Journal of Dentistry 163 (2025) 106109

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Salivary microbiota in children with and without type 1 diabetes mellitus: A one-year follow-up study

Neslihan Yilmaz [a,b,*], Ulvi Kahraman Gürsoy [a], Daniel Belstrøm [c], Recep Polat [d], Mervi Gürsoy [a,e]

[a] Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland
[b] Department of Pediatric Dentistry, Faculty of Dentistry, Sakarya University, Sakarya, Turkiye
[c] Section for Clinical Oral Microbiology, Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[d] Department of Pediatric Endocrinology, Faculty of Medicine, Sakarya University, Sakarya, Turkiye
[e] The Wellbeing Services County of Southwest Finland, Turku, Finland

ARTICLE INFO

Keywords:
Microbiota
Saliva
Diabetes mellitus
Children
16S rRNA

ABSTRACT

Objective: Longitudinal data on the composition of salivary microorganisms in type 1 diabetes mellitus (T1DM) patients are lacking. This study aimed to characterize and compare the salivary microbiota of children with and without T1DM in a longitudinal approach. We hypothesized that the bacterial composition in saliva differs between healthy and T1DM children in a 1-year period.
Methods: Overall, 55 children (4–15 years old; 26 with T1DM, 29 healthy controls) completed the study. Oral examinations (plaque index, bleeding on probing, and Decayed, Missing, Filled Teeth index) and unstimulated saliva sampling were performed at baseline and after 1 year. Microbial composition was assessed via 16S rRNA gene sequencing (V1-V3 region) and referenced against the Human Oral Microbiome Database.
Results: Beta diversity analysis (Principal coordinate analysis (PCoA)) showed greater separation between groups at baseline than at follow-up. Linear discriminant analysis effect size identified that T1DM was associated with *Fusobacterium* species, whereas *Rothia* species associated with health. Alpha diversity indexes (Chao 1, Shannon and Simpson) showed no significant differences between the groups ($P > 0.05$).
Conclusion: Our results demonstrated that the salivary microbiota of T1DM children is significantly distinct from healthy controls during 1-year of follow-up. Future studies are needed to reveal whether improved T1DM management benefits microbial composition.
Clinical significance: The microbial shift in diabetic children may contribute to increased susceptibility to oral diseases, highlighting the importance of preventive dental care in this population.

1. Introduction

The oral microbiota is one of the most complex microbial communities in human body [1]. Recent advances in OMICs technologies have enhanced our knowledge of this diverse ecosystem and paved the way to identify a substantial proportion of microbial species in the oral cavity [2]. Its composition is shaped by the ecological conditions within the oral environment, influenced by oral health, and potentially also by general health status [3,4].

Type 1 diabetes mellitus (T1DM), which is a complex disorder [5], is the most prevalent form of diabetes among young individuals, representing over 90 % of childhood diabetes in most westernized countries [6], with an estimation of over one million children and adolescents under the age of 20 globally [7]. Data indicate that those with T1DM, particularly individuals with poorer glycemic control and longer duration of diabetes, have increased susceptibility to periodontal diseases [8]. In addition, studies have reported that diabetic children have more clinical signs of periodontal disease, including higher plaque index (PI), gingival index (GI), bleeding on probing (BOP), periodontal pocket depth (PPD), and clinical attachment loss (CAL) scores, compared to healthy children [9,10].

While the clinical impact of T1DM on periodontal disease

* Corresponding author.
E-mail addresses: neyilm@utu.fi (N. Yilmaz), ulvi.gursoy@utu.fi (U.K. Gürsoy), dbel@sund.ku.dk (D. Belstrøm), receppolat@hotmail.com (R. Polat), mervi.gursoy@utu.fi (M. Gürsoy).

https://doi.org/10.1016/j.jdent.2025.106109
Received 27 June 2025; Received in revised form 8 September 2025; Accepted 12 September 2025
Available online 13 September 2025

susceptibility is well described in the literature[11], the potential effect of T1DM on oral microbiota remains controversial. This controversy largely arises from variations in study populations such as age, disease duration, glycemic control, and geographic location as well as methodological differences, including sampling protocols and sequencing platforms. Consequently, there is currently no compelling evidence supporting a causal link between the composition of periodontal microbiota and the presence of T1DM [12,13]. Most studies focusing on the relationship between diabetes and oral microbiome have been performed in adult T2DM patients with or without periodontitis[14–17], whereas only limited data are available in children and adolescents with T1DM [18–21].

According to the literature, especially the acute phase of T1DM is characterized by dysbiosis of the salivary microbiota [22] and studies have reported distinct microbial compositions in children with T1DM compared to their healthy counterparts [19]. Although previous studies indicated that the oral microbiota in T1DM patients may differ from that of systemically healthy individuals, the majority have employed cross-sectional designs, thereby limiting the ability to assess temporal microbial dynamics. Given the chronic and dynamic nature of T1DM, a follow-up design allows for the evaluation of whether the microbial composition shifts in response to disease progression or variation in systemic status. Therefore, the aim of the present study was to characterize and compare the salivary microbial profile of children and adolescents with and without T1DM based on paired saliva samples collected over a 1-year period. We tested the hypothesis that the salivary microbiota composition would differ between healthy and T1DM children and adolescents across 1-year follow-up period.

2. Methods

2.1. Study population

The present study is an observational prospective study, in which the patient recruitment was conducted from January 2021 to February 2022. The inclusion criteria were as follows: (1) age between 4–15 years; (2) diagnosis of T1DM by a pediatric endocrinologist at least 6 months prior to the inclusion; (3) no systemic diseases (for the systemically healthy control group). All individuals with any of the following conditions were excluded: (1) systemic complications related to diabetes, such as neuropathy, kidney disease, celiac disease, cardiovascular disease, and obesity; (2) a history of orthodontic treatment, (3) professional periodontal therapy within the 6 months prior to the study, and (4) use of any antimicrobial drugs 3 months prior and during the study. The research protocol was approved by the Ethical committee of Sakarya University Faculty of Medicine (protocol number: E-16,214,662–050.01.04–310,642–170) and was performed in full accordance with Declaration of Helsinki, as revised in 2013.

