein into Lewy bodies disrupts cellular proteostasis, leading to dopaminergic neuron degeneration. Similarly, in Huntington's disease (HD), the accumulation of mutant huntingtin protein forms toxic aggregates that interfere with cellular functions, causing motor, cognitive, and psychiatric symptoms. In these diseases, proteases are often unable to clear the toxic aggregates, highlighting the importance of proteolytic pathways in preventing disease progression [19,20].

2.4. Potential Therapeutic Applications of Proteolytic Enzymes

Given their role in maintaining protein homeostasis, proteolytic enzymes offer potential therapeutic avenues for treating neurodegenerative diseases. By enhancing the activity of proteases or introducing new proteolytic enzymes capable of degrading misfolded proteins, researchers aim to promote the clearance of toxic aggregates from the brain. For example, bacterial proteases produced by gut microbiota have been identified as capable of degrading amyloid-beta and reducing its neurotoxic effects. Similarly, other proteases targeting alpha-synuclein or huntingtin could provide new opportunities for therapeutic intervention. In addition to direct protease therapies,

approaches aimed at enhancing the efficiency of the UPS or autophagy systems could be explored as a way to restore cellular proteostasis and reduce protein aggregation [17,21].

3. Proteolytic Bacteria and Their Interaction with Misfolded Proteins

Proteolytic bacteria have emerged as key players in the regulation of protein homeostasis (proteostasis) due to their ability to degrade misfolded and aggregated proteins. These pathological protein species are central to the development and progression of several neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's disease. Through the production of diverse proteolytic enzymes, these bacteria participate in the maintenance of cellular integrity across various ecosystems and are now being explored for their potential therapeutic applications. This section outlines the ecological distribution and enzymatic classification of proteolytic bacteria, their mechanisms of interaction with misfolded proteins, and their role in gut-brain communication and neurodegenerative disease modulation [22].

3.1. Sources and Classification of Proteolytic Bacteria

Proteolytic bacteria are widely distributed in natural environments, including the human gastrointestinal tract, terrestrial soils, and marine ecosystems. In each of these habitats, they contribute to the degradation of organic proteinaceous matter, including misfolded and denatured proteins [23].

In the human gut, proteolytic species such as *Bacteroides*, *Clostridium*, and *Lactobacillus* play integral roles in protein digestion and metabolic regulation. Recent studies suggest that some of these bacteria may also contribute to the breakdown of neurotoxic aggregates, such as amyloid-β and α-synuclein, thereby potentially influencing the progression of neurodegenerative disorders through local proteostasis regulation.

Soil microbiota represent another rich reservoir of proteolytic activity. Species such as *Bacillus*, *Pseudomonas*, and *Streptomyces* secrete a range of extracellular proteases that facilitate the decomposition of environmental proteins. Their enzymes are not only efficient but also robust, functioning under diverse environmental conditions, which makes them attractive candidates for biotechnological applications aimed at protein aggregate degradation.

In marine environments, proteolytic bacteria such as *Pseudoalteromonas* and *Vibrio* contribute to nutrient cycling and organic matter turnover. Marine-derived proteases often possess unique catalytic properties, including cold activity and salt tolerance, which may be particularly useful for developing therapeutic agents capable of functioning under physiologically challenging conditions [24].

Proteolytic bacteria are further classified based on the types of enzymes they produce:

- **Serine proteases,** such as subtilisin from *Bacillus subtilis*, utilize a serine residue at the active site to hydrolyze peptide bonds and are involved in broad-spectrum protein degradation.
- **Metalloproteases,** including those produced by *Streptomyces* and *Clostridium* species, require divalent metal ions (e.g., Zn^{2+}) for activity and are crucial in extracellular matrix turnover.
- **Cysteine proteases,** found in *Clostridium histolyticum* and *Porphyromonas gingivalis*, use a cysteine thiol group for proteolysis and have demonstrated potential in the breakdown of aggregated proteins.
- **Aspartic proteases,** produced by certain gut and marine bacteria, are active in acidic environments and have been shown to degrade misfolded proteins under conditions similar to the gastrointestinal tract [25].

