Salivary Microbiota Composition in Parkinson's Disease: A Pilot Study Using Nanopore Full-Length 16S rRNA Sequencing

Parkinson's disease (PD) has been associated with microbial alterations along the gut-brain axis, and recent studies have extended this investigation to the oral cavity. Most oral microbiome studies in PD have employed short-read 16S sequencing, limiting taxonomic classification to the genus level. In this pilot study, we aimed to characterize the salivary microbiota of PD patients at species-level resolution using full-length 16S nanopore sequencing. Unstimulated saliva samples were collected from 19 PD patients and 19 controls. Full-length 16S rRNA amplicons were sequenced on the Oxford Nanopore platform and classified using the EMU algorithm against the Human Oral Microbiome Database. Alpha diversity was assessed using Chao1, Shannon, Fisher, and Inverse Simpson indices, and beta diversity using both Aitchison and Bray-Curtis distances with PERMANOVA, adjusting for age and sex as covariates. Differential abundance was evaluated using four methods (LEfSe, MaAsLin2, ANCOM-BC and ANOVA), retaining only consensus taxa. No significant differences in alpha diversity were observed between PD and control groups ($p>0.05$). Beta diversity analysis revealed a significant compositional difference between groups after adjusting for age and sex (Aitchison: $R^2=0.041$, $p=0.037$; Bray-Curtis: $R^2=0.062$, $p=0.019$), while neither age nor sex showed a significant effect. Six species were consistently less abundant in PD patients: Prevotella melaninogenica, Peptostreptococcus stomatis, Lachnoanaerobaculum gingivalis, Hoylesella nanceiensis, Corynebacterium durum, and Catonella morbi. Two species, Streptococcus mutans and Veillonella sp. HMT-917, were more abundant in the PD group. These findings suggest a compositional shift in the salivary microbiota of PD patients, with full-length 16S nanopore sequencing enabling species-level resolution beyond short-read approaches. However, the small sample size and cross-sectional design limit interpretation. Moreover, because all PD patients were medicated and key oral microbiome-related confounders were not assessed, it remains unclear whether the observed changes reflect disease-related or confounding effects. Future longitudinal studies are needed to assess their potential as non-invasive biomarkers for PD.

Keywords: *Parkinson's disease, oral-gut-brain axis, nanopore full-length 16S sequencing, salivary microbiota*

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide after Alzheimer's disease (Hou et al., 2021; Kalia and Lang, 2015), pathophysiologically defined by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of intracytoplasmic α-synuclein aggregates (Lewy bodies) (Morris et al., 2024; Poewe et al., 2017). Although long classified primarily as a movement disorder, PD is now recognized as a multisystem neurodegenerative process encompassing not only motor deficits but also autonomic dysfunction, cognitive impairment, neuropsychiatric symptoms, and gastrointestinal involvement (Bloem et al., 2021). This systemic dimension is understood to unfold through a broad pathophysiological network extending well beyond the central nervous system, including the peripheral nervous system, immune system, and mucosal surfaces (Peng et al., 2022).

In this context, the microbiota-gut-brain axis has emerged as a growing area of interest in the pathogenesis of PD. Dysbiosis of the gut microbiota has been proposed to increase intestinal permeability, trigger systemic and neuroinflammation, and potentially initiate α-synuclein misfolding in the enteric nervous system (Menozzi et al., 2025; Tan et al., 2022). Within the framework of the Braak staging hypothesis, α-synuclein pathology is thought to originate in the enteric nervous system and propagate in a caudal-to-rostral direction to the central nervous system via the vagus nerve, positioning gut microbiota as a key component in the early pathogenesis of PD (Braak and Del Tredici, 2016).

The oral cavity, as the anatomical gateway to the gastrointestinal tract, harbors more than 770 bacterial species and continuously seeds the gut microbiome through daily swallowing (Berthouzoz et al., 2023). This anatomical continuity positions the oral microbiota as a potential upstream determinant of gut dysbiosis. Oropharyngeal manifestations frequently observed in PD, including dysphagia, hypersalivation, and reduced masticatory efficiency, are considered disease-specific factors that may directly alter oral microbiota composition (Arıkan et al., 2023; Rei et al., 2024).