The current study was part of a larger follow-up project, in which the aim was to profile the oral macrophage/T-cell response and microbial composition of T1DM patients [10]. According to the study protocols, all T1DM patients at the Sakarya University, Faculty of Medicine, Department of Pediatric Endocrinology received an invitation to participate to the study. Those who were willing to participate to the study and fit to the inclusion criteria were recruited. As the main study included both immunological and microbiological analyses, the initial sample size calculation was performed using salivary chemokine levels for a two-year-follow-up design. The sample collection for the microbiota analyses terminated after the completion of 1-year follow-up due to high dropouts, and thus present study includes all participants ($n = 55$), who completed the 1-year follow-up. The case group was formed of 26 T1DM patients, who had been using intramuscular insulin injections for their diabetic treatment. The control group was comprised by 29 children and adolescents, who were referred to Sakarya University, Faculty of Dentistry, Department of Pediatric Dentistry for routine dental examinations. All study participants and their parents/guardians received comprehensive verbal disclosures regarding the research protocol and objectives, and each parent/guardian approved the participation by signing a consent form, while children and adolescents provided their assent verbally. Demographic variables, including age, sex, medical and dental treatment history, diabetes duration, and current medications of diabetic participants, were obtained by interviews. Inhalation of environmental tobacco smoke was defined as passive smoking [23]. Hence, individuals whose parent(s) engaged in regular or daily smoking at home were categorized as passive smokers.

2.2. Clinical and medical examination

Periodontal parameters including PI %, BOP %, PPD, and CAL were measured from all sites and recorded at four sites per tooth (buccal, lingual/palatal, mesial, and distal surfaces) by using a manual periodontal probe (PW7, Hu-Friedy, IL, US) excluding primary teeth with mobility. Dental caries status of the participants was assessed using the decayed, missing, and filled teeth (dmft/DMFT) index for primary and permanent teeth, respectively. All clinical examinations were conducted by a single calibrated (kappa: 0.88) pediatric dentist (NY). Gingivitis was diagnosed according to the 2017 Classification of Periodontal and Peri-implant Diseases and Conditions [24]. Briefly, participants were diagnosed as having gingivitis in the presence of BOP $\geq$10 % and PPD $\leq$ 3 mm.

An expert pediatrician (RP) performed metabolic examinations of participants. According to the International Society for Pediatric and Adolescent Diabetes (ISPAD) Clinical Practice Consensus Guidelines 2022 [6], individuals with fasting plasma glucose level of $\geq$126 mg/dl (7.0 mmol/l; fasting defined as no caloric intake for $\geq$8 h) and HbA1c $\geq$6.5 % (48 mmol/mol) were diagnosed as having T1DM. Data regarding the therapeutic regimen, duration of diabetes, and HbA1c were obtained from medical records.

After the baseline examination, detailed oral hygiene instructions were delivered and supragingival prophylaxis was performed. Control visit was performed 1 year after the baseline examination. As none of the participants suffered from periodontitis, professional periodontal treatment was not performed to any study participant during 1-year follow-up period.

2.3. Saliva sampling

Unstimulated saliva samples were collected from all participants at baseline and at 1-year follow-up. Samples were collected before the clinical and metabolic examination between 8 and 11 AM by the same pediatric dentist (NY). Participants refrained from eating, drinking, and tooth brushing for a minimum of 1 h prior to sample collection. Saliva sampling was performed by spitting for 5 min and filling Eppendorf tubes (5 ml). All samples were immediately frozen and stored at −80 °C until further analyses.

2.4. DNA extraction, library preparation and DNA sequencing

DNA extraction, library preparation, and DNA sequencing were performed as previously described in detail [25,26]. In brief, sequencing libraries for the bacteria 16S rRNA gene variable regions 1–3 (bV13-A) were prepared using a custom protocol based on Caporaso et al [27]. Following quality control, only samples exceeding 10,000 reads were selected for downstream analysis. In this project, all samples were included in the subsequent analyses.

2.5. Bioinformatics processing and statistics

The generated sequences were taxonomically classified by comparison with reference sequences in the extended Human Oral Microbiome Reference Sequence Database (eHOMD) version 15.2 [28], allowing for identification at the highest possible taxonomic level. All data were

assessed for normality using box plots, histograms, and skewness and kurtosis z-scores. Data distribution of PI % and dmft/DMFT were normal, and thereby, the data are presented as means ± standard deviations. All categorical variables were evaluated by Chi-Square tests and are given as frequencies and percentages. Data distributions of BOP % were skewed. Intragroup comparisons were performed either using parametric independent *t*-tests or with the nonparametric Mann-Whitney U test, depending on the data distribution of the tested parameter. A *P* value of <0.05 was considered statistically significant.

The salivary microbiota was characterized and compared by relative abundance, alpha diversity, and beta diversity (visualizing of data by Principal coordinate analysis (PCoA) based on Bray-Curtis distance). Alpha diversity measurement was performed according to richness (Chao 1) and diversity (Shannon and Simpson index). Differences in alpha diversity were evaluated by independent *t*-test, as the data met the assumptions of normality. Linear discriminant analysis effect size (LEfSe) was used to identify the most discriminatory bacterial genera and species among the groups [29]. LEfSe analysis was performed using version 1.1.2 of the tool via the Bioconda distribution on a local server. The analysis was applied with default settings to the raw sequence count tables augmented with experiment metadata for groupings. Significant differences were identified by the combined criteria of Kruskal-Wallis $P < 0.05$, Wilcoxon *P*-adjusted< 0.05, and Linear Discriminant Analysis (LDA) score > 2.

3. Results

3.1. Characteristics of study participants

A population of 26 T1DM patients (57 % female, 43 % male) with mean (±SD) age of 10.7 ± 3.1 years and 29 systemically healthy individuals (34.5 % female, 65.5 % male) of 9.2 ± 2.5 years, respectively, completed the study. Table 1 presents the demographic, medical, and periodontal characteristics of the study participants at each assessment point. The mean diabetes duration was 3.07±2.7 years for participants with T1DM. At baseline, the T1DM group had significantly higher mean PI % ($P = 0.019$) than healthy controls, and at 1-year follow-up mean BOP % was significantly higher in the T1DM group ($P = 0.001$). There was no CAL or PPD higher than 3 mm in any of the participants at each assessment point. No significant difference was detected in passive smoking rates between the two groups at baseline and 1-year follow-up ($P = 0.635$).