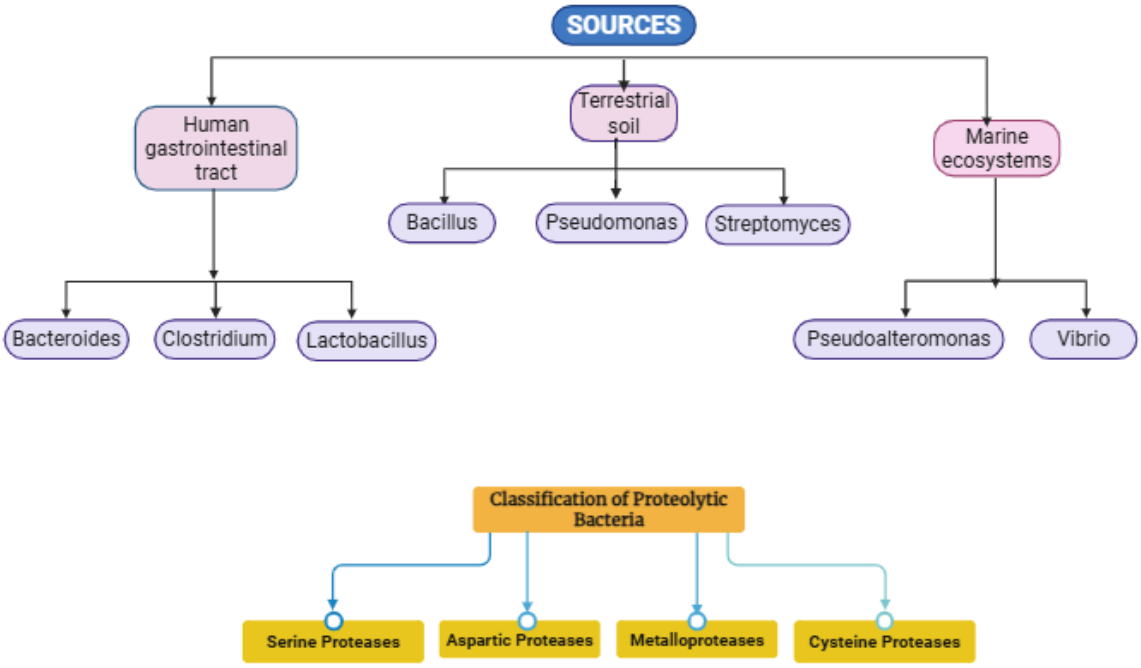

Figure 3: Sources and classification of proteolytic bacteria. The diagram shows that these bacteria are found in various environments, including the human gut (Bacteroides, Clostridium, Lactobacillus), terrestrial soil (Bacillus, Pseudomonas, Streptomyces), and marine ecosystems (Pseudoalteromonas, Vibrio). Bacteria are classified based on their primary proteolytic enzymes: serine proteases, aspartic proteases, metalloproteases, and cysteine proteases.

3.2. Mechanisms of Interaction with Misfolded Proteins

Proteolytic bacteria employ several complementary strategies to interact with and degrade misfolded or aggregated proteins. These mechanisms can be broadly divided into extracellular and intracellular pathways.

Extracellular proteolysis is a primary route by which many bacteria exert their degradative functions. Secreted proteases can access and cleave protein aggregates within the surrounding environment, such as the gut lumen or biofilm matrices. This is particularly significant in the gastrointestinal tract, where secreted enzymes may degrade amyloidogenic peptides before they enter systemic circulation [26].

Cell-associated proteolysis represents another mechanism, in which proteases remain tethered to the bacterial surface. This allows for localized degradation of misfolded proteins that come into direct contact with bacterial cells. Such proximity-driven interactions enhance enzymatic efficiency and specificity.

