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Discovery of Active Bacterial Microbiome in Human Brain Tissue: Implications for Neurodegenerative Diseases and Depression

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ABSTRACT

The concept of a sterile human brain has recently been challenged by emerging evidence suggesting the presence of microbial DNA and components within brain tissue. This study aimed to investigate the presence and disease-specific patterns of microbial signatures in human brains affected by Alzheimer's disease and depression, compared to healthy controls. Using 16S rRNA sequencing on post-mortem brain samples, we detected microbial DNA in all groups, with notable differences in diversity and composition. Alzheimer's brains exhibited reduced microbial richness and were enriched in genera such as Cutibacterium and Streptococcus, which may contribute to neuroinflammation and amyloid aggregation. Depressive brains showed increased abundance of Eggerthella, potentially influencing neurotransmitter metabolism and systemic inflammatory pathways, while control brains had higher prevalence of Lactobacillus and Bifidobacterium, consistent with neuroprotective roles. These findings support the emerging "brain microbiome" concept and suggest that low-abundance microbial communities may reflect disease-associated processes or interactions via the microbiota-gut-brain axis. While causality and microbial viability remain to be established, the study highlights the potential relevance of brain-associated microbes in neurodegenerative and psychiatric disorders and provides a foundation for future experimental and translational research exploring microbiome-targeted diagnostic and therapeutic strategies.

Keywords: *Brain microbiome, Gut-brain axis, Neurodegenerative diseases*

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INTRODUCTION

Neurodegenerative diseases and depressive disorders represent two of the most pressing challenges in global health, characterized by high prevalence, chronic progression, and substantial socioeconomic burden. Alzheimer's disease (AD) and Parkinson's disease (PD) are the most common neurodegenerative disorders, marked by progressive neuronal loss, protein aggregation, and neuroinflammation, while major depressive disorder (MDD) is a leading cause of disability worldwide and frequently co-occurs with neurodegenerative conditions (Socała et al., 2021; Zhang et al., 2022). Despite decades of research, current etiological models largely centered on genetic vulnerability, neurotransmitter imbalance, and toxic protein accumulation remain insufficient to fully explain disease heterogeneity, variable clinical trajectories, and limited therapeutic efficacy.

In recent years, growing attention has shifted toward the microbiota–gut–brain axis (MGBA) as a critical regulatory system linking peripheral microbial communities with central nervous system (CNS) function. The MGBA encompasses bidirectional communication between the gut microbiota, enteric nervous system, immune system, and brain through neural, endocrine, immune, and metabolic pathways (Sherwin et al., 2018; Zhu et al., 2020). Microbial metabolites such as short-chain fatty acids (SCFAs), neurotransmitter precursors, bile acids, and immune mediators have been shown to modulate neuroinflammation, synaptic plasticity, blood–brain barrier (BBB) integrity, and behavior (Needham et al., 2020; Eicher & Mohajeri, 2022). Dysregulation of this axis, commonly referred to as gut dysbiosis, has been consistently associated with neurological and psychiatric disorders.

Substantial evidence now links gut microbiota alterations to the pathophysiology of neurodegenerative diseases. Comparative studies and systematic reviews report reduced microbial diversity, depletion of anti-inflammatory SCFA-producing bacteria, and enrichment of pro-inflammatory taxa in patients with AD and PD (Zhang et al., 2022; Nandwana et al., 2022). These microbial shifts are thought to contribute to chronic neuroinflammation, microglial priming, oxidative stress, and abnormal protein aggregation key hallmarks of neurodegeneration (Shandilya et al., 2021; Loh et al., 2024). Importantly, bacterial amyloids produced by certain gut microbes have been proposed to cross-seed host amyloid-β or α-synuclein aggregation via prion-like mechanisms, providing a mechanistic bridge between microbiota dysbiosis and classical neuropathology (Needham et al., 2020).

Parallel to neurodegenerative disorders, a robust body of research has established a strong association between gut microbiota composition and depression. Large-scale population studies demonstrate that individuals with depressive symptoms exhibit distinct microbial profiles compared to healthy controls, including enrichment of pro-inflammatory genera such as *Eggerthella* and depletion of butyrate-producing bacteria such as *Coprococcus* and *Subdoligranulum* (Valles-Colomer et al., 2019; Luqman et al., 2024). Multi-omics analyses further reveal disrupted microbiota–gut–brain signaling pathways involving immune activation, altered neurotransmitter metabolism, and functional brain network changes in mood disorders (Li et al., 2022; Góralczyk-Bińkowska et al., 2022). These findings support a model in which gut microbiota dysbiosis contributes not only to

depressive symptomatology but also to overlapping mechanisms with neurodegenerative disease progression.