Table 1
Demographic, medical, and periodontal characteristics of the study participants.

	Baseline		P value	1 Year Follow-up		P value
	T1DM (n = 26)	Healthy (n = 29)		T1DM (n = 26)	Healthy (n = 29)	
Age (years)	12 (9.4, 12)	9 (8.2, 10.2)	0.061*	13 (10.4, 13)	10 (9.2, 11.2)	0.06*
Female (%)	57.7 %	34.5 %	0.114#	57.7 %	34.5 %	0.114#
Passive Smoker (%)	53.8 %	41.4 %	0.635#	53.8 %	41.4 %	0.635#
Duration of T1DM (years)	3.07 ±2.7	–	–	3.5 ± 2.7	–	–
HbA1c (%)	9.13 ±2.9	–	–	9.18 ±2.4	–	–
PI (%)	44.2 ± 24	30.7 ± 16.7	**0.019***	42 ±22.7	32.7 ± 16.4	0.088*
BOP (%)	12 ±13.7	5.3 ± 7.9	0.064¶	14.1 ± 17.6	4.2 ± 5.9	**0.001**¶
Gingivitis (%)	42.3 %	20.7 %	**0.004**#	53.8 %	13.8 %	**0.004**#
dmft/DMFT	7.6 ± 3.7	9.6 ± 3.9	0.066*	9.8 ± 4.1	9.1 ± 3.4	0.515*

Bold P values indicate statistically significant difference.
* Independent t-test.
# Chi-Square.
¶ Mann-Whitney U.

3.2. Sequencing metadata

DNA extraction and sequencing library preparation was successful for all samples analyses (100 %) and yielded between 21,824 and 651,429 DNA reads after QC and bioinformatic processing. A total of 3700 OTUs were identified from all samples with a total of 392 different bacterial species and 107 bacterial genera identified corresponding to 63 % and 97.7 % coverage of generated sequences, respectively. Sample sequencing rarefaction curves for evaluation of the obtained sequencing depth relative to sample complexity are represented in Supplementary File 1, indicating that the sequencings depth of the study was sufficient.

3.3. Microbial diversity

Alpha diversity of T1DM and healthy controls was compared using Chao1, Shannon, and Simpson index at baseline and 1 year follow-up. No statistically significant differences were found between groups at both sampling time ($P > 0.05$) (Supplementary File 2). As per the beta diversity analysis, PCoA utilizing the two principal components (PCo1 and PCo2), which collectively represented about 15 % of the dataset, revealed a more distinct separation between the healthy and T1DM groups at the baseline compared to the 1-year follow-up (Fig. 1A, B).

3.4. Taxonomic profiles

The relative abundance of top 20 predominant bacterial phyla, genera and species in both groups during the follow-up is presented in Supplementary File 3, Figs. 2 and 3, respectively. The relative abundance of predominant *Streptococcus* species and *Prevotella* species are demonstrated in Supplementary File 4. The predominant bacterial genera at baseline and after 1 year in both T1DM and healthy controls were *Streptococcus, Prevotella, Neisseria*, and *Veillonella* with no significant differences between the groups. Considering the abundance at the species level, the most common bacterial species in both T1DM group and healthy controls were *Prevotella melaninogenica, Granulicatella adiacens, g_Neisseria_ASV2,* and *Rothia mucilaginosa.*

LefSe analysis of the baseline data showed that T1DM was associated with *Prevotella, Fusobacterium, Capnocytophaga, Alloprevotella*, and *Campylobacter* genera (Fig. 4A). At 1-year follow-up, T1DM was associated with *Leptotrichia* and *Fusobacterium* genera, while the healthy controls were associated with *Rothia* and *Abiotrophia* genera (Fig. 4B). Notably, no genera met the significance threshold (LDA score > 2, $P < 0.05$) for the healthy group at baseline, which is reflected in the absence of bars in Fig. 4A. Figs. 5A, B display the LefSe analysis at species level between the groups at baseline and 1-year follow-up. At baseline, LefSe identified 13 species (Fig. 5A) including *Fusobacterium periodonticum, Gemella morbilorum*, and *Prevotella pallens,* which were significantly more abundant in the T1DM group versus healthy controls. At 1-year follow-up, T1DM was associated with a significantly higher abundance of 5 species including *Bergeyella_sp_HMT_206, Leptotrichia_sp_HMT_498, Veillonella parvula, Leptotrichia hongkongensis*, and *Streptoccoccus oralis_subsp_tigurinus_clade_071*. In healthy group, LefSe revealed an increased abundance of *Rothia mucilaginosa, Granulicatella elegans, Streptococcus_sp_HMT_061, Rothia aeria*, and *Abiotrophia defective* compared to the T1DM group.

4. Discussion

In the present study, we confirmed our hypothesis and demonstrated that the salivary microbiota of children and adolescents with T1DM is