Certain bacteria also engage in endocytosis and intracellular degradation of misfolded proteins. After uptake via endocytic processes, aggregates are delivered to protease-rich intracellular compartments for processing. This has been observed in select gut-resident species and may represent a controlled strategy to neutralize toxic protein species.

Finally, biofilm-associated degradation plays a supportive but important role. Biofilms provide a stable microenvironment where proteolytic activity is concentrated, facilitating the gradual breakdown of otherwise resistant protein aggregates. This may have relevance not only in environmental contexts but also in microbial-host interactions, including gut-brain dynamics [27].

3.3. Gut-Brain Communication and Therapeutic Potential of Proteolytic Bacteria

The gut-brain axis is an integrated signaling network connecting the gastrointestinal system and the central nervous system. Recent findings suggest that proteolytic bacteria residing in the gut can influence neurological function and disease progression through multiple interconnected mechanisms, which have been supported by experimental data from in vitro systems and animal models as seen in Fig.2 [26].

One major mechanism involves the local degradation of neurotoxic proteins within the gut. Proteolytic enzymes from resident bacteria can break down amyloidogenic species before they translocate into systemic circulation. In vitro studies using proteases from *Bacillus* and *Streptomyces* have demonstrated the successful degradation of amyloid-β fibrils and hyperphosphorylated tau, indicating a direct therapeutic application in the clearance of misfolded proteins [28].

In animal models of neurodegeneration, modulation of the gut microbiome through the introduction of proteolytic bacterial strains has shown promising results. Transgenic mice colonized with such strains exhibited reduced protein aggregation in the brain, improved behavioral performance, and attenuation of neuroinflammatory markers. These outcomes suggest that proteolytic bacteria may exert neuroprotective effects through both direct proteolysis and immune modulation [29].

Another relevant pathway is the modulation of inflammatory signaling. Gut dysbiosis is commonly associated with increased intestinal permeability and chronic inflammation, both of which are implicated in neurodegenerative disease progression [30]. Proteolytic bacteria may help restore homeostasis by degrading pro-inflammatory mediators or by producing anti-inflammatory metabolites, such as short-chain fatty acids (SCFAs). These compounds are known to influence microglial activation, blood-brain barrier integrity, and cytokine production [31].

In addition to immunomodulation, neuroactive metabolite production further links proteolytic bacteria to brain health. Some species are capable of producing SCFAs, neurotransmitter precursors, and other signaling molecules that directly influence neuronal survival and function. By simultaneously reducing the pool of misfolded proteins and enhancing the neurochemical environment, proteolytic bacteria may support cognitive resilience [32].

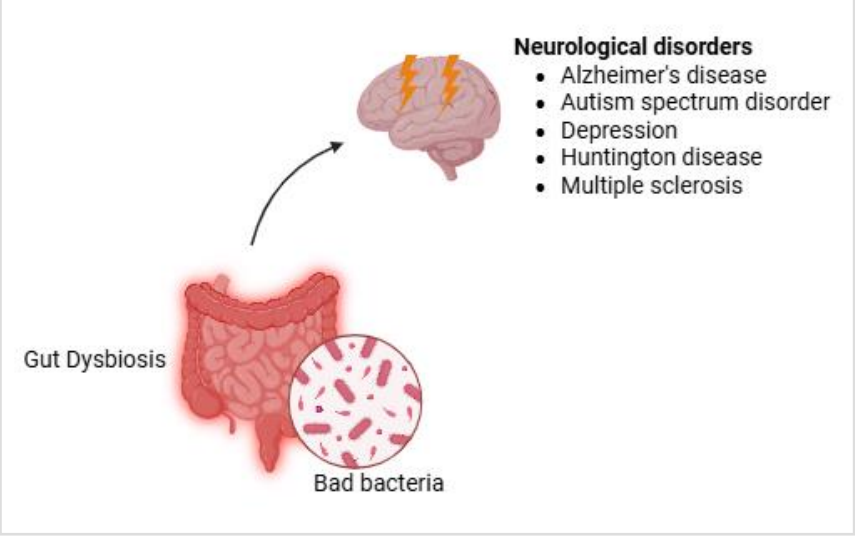

Figure 4: Gut dysbiosis, characterized by an imbalance in the gut microbiome, is associated with several neurological disorders, including Alzheimer's disease, autism spectrum disorder, depression, Huntington's disease, and multiple sclerosis.