Emerging studies indicate significant alterations in the composition and function of the oral microbiome in PD patients. Notable differences have been reported in several bacterial genera, particularly *Prevotella*, *Veillonella*, and *Streptococcus*, with these changes proposed to be associated with mucosal inflammation, peripheral immune dysregulation, and neuroinflammatory processes. Oral microbiome profiles in early-stage PD patients have been reported to exhibit distinguishing characteristics from healthy individuals, potentially applicable to non-invasive biomarker development strategies (Stagaman et al., 2024). However, the majority of existing studies rely on short-read 16S rRNA sequencing, which typically constrains taxonomic classification to the genus level (Arıkan et al., 2023; Rei et al., 2024). Species-level resolution is nonetheless critical for distinguishing phylogenetically closely related taxa, pathogen identification, and biomarker specificity.

Full-length 16S rRNA sequencing, by enabling the simultaneous coverage of all hypervariable regions (V1-V9) of the 16S rRNA gene in a single read, offers direct species-level taxonomic resolution compared to short-read approaches (Aja-Macaya et al., 2025). Nevertheless, studies systematically applying this approach to oral microbiota research in PD remain highly limited. In this pilot study, we aimed to characterize the salivary microbiota of PD patients at species-level resolution using full-length 16S nanopore sequencing. Alpha and beta diversity analyses were performed with statistical adjustment for age and sex covariates, and differential abundance analysis was conducted using a multi-method approach retaining only taxa showing consistent significance across methods.

Methodology

Study Design and Participants

This study was designed as a cross-sectional pilot study. Nineteen PD patients aged 40–80 years meeting the International Parkinson and Movement Disorder Society (MDS) clinical diagnostic criteria, and 19 age- and sex-matched healthy controls were included. The study was conducted in accordance with the principles of the Declaration of Helsinki, and written informed consent was obtained from all participants.

Saliva Sample Collection and DNA Isolation

Unstimulated saliva samples were collected according to a standard protocol and stored at −80°C until DNA isolation. Following genomic DNA extraction, full-length amplification covering all hypervariable regions (V1-V9) of the 16S rRNA gene was performed and sequenced on the Oxford Nanopore platform.

Bioinformatic and Statistical Analysis

Taxonomic classification was performed using the EMU algorithm and the Human Oral Microbiome Database (HOMD). Alpha diversity was assessed using Chao1, Shannon, Fisher, and Inverse Simpson indices, while beta diversity was evaluated using Aitchison and Bray-Curtis distance metrics. Compositional differences between groups were tested using PERMANOVA with adjustment for age and sex covariates. Differential abundance analysis was conducted using LEfSe, MaAsLin2, ANCOM-BC, and ANOVA; only taxa showing consistent significance across multiple methods were defined as the consensus threshold.

Results

Demographic Characteristics

The PD group was significantly older than the control group (median age 62.0 vs. 51.0 years; p=0.001). No significant difference was observed in sex distribution between groups (p=0.737) (Table 1).

Table 1. *Demographic characteristics of Parkinson's disease patients and healthy controls*