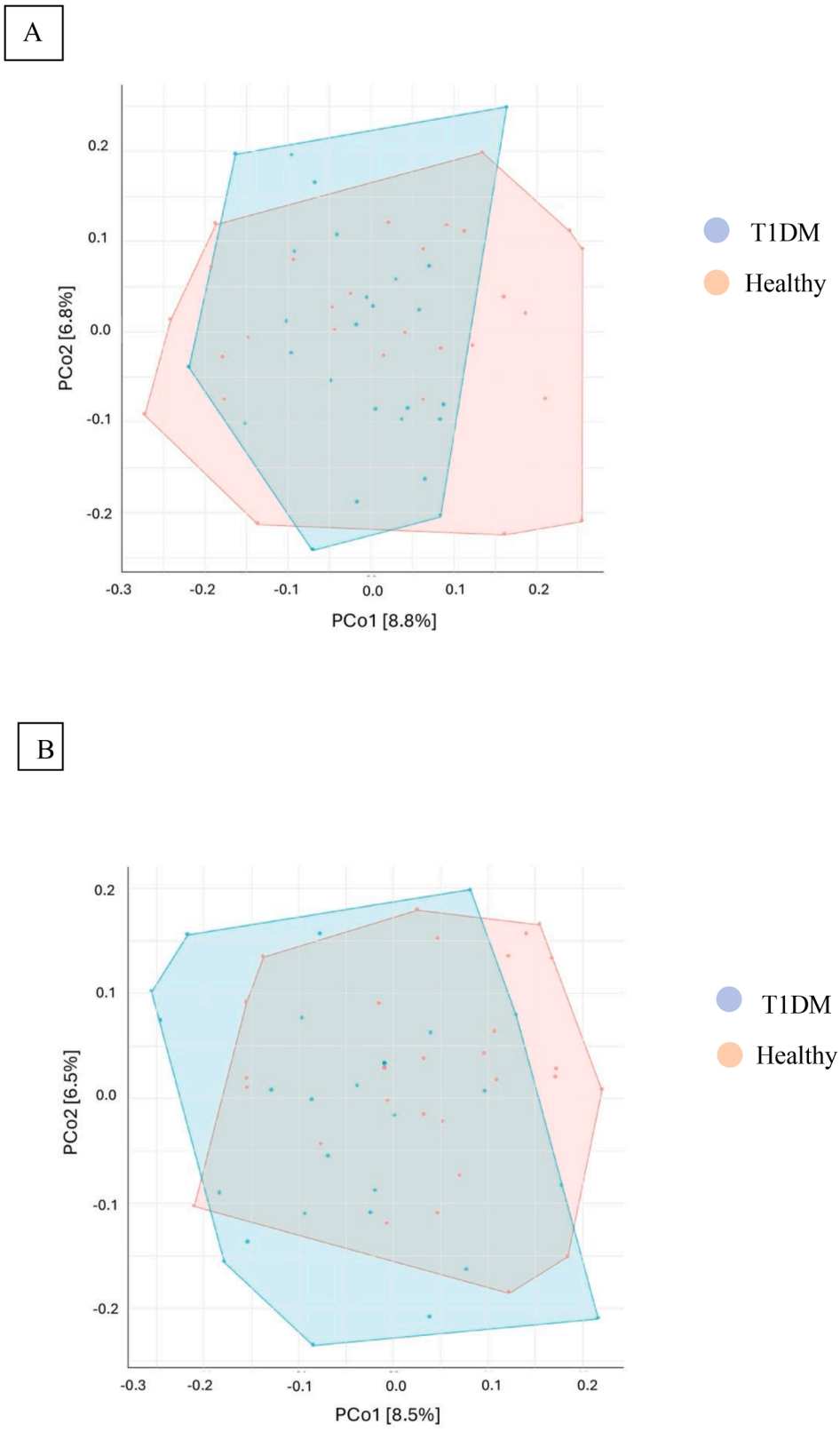

Fig. 1. Principal coordinate analysis (PCoA) of the T1DM and healthy groups. PCoA expressed by to most decisive variable (PCo1 and PCo2) accounting for approximately 15 % of the variation of the dataset in study groups. Comparison between type 1 diabetes (T1DM) and healthy groups at baseline (A) and at 1-year follow-up (B).

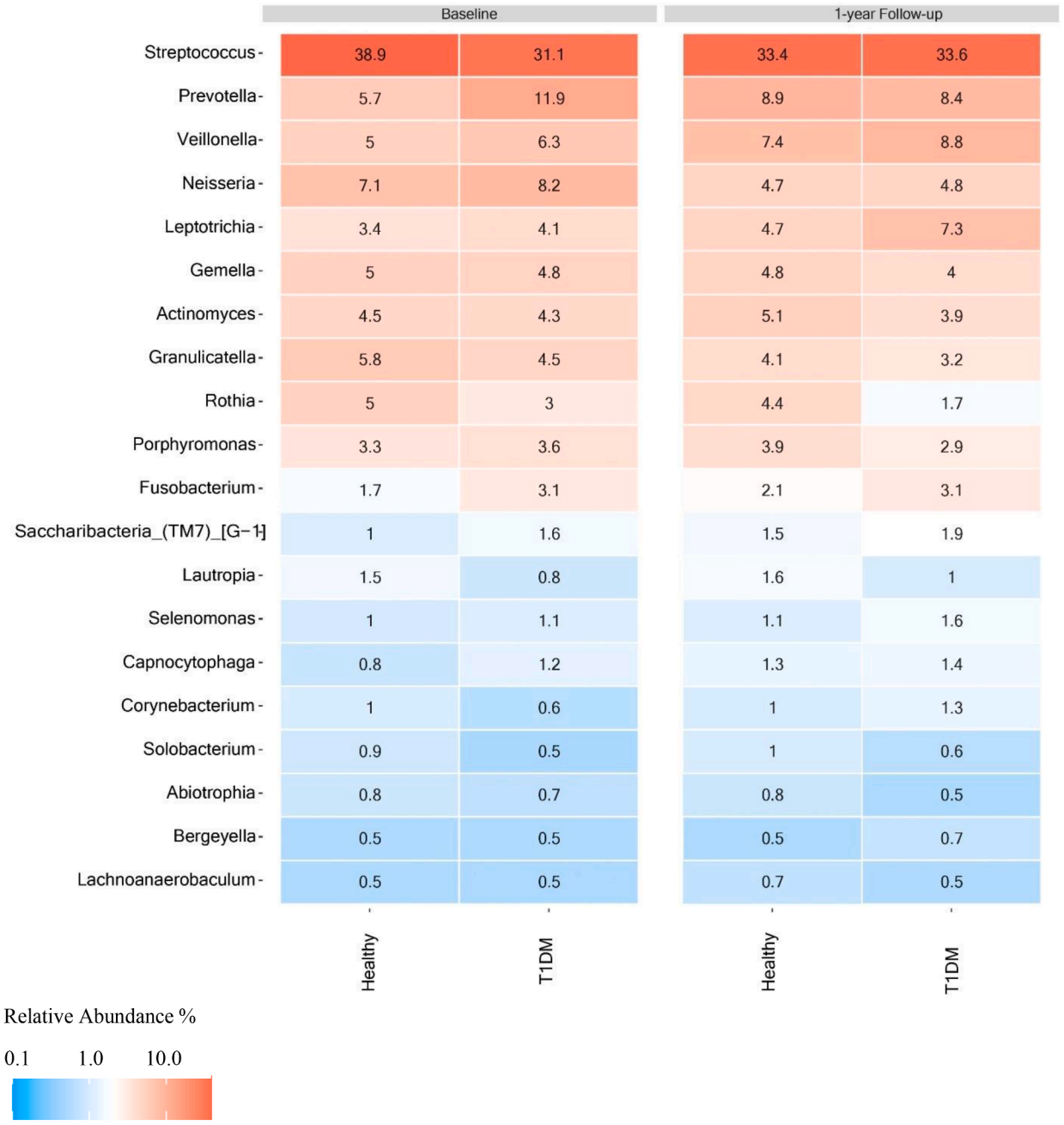

Fig. 2. Heatmap of the relative abundances (expressed as mean %) of top 20 predominant genera at baseline and 1-year follow-up. Comparison between type 1 diabetes (T1DM) and healthy groups at baseline and at 1-year follow-up.