Collectively, these mechanisms highlight the therapeutic potential of proteolytic bacteria and their enzymes. Emerging strategies include the development of probiotic formulations containing live proteolytic strains and biopharmaceuticals based on purified bacterial proteases [33]. These approaches offer a novel, non-invasive

means of targeting the underlying causes of neurodegenerative diseases. However, their clinical application will require further investigation into enzyme specificity, delivery methods, safety, and long-term effects in human populations.

4. Implications for Brain Health and Neurodegenerative Diseases

Bacterial proteases have emerged as a compelling area of research in neurodegenerative diseases due to their capacity to modulate protein homeostasis, neuroinflammation, and neuronal integrity [34]. Given that proteolytic enzymes govern crucial cellular processes, their dysregulation or therapeutic utilization could significantly impact the pathophysiology of conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD). These enzymes present both opportunities and challenges in the context of neurodegeneration—acting as potential neuroprotective agents by degrading toxic protein aggregates while also posing risks if they induce neurotoxicity or exacerbate inflammation.

4.1 How Bacterial Proteases Cross the Blood-Brain Barrier (BBB) and Evidence from Experimental and Clinical Studies

The blood-brain barrier (BBB) is a highly selective endothelial interface that regulates molecular entry into the central nervous system (CNS). However, bacterial proteases can breach this barrier through multiple mechanisms, potentially influencing neurological health. Several bacterial proteases, particularly metalloproteases and serine proteases, have been shown to degrade endothelial tight junction proteins such as occludin and claudin, increasing BBB permeability. Pathogens such as *Pseudomonas aeruginosa* produce elastases that compromise vascular integrity, enabling bacterial components, including proteases, to infiltrate the CNS. Some bacterial molecules may also hijack endogenous transport mechanisms to cross the BBB. For instance, *Listeria monocytogenes* utilizes internalin-A to interact with E-cadherin on endothelial cells, facilitating transcytosis [35]. It is plausible that bacterial proteases may exploit similar pathways for CNS entry. Additionally, bacterial infections elevate systemic levels of pro-inflammatory cytokines such as TNF-α and IL-6, which can weaken BBB integrity, indirectly allowing proteases and other bacterial products to penetrate the brain parenchyma. Emerging research also suggests that bacterial proteases can be encapsulated within exosomes or extracellular vesicles (EVs), providing a novel mechanism for their transport across the BBB without direct permeability disruption [38 liu].

Experimental and clinical findings support the role of bacterial proteases in neurodegeneration. *In vitro* studies have shown that bacterial proteases such as subtilisins and metalloproteases exhibit amyloid-degrading properties, suggesting their potential to clear pathological protein aggregates in AD and PD [39 hsu]. However, excessive protease activity may also degrade essential neuronal proteins, leading to neurotoxicity. Preclinical studies in animal models have shown that proteases from probiotic *Lactobacillus* species may reduce amyloid-beta (Aβ) burden in AD models [40kwon]. Conversely, proteases from periodontal bacteria (*Porphyromonas gingivalis*) have been implicated in tau hyperphosphorylation and neuroinflammation, worsening AD pathology. Epidemiological evidence also links chronic bacterial infections, particularly those associated with *P. gingivalis* and *Helicobacter pylori*, to increased dementia risk, potentially due to bacterial proteases modulating neuroinflammatory pathways [41,dominy,olsen]