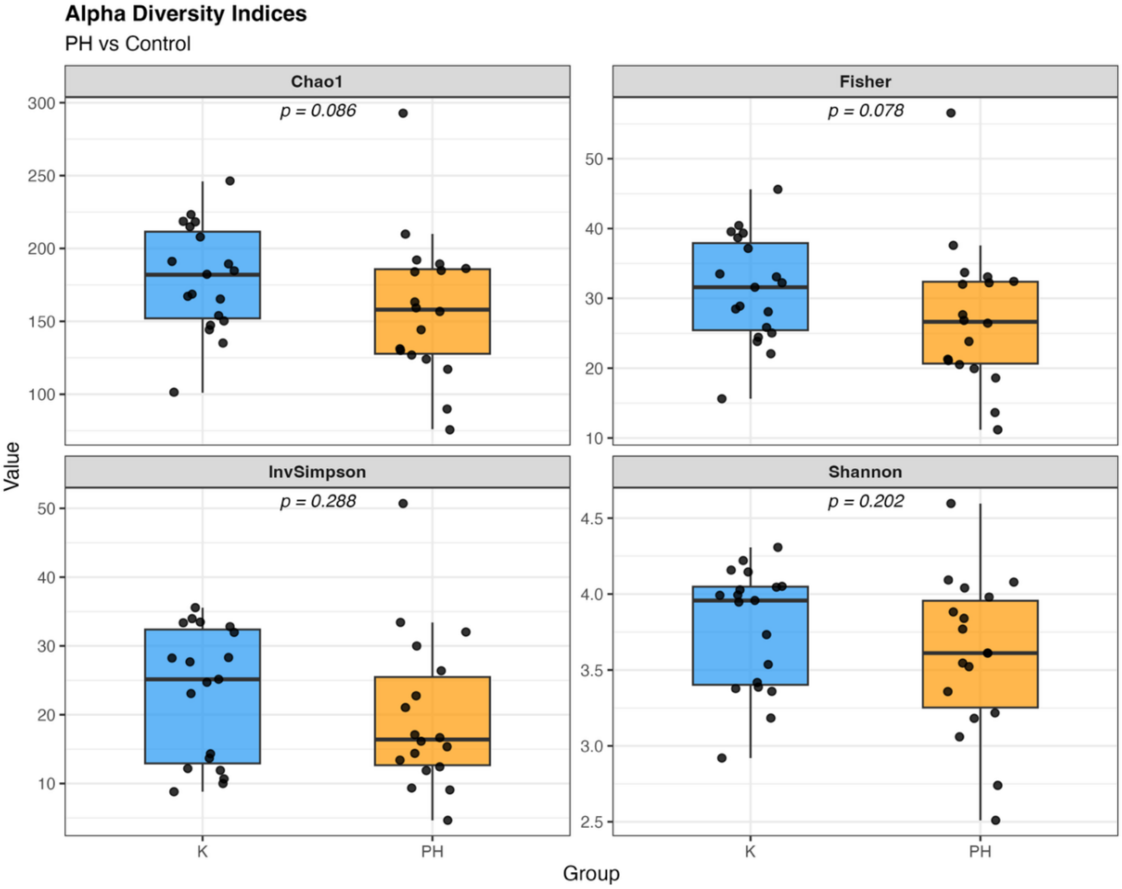
Variable	PD (n=19)	Control (n=19)	p value
Age, median (IQR)	62.0 (59.0–65.0)	51.0 (44.5–59.5)	0.001*
Sex, n (%)			0.737
Male	13 (68.4%)	10 (52.6%)	
Female	6 (31.6%)	9 (47.4%)	

*IQR: Interquartile range. *Mann-Whitney U test; †Chi-square test. p<0.05 considered statistically significant.*

Alpha Diversity

Alpha diversity analysis using Chao1, Fisher, Shannon, and Inverse Simpson indices revealed no statistically significant difference between the PD and control groups (p=0.086, p=0.078, p=0.202, and p=0.288, respectively). Similar species richness and evenness values were obtained in both groups (Figure 1).

Figure 1. *Alpha diversity indices (Chao1, Fisher, Shannon, and Inverse Simpson) comparing Parkinson's disease (PH) and control (K) groups*