significantly distinct from that of healthy controls during 1-year follow-up. Specifically, LEfSe analysis revealed that, in the T1DM group, potential opportunistic pathogens such as *Fusobacterium* and *Prevotella* were enriched. To the best of authors' knowledge, this is the first study to highlight the characteristics of the oral microbiota among diabetic children and adolescents within a 1-year observational period.

The primary strength of this study lies in its follow-up design with paired sampling over a one-year interval. While this approach does not capture short-term fluctuations in glycemic status, it provides insight into whether observed microbiota differences persist across broader timeframes. Although mean HbA1c values were similar at both sampling points, longitudinal monitoring helps to account for the chronic metabolic environment of T1DM beyond single-time point measures. Moreover, use of next-generation gene sequencing, which offers several advantages over traditional culture-based studies in microbial analyses, brought additional power to the presented study. Finally, we used saliva as sample material, as shifts in the oral microbiota, which are associated with inflammatory diseases of the oral cavity, are reflected in saliva [3]. In addition to the relatively small sample size, the limited number of timepoints restricted the longitudinal interpretation of our findings. Furthermore, the higher rate of gingivitis observed in the T1DM group, along with the wide age range covering different dentition phases, may have influenced the salivary microbial composition. Although the proportion of participants with gingivitis in the T1DM group increased from 42.3 % to 53.8 % during follow-up, this difference was not statistically significant (Data not shown.). Interestingly, this occurred despite a slight reduction in plaque index scores, suggesting that factors beyond plaque accumulation may have contributed to gingival inflammation. The concurrent increase in bleeding on probing and shifts in microbial composition may reflect an enhanced host susceptibility to

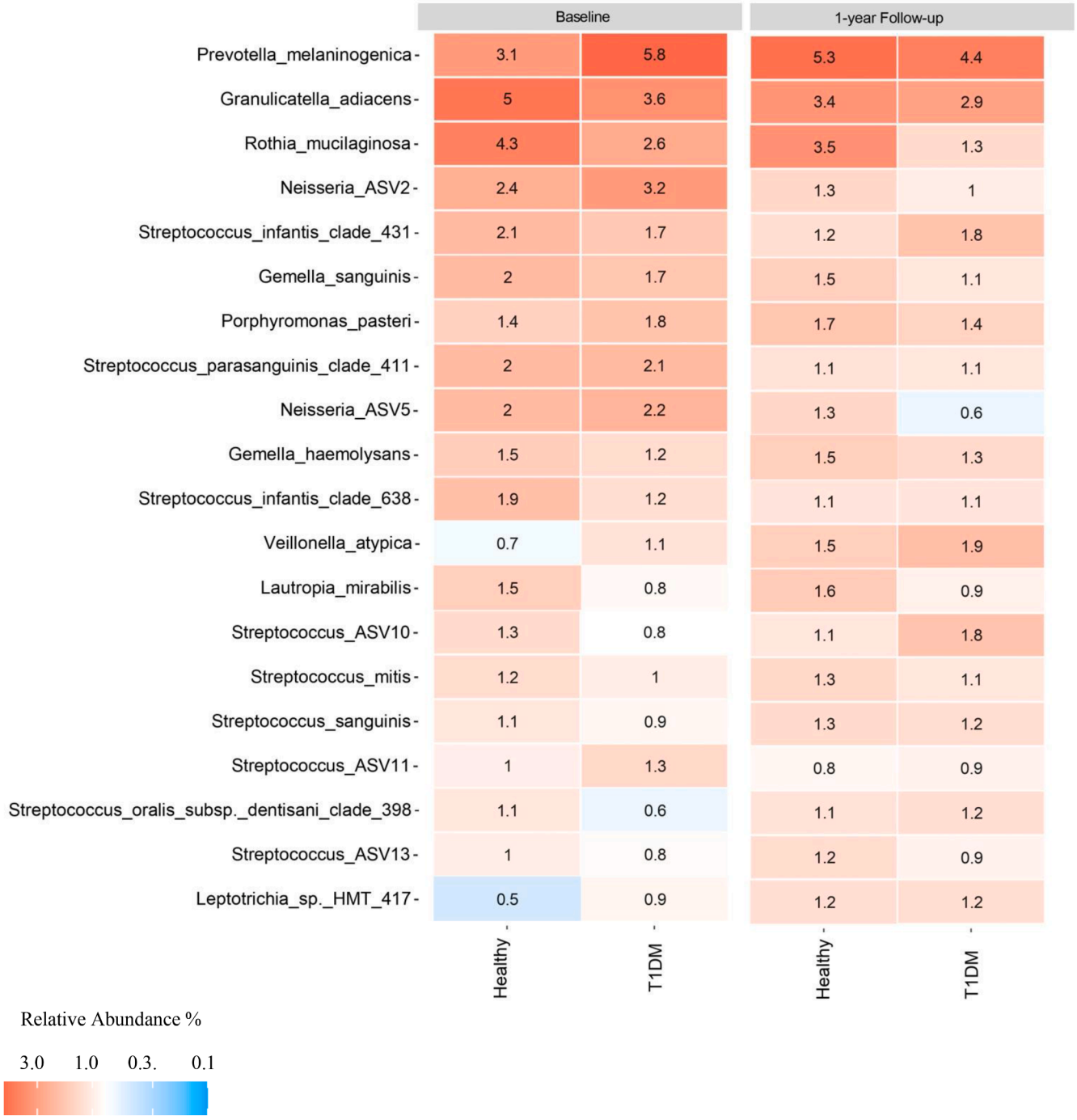

Fig. 3. Heatmap of the relative abundances (expressed as mean %) of top 20 predominant species at baseline and 1-year follow-up. Comparison between type 1 diabetes (T1DM) and healthy groups at baseline and at 1-year follow-up.

plaque-induced inflammation in children with T1DM. Subgroup analyses excluding participants with gingivitis or stratifying by dentition phase could not be conducted due to sample size constraints but should be considered in future studies. Moreover, future research would benefit from using plaque indices that quantify plaque amounts more precisely, enabling better exploration of the relationship between plaque burden, host inflammatory response, and salivary microbiota. Therefore, larger-scale studies are still needed to better demonstrate the effects of T1DM on the salivary microbiota in pediatric populations.