4.2 Potential Neuroprotective or Neurotoxic Effects of Bacterial Proteases

Bacterial proteases present a dualistic nature in the context of neurodegenerative diseases, exhibiting both potential benefits and risks. On one hand, certain bacterial proteases offer promising neuroprotective capabilities. For instance, nattokinase and serrapeptase have demonstrated the ability to degrade fibrillar Aβ, a hallmark of Alzheimer's disease, and to mitigate neuroinflammation, a key factor in various neurodegenerative processes. Furthermore, proteases derived from *Bacillus* species have shown fibrinolytic activity, which may counteract the formation of amyloid plaques. The development of engineered proteases with enhanced substrate specificity holds potential for their use as biotherapeutics, allowing for targeted amyloid degradation while minimizing off-target effects on other proteins [39hsu]

Conversely, the activity of other bacterial proteases can be detrimental. Proteases from pathogens such as *P. gingivalis*, including gingipains, have been detected in AD brain samples, strongly implicating them in the pathogenesis of the disease. These proteases are involved in tau hyperphosphorylation, a process that disrupts neuronal function and contributes to synaptic dysfunction. Excessive proteolysis, in general, can lead to the degradation of neuronal membranes, the disruption of essential synaptic proteins, and the promotion of chronic neuroinflammation, all of which exacerbate neurodegeneration. Thus, while some bacterial proteases may offer therapeutic potential, others pose a significant threat to neuronal health and may contribute to the progression of neurodegenerative conditions [43kanagasingam]

5. Diagnostic Tools and Therapeutic Strategies

5.1 Diagnostic Tools

5.1.1 Mass Spectrometry and Immunoassays

Recent advancements in mass spectrometry, particularly tandem mass spectrometry (LC-MS/MS), enable the precise identification of bacterial proteases in cerebrospinal fluid (CSF) and blood samples. These techniques allow the differentiation of proteolytic signatures associated with neurodegenerative diseases, improving early diagnostic accuracy. Highly specific enzyme-linked immunosorbent assays (ELISA) have also been designed to detect bacterial proteases and their substrates in CSF. Multiplex immunoassays further enhance detection capabilities by simultaneously measuring protease activity and associated inflammatory markers [44,45azevedo, therrialt]

5.1.2 Activity-Based Probes and Metaproteomics

Additionally, novel fluorogenic and bioluminescent activity-based probes (ABPs) are being developed to selectively label active bacterial proteases in biological fluids, allowing for real-time monitoring of protease activity in neurodegenerative conditions. High-throughput metaproteomic approaches are being employed to profile gut-derived bacterial proteases and their systemic impact on neurodegeneration, shedding light on gut-brain axis interactions. [46,47haditsch, marcin]

5.2 Therapeutic Strategies

5.2.1 Enzyme Therapy and Inhibitors

The therapeutic potential of bacterial proteases is also being explored. Recombinant bacterial proteases engineered for targeted amyloid degradation are being investigated as potential therapeutics for AD. Advanced bioengineering techniques are being used to modify proteases to selectively degrade amyloid plaques while avoiding essential neuronal proteins. This includes structure-guided mutagenesis to enhance enzyme specificity and reduce immunogenicity. Furthermore, the development of novel small-molecule inhibitors targeting neurotoxic bacterial proteases, such as gingipain inhibitors, is underway. These compounds aim to mitigate neuroinflammatory responses while preserving endogenous protease balance in the CNS. Preclinical studies indicate that selective gingipain inhibitors can significantly reduce tau phosphorylation and synaptic loss in AD models [48sabbagh]

5.2.2 Nanocarrier-Based Delivery

Advanced nanotechnology is being utilized to deliver beneficial proteases across the BBB while minimizing off-target effects. Strategies include the use of lipid-based nanoparticles, such as liposomal carriers, to encapsulate therapeutic proteases and enhance BBB penetration and protease stability in circulation. Biodegradable polymers, such as PLGA (poly(lactic-co-glycolic acid)), are also being employed to deliver proteases in a controlled manner, reducing systemic toxicity. Engineered exosomes derived from mesenchymal stem cells are being explored as carriers for proteases, allowing targeted delivery to neurons and astrocytes without triggering an immune response. Additionally, functionalized magnetic nanoparticles are being investigated for targeted protease delivery, enabling externally controlled localization and release of therapeutic enzymes in neurodegenerative regions [49]

6. Challenges and Limitations

Despite the promising potential of bacterial proteases in addressing neurodegenerative diseases, several significant challenges and limitations must be overcome before clinical translation can be realized.