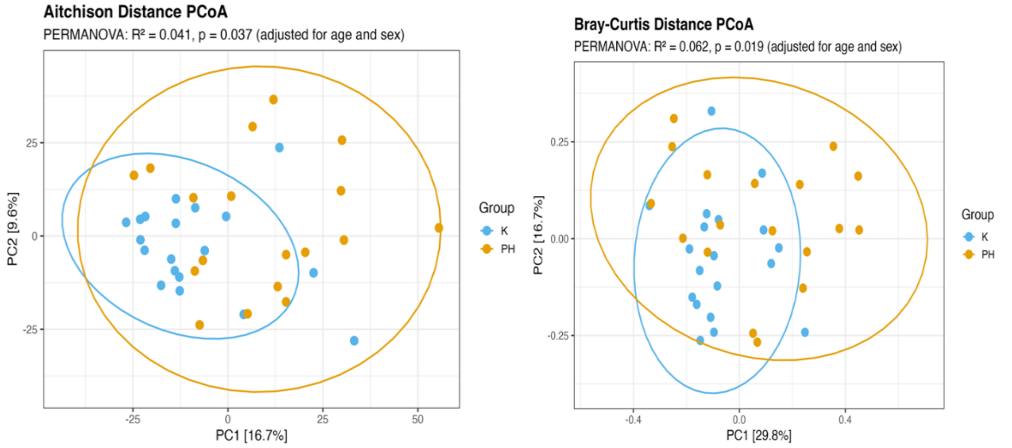


Boxes represent interquartile range, horizontal lines indicate median values, and dots represent individual data points. No statistically significant difference was observed between groups for any index (Chao1: p=0.086; Fisher: p=0.078; Shannon: p=0.202; Inverse Simpson: p=0.288).
Source: Created by authors.

Beta Diversity

PERMANOVA analysis, after adjusting for age and sex covariates, revealed a statistically significant compositional difference between the PD and control groups. Aitchison distance-based analysis identified a significant separation explaining 4.1% of intergroup variance (R^2=0.041, p=0.037). Bray-Curtis distance-based analysis yielded 6.2% explained variance (R^2=0.062, p=0.019). Neither age nor sex covariates showed an independent significant effect on microbiota composition (Figure 2).

Figure 2. *Beta diversity analysis using Aitchison Distance PCoA and Bray-Curtis Distance PCoA*



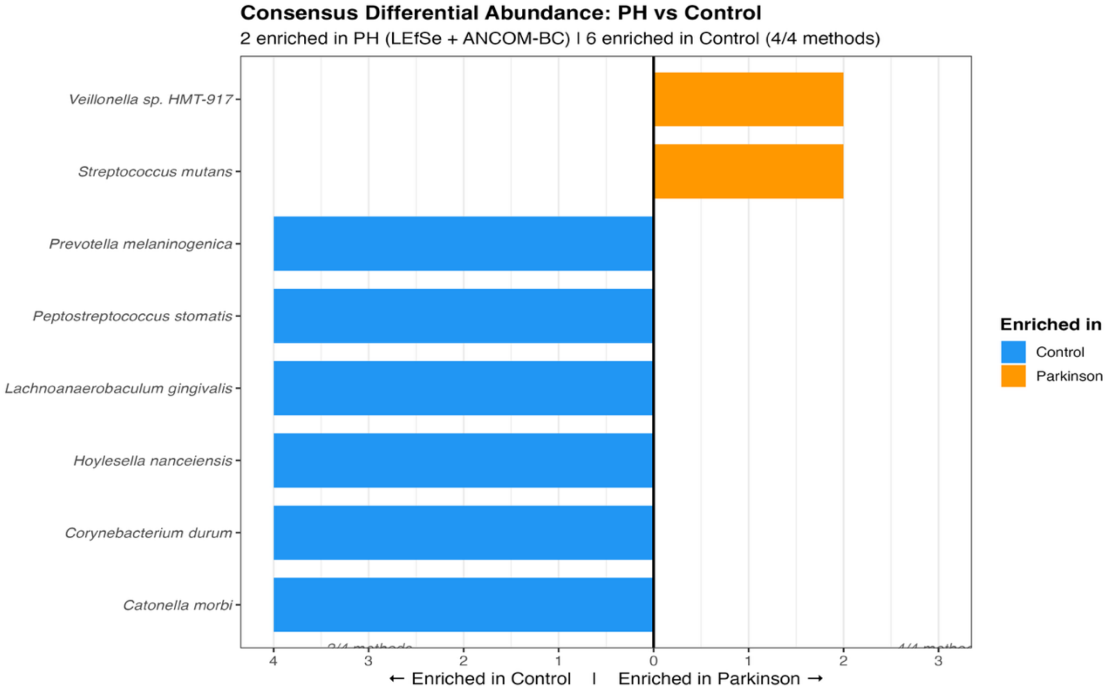
Ellipses represent 95% confidence intervals for each group. (Aitchison: R^2=0.041, p=0.037; Bray-Curtis: R^2=0.062, p=0.019).