Evidence has highlighted the prevalence of oral health complications in individuals with diabetes, however, available data pertaining to the oral microbiota in children with T1DM relies predominantly on cross-sectional studies [30,31]. The comprehensive characterization of the oral bacterial community and the structural modifications in the oral microbiota of children with T1DM using repeated sampling over time remains scarce. The prevalence of periodontal disease is more than doubled in individuals with T1DM compared to those without T1DM [32]. Furthermore, Lalla et al [33] demonstrated an association between T1DM and periodontal destruction even in early life, and this association becomes more prominent as children become adolescents. In line with these findings, in the present study, T1DM subjects exhibited higher proportion of gingivitis than systemically healthy controls at both examination times, confirming that diabetes is associated with gingival inflammation. Notably, the proportion of T1DM participants with

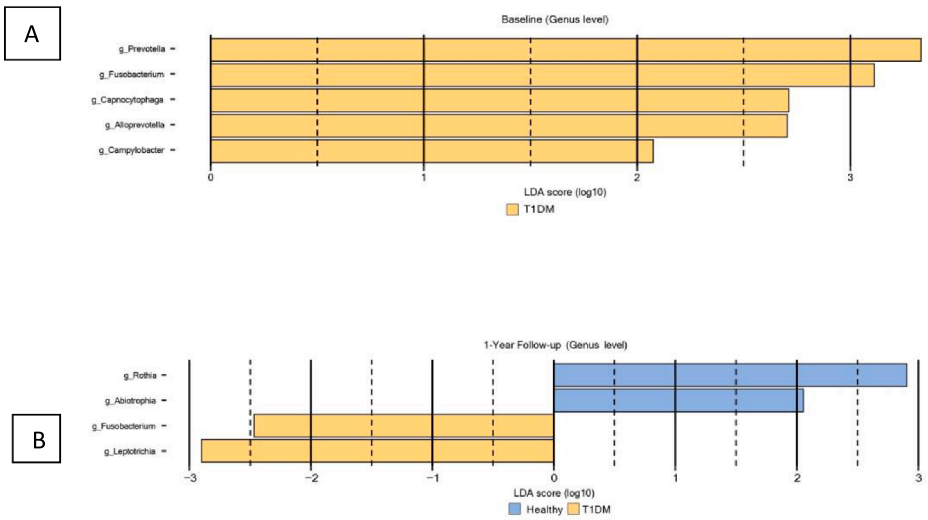

Fig. 4. Linear discriminant analysis effect size (LEfSe) analysis expressed by significant genera at baseline (A) and 1-year follow-up (B) in type 1 diabetes (T1DM) and healthy groups.

gingivitis increased over the 1-year follow-up, whereas the percentage of sites with plaque in this group remained relatively stable. This pattern suggests that factors beyond plaque accumulation such as alterations in host immune response and shifts in microbial composition may contribute to the progression of gingival inflammation in children with T1DM.

There was no significant difference in alpha diversity between the T1DM and healthy groups. Previous studies reported either a lower alpha diversity [19,22] or no discernible difference [20] in the oral microbiota of individuals with T1DM in comparison to their healthy counterparts. This variation could be attributed to the diversity among participants, encompassing factors such as age, diabetes duration, medication usage, and dietary patterns. Beta diversity analysis based on Bray-Curtis distance demonstrated more distinct segregation between the healthy and T1DM groups at the baseline compared to the 1-year follow-up, signifying that the composition of the salivary microbiota differed between the two groups. These results are in line with previous studies conducted on individuals with T1DM across various age groups [22,34]. The reduced separation over time may be explained by the significantly higher plaque index observed in the T1DM group compared to healthy controls at baseline, a difference that was no longer present at the 1-year follow-up.

Our findings revealed that the predominant bacterial genus in both groups at baseline and 1-year follow-up was *Streptococcus*, which is frequently acknowledged as a resident member of oral microbiota [20]. LEfSe analysis showed notable differences in the abundance of specific taxa between the T1DM and control groups at baseline and follow-up. At the genus level, the T1DM group at baseline associated with higher abundance of bacteria including *Prevotella, Fusobacterium, Capnocytophaga, Alloprevotella,* and *Campylobacter*. One year later, the T1DM group continued to exhibit a predominance of the bacterial genera *Fusobacterium* and *Leptotrichia*.

Gram-negative anaerobes, *Prevotella* and *Fusobacterium,* are common inhabitants of the oral cavity. While many species within these genera are commensal and act as a part of the healthy oral microbiota, an imbalance or increased abundance of *Fusobacterium* and *Prevotella* species has been associated with various oral health conditions including periodontal and endodontic diseases [35–37]. Human *Capnocytophaga* species are Gram-negative rods detected frequently in the oral cavity. Variations in the abundance of *Capnocytophaga* species have been associated with the presence of local or systemic diseases such as periodontal diseases, diabetes, or certain types of cancer [38]. Consistent with our study, a culture-based study concluded that the presence of some *Capnocytophaga* species were associated with gingivitis in children with T1DM [39]. *Campylobacter* is a spiral-shaped, Gram-negative bacteria that includes several species known to cause gastrointestinal infections in humans. Although there are studies indicating that genus *Campylobacter* can be an indicator of ecological changes in the subgingival or salivary microbiota, it is not commonly linked with autoimmune conditions like T1DM [40,41]. *Leptotrichia* is Gram-negative, anaerobic, rod-shaped, and opportunistic member of the oral cavity. *Leptotrichia* species have been associated with various infections, including oral infections, abscesses, and systemic infections, particularly in individuals with compromised immune systems [42]. Similar to the findings reported by Abola et al [20], our study identified an enrichment of *Leptotrichia* in the group with T1DM, whereas Yuan et al [22] observed decreased abundance, highlighting potential variation due to age, periodontal status, and geography.