6.1. Specificity and Selectivity

A primary concern is the inherent broad substrate specificity of many bacterial proteases. This lack of selectivity increases the risk of off-target effects, whereby beneficial proteases degrade unintended neuronal proteins, potentially leading to neuronal damage or exacerbating existing neurotoxicity. Therefore, a critical focus of future research must be on enhancing protease specificity and selectivity. Strategies such as directed evolution and computational modeling offer promising avenues for engineering proteases with tailored substrate preferences, minimizing unintended consequences [50]

6.2 Blood-Brain Barrier Penetration and Delivery

Efficient and targeted delivery of therapeutic proteases across the blood-brain barrier (BBB) represents another major hurdle. The BBB's restrictive nature, designed to protect the central nervous system, also hinders the entry of many therapeutic agents. Simultaneously, preventing the entry of potentially neurotoxic bacterial enzymes into the CNS is crucial. To address these challenges, innovative delivery strategies are needed. Potential approaches include the development of receptor-mediated delivery systems to exploit specific transport mechanisms across the BBB, the use of exosome-based transport to leverage natural intercellular communication pathways, and nanoparticle encapsulation to protect proteases and enhance their permeability and targeted release [51,52]

6.3 Safety and Long-Term Effects

Thorough evaluation of the long-term safety implications of bacterial protease-based therapies is paramount. The prolonged effects of protease exposure on neuronal function, immune responses, and overall CNS homeostasis require extensive study. Potential risks that need careful consideration include the possibility of immune sensitization, the induction or exacerbation of chronic neuroinflammation, and unintended interactions with endogenous proteases and their natural inhibitors [53]

6.4 Regulatory and Translational Challenges

The development and clinical translation of bacterial protease-based therapies are also subject to stringent regulatory hurdles. Concerns related to safety, stability, immunogenicity, and large-scale manufacturing must be adequately addressed. To facilitate a smooth transition from preclinical studies to clinical applications, it is essential to establish standardized protocols for protease engineering, purification, characterization, and validation [53]

6.5 Future Perspectives

Looking ahead, several novel perspectives and future directions hold promise. Advances in synthetic biology may allow for the engineering of bacterial proteases with highly specific catalytic properties tailored for therapeutic applications. Modulating the gut microbiota to influence bacterial protease production through gut-brain axis and microbiome engineering could also serve as an indirect therapeutic strategy for neurodegenerative diseases. Future research may also focus on patient-specific protease profiling to develop precision medicine approaches that target individual proteolytic imbalances in neurodegenerative diseases [54,55]

7. Conclusion

In summary, the intricate relationship between bacterial proteases and neurodegenerative diseases presents both a formidable challenge and an exciting frontier in biomedical research. While the capacity of certain bacterial proteases to disrupt the blood-brain barrier and exacerbate neuroinflammation poses a significant threat, others offer promising avenues for targeted therapeutic intervention. The ongoing development of advanced diagnostic tools and innovative therapeutic strategies, including precision-engineered proteases and nanocarrier-based delivery systems, holds the potential to revolutionize our approach to treating these debilitating conditions. As we continue to unravel the complex interplay between the microbiome, the central nervous system, and

neurodegeneration, future research should prioritize addressing key challenges related to protease specificity, safety, and regulatory compliance. Ultimately, a deeper understanding of these multifaceted interactions may pave the way for novel, patient-specific therapies that restore proteolytic balance and improve the lives of those affected by neurodegenerative disorders.