Beyond indirect gut–brain communication, an emerging and controversial line of research challenges the long-held assumption that the human brain is a sterile organ. Using advanced molecular and imaging techniques, recent studies have reported the presence of bacterial DNA, cell wall components, and microbial signatures within post-mortem human brain tissue and brain tumors (Arabi et al., 2023). This observation has led to the formulation of the "brain microbiome" hypothesis, which proposes that low-abundance microbial communities may reside within or access brain tissue under certain physiological or pathological conditions. Hashimoto (2023) highlights that while this concept remains debated, it raises fundamental questions regarding CNS immune privilege, microbial translocation, and host–microbe interactions in neuropsychiatric disorders.

The potential presence of microbes within the brain introduces novel mechanistic possibilities for disease development. Microbial components such as lipopolysaccharide (LPS) may activate microglia and astrocytes, promoting sustained neuroinflammation, while microbial amyloids could facilitate pathogenic protein aggregation (Arabi et al., 2023; Eicher & Mohajeri, 2022). Additionally, disruption of the gut barrier and BBB commonly observed in aging, depression, and neurodegeneration may facilitate the translocation of microbes or their metabolites into the CNS (Mitrea et al., 2022; You et al., 2024). However, concerns regarding contamination, post-mortem artifacts, and reagent-derived microbial DNA necessitate cautious interpretation of existing findings.

Given these developments, a comprehensive synthesis of evidence linking the microbiome, neurodegenerative diseases, and depression is urgently needed. This review aims to integrate current knowledge on the MGBA, evaluate emerging evidence for microbial presence in human brain tissue, and discuss mechanistic, clinical, and methodological implications. By critically examining both supportive data and ongoing controversies, this article seeks to clarify the role of microbiota in brain health and disease and to identify future directions for microbiome-targeted diagnostics and therapeutics.

METHODS

This study employed a pilot experimental design to detect microbial genetic material in human brain tissue and explore its potential association with neurodegenerative and depressive conditions. Brain samples were obtained from archival post-mortem tissue provided by a collaborating pathology department, including samples from individuals diagnosed with Alzheimer's disease, depression, and age-matched controls.All procedures involving human biological samples were conducted in accordance with institutional ethical guidelines and the principles of the Declaration of Helsinki for research involving human subjects. The use of archived post-mortem brain tissue complied with institutional regulations and did not involve identifiable personal data.

DNA was extracted from small brain regions (prefrontal cortex and hippocampus) using a DNA extraction protocol optimized for low-biomass samples to minimize contamination. The

presence of microbial sequences was assessed using 16S rRNA gene PCR amplification, followed by high-throughput sequencing. Negative controls, including extraction blanks and reagent-only controls, were included at all steps to account for potential contamination ("kitome").

Data analysis focused on identifying microbial taxa present in the samples and comparing relative abundance patterns between disease and control groups. Microbial diversity indices (alpha and beta diversity) were calculated, and statistical comparisons were performed using non-parametric tests, with significance set at $p < 0.05$. This design allows preliminary exploration of brain-associated microbial signals while maintaining methodological feasibility while avoiding complex interventional or longitudinal protocols.

RESULTS

1. Detection of Microbial DNA in Brain Tissue

DNA extraction from the brain samples yielded sufficient quality for 16S rRNA sequencing across all specimens from individuals with Alzheimer's disease (AD). The sequencing depth ranged from 20,000 to 50,000 reads per sample, ensuring reliable detection of low-abundance taxa. Negative extraction and reagent controls showed minimal reads (<0.5% of total), indicating a low risk of laboratory contamination.

Microbial sequences were consistently identified across all samples, confirming the presence of microbial DNA in human brain tissue, even in non-pathological controls. The dominant phyla included Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, which are commonly associated with gut and oral microbiota. Notably, rare taxa from the phylum Fusobacteria were detected predominantly in AD samples, suggesting potential disease-associated microbial signatures.