In the healthy group, LEfSe analysis revealed that the genera *Rothia* and *Abiotrophia* dominated. Both genera are considered as a part of the resident oral microbiota and are often found on the surfaces of teeth, mucous membranes, and other oral tissues [43]. At species level, at both baseline and follow-up, the T1DM group exhibited an enrichment of opportunistic bacterial species such as *Fusobacterium periodonticum,* whereas the healthy group demonstrated enrichment of bacterial species commonly regarded as commensal in the oral cavity such as *Rothia mucilaginosa* and *Granulicatella elegans*.

These taxonomic shifts may reflect the altered ecological balance associated with T1DM. Hyperglycemia is known to impair neutrophil function, enhance oxidative stress, and upregulate pro-inflammatory cytokines [44–46], creating an environment that favors the persistence of opportunistic pathogens such as *Fusobacterium* and *Leptotrichia*. These microorganisms produce lipopolysaccharides and proteolytic enzymes that can intensify gingival inflammation [37,47], potentially contributing to the increased BOP % and gingivitis observed in our T1DM participants. Importantly, the increase in gingivitis prevalence occurred despite relatively stable plaque levels, underscoring that inflammation and host–microbe interactions, rather than plaque accumulation alone, may drive disease susceptibility in T1DM. While some of these taxa are opportunistic rather than classical periodontal bacteria, their enrichment may indicate a T1DM-specific dysbiotic signature beyond gingivitis alone. Future studies including gingivitis-free T1DM patients and incorporating immune profiling are warranted to confirm this possibility.

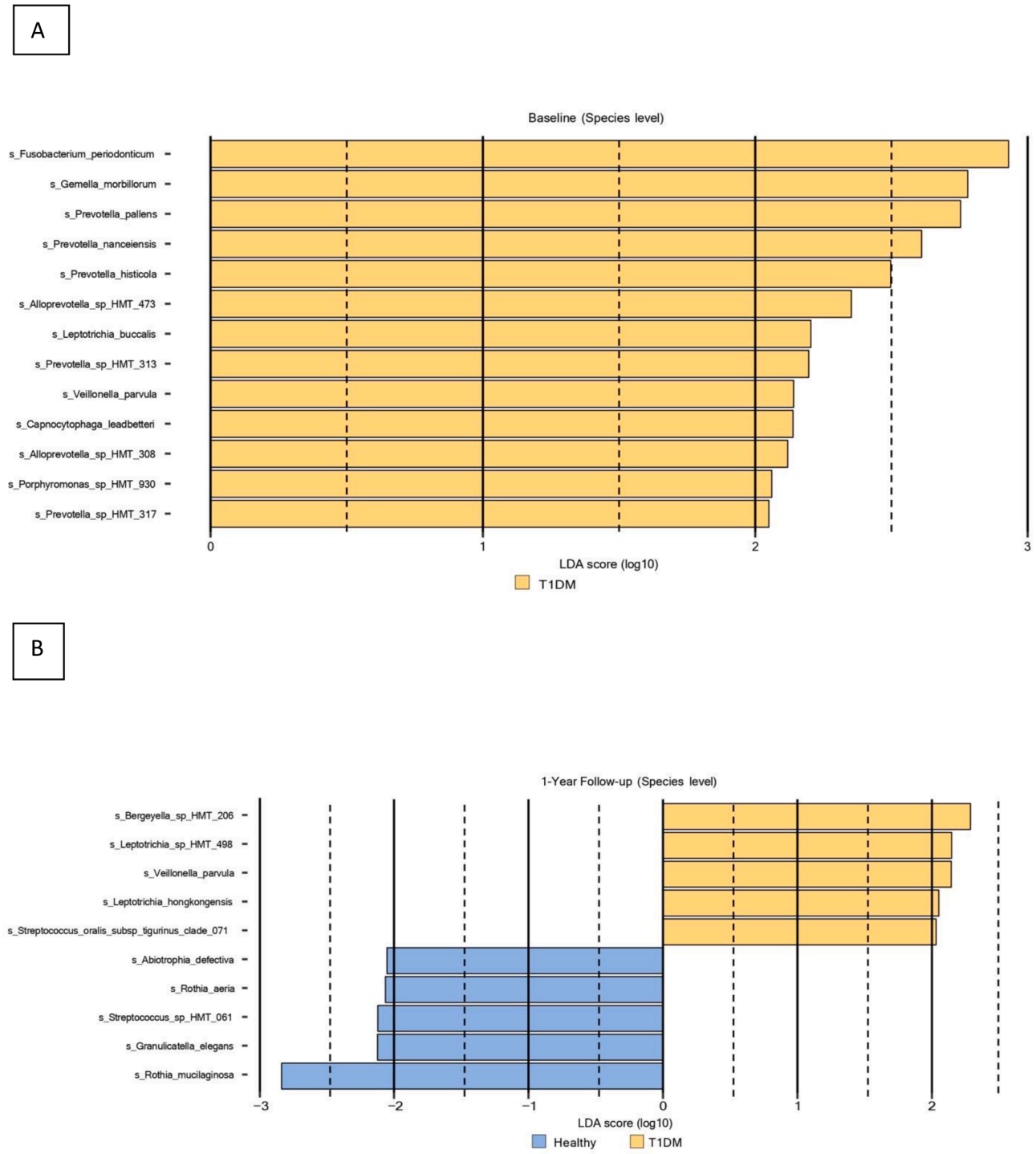

Fig. 5. Linear discriminant analysis effect size (LEfSe) analysis expressed by significant species at baseline (A) and 1-year follow-up (B) in type 1 diabetes (T1DM) and healthy groups.

5. Conclusion

Within the limitations of the current study, we conclude that the salivary microbiota of children and adolescents with T1DM differs from that of systemically healthy individuals. Enrichment of potential opportunistic pathogens in the oral cavity of T1DM patients may explain the susceptibility to develop oral inflammatory diseases. Notably, in our study participants, gingivitis prevalence and BOP % tended to increase over time in the T1DM group despite relatively stable plaque scores, suggesting a heightened inflammatory response that may be linked to both microbial shifts and host immune dysregulation. These findings underscore the importance of early periodontal monitoring and preventive care in pediatric T1DM populations. Future studies with larger sample sizes and longer observation periods are warranted to better characterize the interplay between microbial alterations, host responses, and oral health outcomes in pediatric T1DM.