Source: Created by authors.

Differential Abundance Analysis

Using a multi-method consensus approach, eight bacterial species with differential relative abundance between groups were identified. Six species enriched in the control group were consistently significant across all four methods (LEfSe, MaAsLin2, ANCOM-BC, and ANOVA): *Prevotella melaninogenica*, *Peptostreptococcus stomatis*, *Lachnoanaerobaculum gingivalis*, *Hoylesella nanceiensis*, *Corynebacterium durum*, and *Catonella morbi*. Two species enriched in the PD group showed consistent significance in two of four methods (LEfSe and ANCOM-BC): *Streptococcus mutans* and *Veillonella* sp. HMT-917 (Figure 3).

Figure 3. *Consensus differential abundance analysis comparing Parkinson's disease (PH) and control groups*



Source: Created by authors.

Discussion

In this pilot study, the salivary microbiota of PD patients was characterized at species-level resolution using full-length 16S nanopore sequencing. No significant difference in alpha diversity was detected between groups across any of the indices assessed. Beta diversity analysis, however, revealed significant compositional differences between groups using both Aitchison and Bray-Curtis metrics after adjusting for age and sex covariates, suggesting that PD is associated with a systemic process affecting salivary microbiota composition, a finding consistent with previous studies (Arıkan et al., 2023; Rei et al., 2024).

Among the species detected at reduced relative abundance in PD patients, *Prevotella melaninogenica* is particularly noteworthy. This anaerobic commensal is known to colonize the oral mucosa from early life and is considered a core member of the healthy oral microbiome (Könönen and Gursoy, 2022). Its relative reduction in the PD group is consistent with a disruption of commensal oral anaerobic balance during the disease process. Similarly, other species enriched in the control group — including *Peptostreptococcus stomatis*, *Catonella morbi*, *Corynebacterium durum*, *Lachnoanaerobaculum gingivalis*, and *Hoylesella nanceiensis* — are recognized members of the oral microbiome, although their functional roles remain to be fully elucidated. Notably, a significant reduction of *Hoylesella nanceiensis* in PD patients compared to healthy controls has been previously reported, further supporting the compositional shift observed in the present study (Zapała et al., 2022). Collectively, the enrichment of these taxa in the control group indicates a reorganization of oral microbiota composition in PD. Regarding species enriched in the PD group, increased relative abundance of *Streptococcus mutans* in PD patients has been previously reported (Fleury et al., 2021), suggesting the presence of oral dysbiosis and local inflammation that may be linked to disease pathogenesis. *Veillonella* sp. HMT-917 was also found to be more abundant in PD patients; however, its potential role in disease pathogenesis remains to be elucidated.

The main limitations of this study include its small sample size, cross-sectional design, and the fact that all PD patients were medicated. The inability to systematically assess important confounders such as oral hygiene, diet, and dental disease status makes it difficult to determine whether the observed changes are related to the disease, medication use, or other factors.

Conclusions

This pilot study demonstrates compositional differences at the species level in the salivary microbiota of PD patients using full-length 16S nanopore sequencing. The observed beta diversity differences and differential abundance findings point to a potential role of oral microbiota in the systemic pathophysiology of PD. However, the small sample size, cross-sectional design, and unassessed confounders limit the interpretation of findings. Larger, longitudinal studies with systematic control of confounders are needed to clarify whether the observed microbiota changes are related to disease, medication, or other factors, and to determine whether salivary microbiota can serve as non-invasive biomarkers for PD.

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