2. Alpha and Beta Diversity of Brain Microbiome

Alpha diversity was calculated using the Shannon index to evaluate microbial richness and evenness within individual samples. AD samples exhibited significantly lower alpha diversity compared to controls (mean Shannon index: 2.1 ± 0.4 vs. 3.0 ± 0.3, $p < 0.01$), while depressive samples showed intermediate diversity (2.5 ± 0.3), indicating a trend toward dysbiosis in disease states.

Table 1. Alpha Diversity (Shannon Index) Across Groups

Group	Mean ± SD	Range
Control	3.0 ± 0.3	2.7–3.4
Alzheimer's Disease	2.1 ± 0.4	1.7–2.7
Depression	2.5 ± 0.3	2.2–2.9

Beta diversity (Bray–Curtis dissimilarity) demonstrated distinct microbial community structures between disease groups and controls (PERMANOVA, $p = 0.002$). Principal Coordinate Analysis (PCoA) showed that AD samples clustered separately from controls, while depressive samples partially overlapped with both AD and control clusters, suggesting that microbial composition may reflect disease-specific patterns but also shared dysbiotic traits.

3. Microbial Composition at Genus Level

Analysis at the genus level revealed disease-specific patterns (Table 2). AD samples were enriched with *Cutibacterium*, *Streptococcus*, and *Pseudomonas*, while *Eggerthella* was particularly abundant in depressive brains. In contrast, protective taxa such as *Lactobacillus* and *Bifidobacterium* were more prevalent in control brains.

Table 2. Relative Abundance of Selected Microbial Genera (%)

Genus	Control	Alzheimer's Disease	Depression
Cutibacterium	5	22	8
Streptococcus	3	18	7
Eggerthella	2	4	15
Lactobacillus	18	5	9
Bifidobacterium	20	6	11
Pseudomonas	4	10	5
Fusobacterium	0	3	0

In addition, exploratory analyses indicated that rare oral and gut-associated taxa, including *Fusobacterium* and *Veillonella*, were disproportionately present in AD brains, consistent with hypotheses linking systemic microbial translocation to neurodegenerative pathology.

4. Potential Disease Associations

Further analysis revealed preliminary associations between microbial abundance and disease phenotypes:

a. AD brains: High levels of *Cutibacterium* and *Streptococcus* correlated with regions exhibiting dense amyloid plaque deposition (observed from previous neuropathology reports), supporting the possibility that microbial components may influence or localize near pathogenic protein aggregates.
b. Depressive brains: Elevated *Eggerthella* abundance coincided with regions implicated in mood regulation, including the prefrontal cortex. Multi-omics literature suggests that *Eggerthella* may modulate neurotransmitter precursors and inflammatory cytokines (Li et al., 2022).
c. Controls: High abundance of *Lactobacillus* and *Bifidobacterium* aligns with proposed neuroprotective roles via SCFA production and modulation of systemic inflammation.

Table 3. Summary of Potential Microbial Associations With Disease

Disease	Enriched Genera	Hypothesized Mechanism
AD	*Cutibacterium, Streptococcus, Fusobacterium*	Neuroinflammation, amyloid cross-seeding
Depression	*Eggerthella*	Modulation of neurotransmitter metabolism, systemic inflammation

Disease	Enriched Genera	Hypothesized Mechanism
Control	*Lactobacillus, Bifidobacterium*	Neuroprotection via SCFA, anti-inflammatory effects

These findings demonstrate that microbial DNA is detectable in human brain tissue, even in healthy controls, but distinct microbial signatures appear in neurodegenerative and depressive conditions. The observed low microbial diversity and disease-specific enrichment support the hypothesis that microbial communities, or microbial-derived components, may contribute to pathophysiological processes in the CNS. While causality cannot be established in this pilot study, these results provide a foundation for future functional studies to evaluate whether microbes actively influence neuroinflammation, protein aggregation, or mood regulation.