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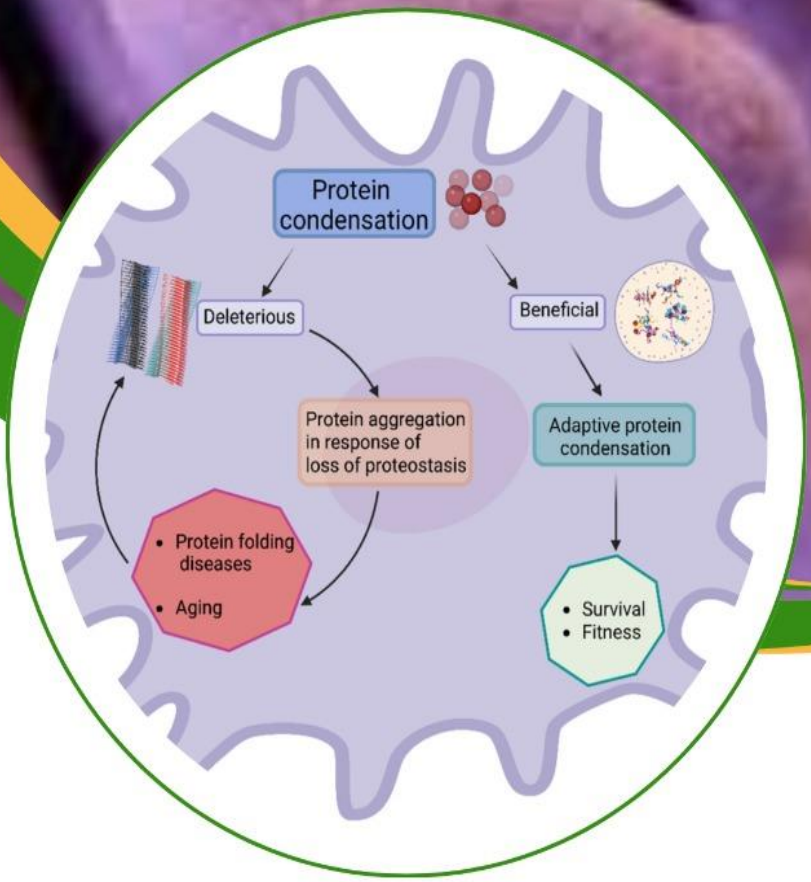

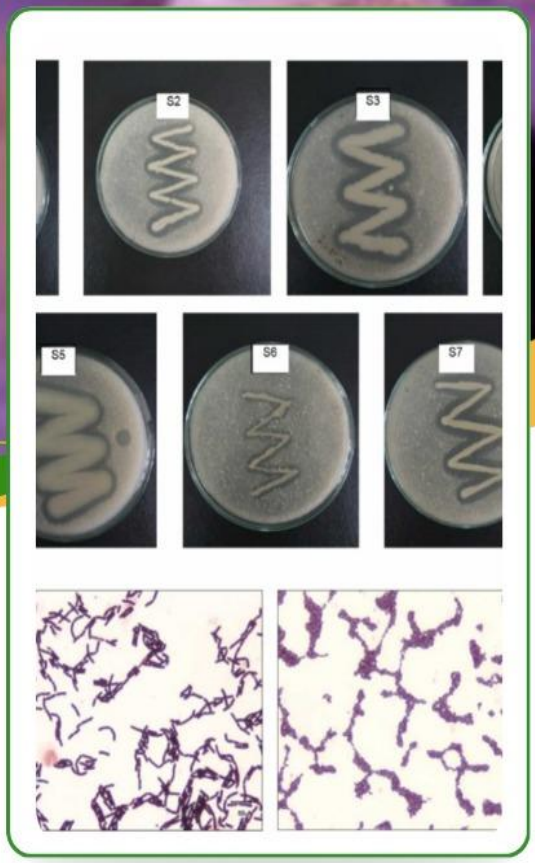

Investigating the Role of **Proteolytic Bacteria** *in Degrading Misfolded Proteins Associated with* **Neurodegenerative Disease**

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