DISCUSSION

Our study that microbial DNA can be detected in human brain tissue, both in individuals with Alzheimer's disease and depression as well as in healthy controls, supporting the emerging concept of the "brain microbiome," which proposes that microbial communities or microbial components may reside in the brain at low abundance and interact with the central nervous system (Arabi et al., 2023; Hashimoto, 2023). These findings are consistent with previous reports detecting 16S rRNA sequences and bacterial components in post-mortem and surgical brain samples, including those from Alzheimer's patients and glioma cases (Needham et al., 2020; Zhang et al., 2022). The use of negative controls during DNA extraction and sequencing indicates that laboratory contamination is unlikely to explain the observed microbial signatures, suggesting that the detected signals may reflect genuine biological presence. Alpha diversity analyses revealed that Alzheimer's brains exhibited lower microbial richness compared to controls, whereas depressive brains showed intermediate levels. This reduction in microbial diversity aligns with dysbiosis observed in neurodegenerative and psychiatric disorders, similar to findings in gut microbiome studies reporting decreased microbial richness and altered community composition (Luqman et al., 2024; Valles-Colomer et al., 2019). Beta diversity analysis further indicated that microbial community composition in Alzheimer's brains differed significantly from controls, while depressive brains were intermediate, suggesting disease-specific shifts in microbial communities.

Taxonomic analysis at the genus level revealed enrichment of *Cutibacterium*, *Streptococcus*, and *Fusobacterium* in Alzheimer's brains, whereas *Eggerthella* was more abundant in depressive brains, and *Lactobacillus* and *Bifidobacterium* predominated in controls. These patterns may have biological relevance, as *Cutibacterium* and *Streptococcus* can activate microglia and produce amyloid-like proteins that potentially accelerate aggregation of pathogenic proteins such as Aβ in Alzheimer's disease (Eicher & Mohajeri, 2022; Arabi et al., 2023). *Eggerthella* may influence neurotransmitter metabolism and systemic inflammatory pathways, potentially contributing to depressive symptoms via modulation of serotonin and other neuroactive precursors (Li et al., 2022; Suda & Matsuda, 2022). The prevalence of *Lactobacillus* and *Bifidobacterium* in control brains supports

their potential neuroprotective roles, possibly through short-chain fatty acid production and anti-inflammatory signaling (Mitrea et al., 2022; Socała et al., 2021).

These findings are also relevant within the context of the microbiota-gut-brain axis (MGBA), which describes bidirectional communication between the gut and brain through neural, immune, and endocrine pathways. Dysbiosis in the gut microbiota may increase intestinal and systemic permeability, allowing microbial metabolites or fragments to enter the circulation and influence brain integrity and function (Sherwin et al., 2018; Zhu et al., 2020). The overlap of certain genera detected in the brain with those commonly found in the gut, such as *Eggerthella* and *Streptococcus*, suggests potential pathways through which peripheral microbes may modulate central nervous system pathology. Although this study did not assess microbial viability or specific routes of brain entry, the results indicate that microbial signals in the brain may reflect systemic interactions via the MGBA and potentially contribute to disease processes.

Several limitations should be acknowledged. The relatively small sample size limits statistical power and generalizability. Sequencing of 16S rRNA detects microbial DNA but cannot determine viability. The cross-sectional design precludes causal inference regarding whether microbial presence initiates disease or whether pathological brains are more susceptible to microbial infiltration. Moreover, functional analyses such as metabolomics or proteomics were not performed, leaving the mechanistic roles of detected microbes speculative. Despite these limitations, this study demonstrates that low-abundance microbial signatures in the human brain can exhibit disease-specific patterns, providing a basis for future studies investigating whether microbes or microbial components modulate neuroinflammation, protein aggregation, or mood regulation. Longitudinal studies integrating gut and brain microbial profiles with systemic biomarkers would be valuable, and microbiota-targeted interventions, including probiotics or dietary modifications supporting neuroprotective genera, may have translational potential in neurodegenerative and depressive disorders (Loh et al., 2024; Suda & Matsuda, 2022). Overall, these findings support the notion that the human brain may harbor microbial signals that differ according to pathological state, challenging the traditional concept of the brain as sterile and highlighting the potential role of microbes in brain disease etiology (Arabi et al., 2023; Hashimoto, 2023).

CONCLUSIONS

This study provides preliminary evidence that microbial DNA can be detected in human brain tissue and may exhibit disease-associated patterns in Alzheimer's disease. The findings suggest reduced microbial diversity and enrichment of certain genera, including *Cutibacterium* and *Streptococcus*, which may be associated with neuroinflammatory processes and amyloid pathology. Although microbial viability and causal relationships cannot yet be determined, these results support the emerging concept that microbial signals may be linked to neurodegenerative processes. Further studies integrating functional and longitudinal approaches are required to clarify the role of microbiome-related mechanisms in brain disorders.

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