Funding

This study was funded by University of TURKU (2022-2023/

FINDOS/N.Y.), by Minerva Foundation, Finland (2023/M.G, and 2024/246/N.Y.), by The Diabetes Research Foundations, Finland (2024/U.K.G.), and by SHS Apollonia, Finland (2024/U.K.G.).

Data availability

The sequencing data for project uploaded and registered in the European Nucleotide Archive (ENA) database. The accession number is PRJEB89884.

CRediT authorship contribution statement

Neslihan Yilmaz: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Ulvi Kahraman Gürsoy:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Daniel Belstrøm:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Recep Polat:** Writing – review & editing, Data curation. **Mervi Gürsoy:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to acknowledge all participants for their invaluable contribution to the present study. We sincerely thank Elif Gül Aydin for the kind assistance during patient examinations and saliva collection.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jdent.2025.106109.

References

[1] F.E. Dewhirst, T. Chen, J. Izard, B.J. Paster, A.C.R. Tanner, W.H. Yu, A. Lakshmanan, W.G. Wade, The human oral microbiome, J. Bacteriol. 192 (2010) 5002–5017, https://doi.org/10.1128/JB.00542-10/SUPPL_FILE/TABLE_S7_DEGREE_OF_HOST_ASSOCIATION_OF_GENERA.PDF.

[2] P.S. Kumar, S.M. Dabdoub, S.M. Ganesan, Probing periodontal microbial dark matter using metataxonomics and metagenomics, Periodontol. 2000 85 (2021) 12–27, https://doi.org/10.1111/PRD.12349.

[3] D. Belstrøm, The salivary microbiota in health and disease, J. Oral. Microbiol. 12 (2020), https://doi.org/10.1080/20002297.2020.1723975.

[4] L. Gao, T. Xu, G. Huang, S. Jiang, Y. Gu, F. Chen, Oral microbiomes: more and more importance in oral cavity and whole body, Protein. Cell 9 (2018) 488–500, https://doi.org/10.1007/s13238-018-0548-1.

[5] The American Diabetes Association, Classification and diagnosis of diabetes: standards of medical care in Diabetesd 2018, Diabetes. Care 41 (2018) S13–S27, https://doi.org/10.2337/dc18-S002.

[6] I. Libman, A. Haynes, S. Lyons, P. Pradeep, E. Rwagasor, J.Y. ling Tung, C. A. Jefferies, R.A. Oram, D. Dabelea, M.E. Craig, ISPAD clinical practice consensus guidelines 2022: definition, epidemiology, and classification of diabetes in children and adolescents, Pediatr. Diabet. 23 (2022) 1160–1174, https://doi.org/10.1111/pedi.13454.

[7] H. Sun, P. Saeedi, S. Karuranga, M. Pinkepank, K. Ogurtsova, B.B. Duncan, C. Stein, A. Basit, J.C.N. Chan, J.C. Mbanya, M.E. Pavkov, A. Ramachandaran, S.H. Wild, S. James, W.H. Herman, P. Zhang, C. Bommer, S. Kuo, E.J. Boyko, D.J. Magliano, IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045, Diabetes. Res. Clin. Pr. 183 (2022) 109119, https://doi.org/10.1016/J.DIABRES.2021.109119.

[8] R.J. Genco, W.S. Borgnakke, Diabetes as a potential risk for periodontitis: association studies, Periodontol. 2000 83 (2020) 40–45, https://doi.org/10.1111/PRD.12270.

[9] E. Jensen, G. Allen, J. Bednarz, J. Couper, A. Peña, Periodontal risk markers in children and adolescents with type 1 diabetes: a systematic review and meta-analysis, Diabetes. Metab. Res. Rev. 37 (2021), https://doi.org/10.1002/dmrr.3368.

[10] N. Yilmaz, R. Polat, M. Gürsoy, W. Kaman, E. Gül Aydin, D. Fteita, D. Yilmaz, F. Bikker, U.K. Gürsoy, Salivary macrophage activation-related chemokines and mitogen-activated kinase kinase (MAPKK)-degrading proteolytic activity in type 1 diabetes mellitus, J. Periodontol. (2023) 1–9, https://doi.org/10.1002/JPER.22-0314.

[11] D.T. Graves, Z. Ding, Y. Yang, The impact of diabetes on periodontal diseases, Periodontol. 2000 82 (2020) 214–224, https://doi.org/10.1111/PRD.12318.

[12] U.K. Gürsoy, N. Yilmaz, D. Yilmaz, S. Grönroos, M. Gürsoy, Type 1 diabetes mellitus and host–bacterial interactions in the oral cavity, Curr. Opin. Endocr. Metab. Res. 36 (2024) 100536, https://doi.org/10.1016/J.COEMR.2024.100536.

[13] M. Sanz, A. Ceriello, M. Buysschaert, I. Chapple, R.T. Demmer, F. Graziani, D. Herrera, S. Jepsen, L. Lione, P. Madianos, M. Mathur, E. Montanya, L. Shapira, M. Tonetti, D. Vegh, Scientific evidence on the links between periodontal diseases and diabetes: consensus report and guidelines of the joint workshop on periodontal diseases and diabetes by the International diabetes Federation and the European Federation of Periodontology, Diabetes. Res. Clin. Pr. 137 (2018) 231–241, https://doi.org/10.1016/j.diabres.2017.12.001.

[14] L.S. Liu, N. Gkranias, B. Farias, D. Spratt, N. Donos, Differences in the subgingival microbial population of chronic periodontitis in subjects with and without type 2 diabetes mellitus-a systematic review, Clin. Oral. Investig. 22 (2018) 2743–2762, https://doi.org/10.1007/S00784-018-2660-2.

[15] D.N. de A. Silva, M. Casarin, S. Monajemzadeh, B. de B. Bezerra, R. Lux, F.Q. Pirih, The microbiome in periodontitis and diabetes, Front. Oral. Health 3 (2022), https://doi.org/10.3389/FROH.2022.859209.

[16] J.L. Ebersole, S.S. Kirakodu, X. Zhang, D. Dawson, C.S. Miller, Salivary microbiome and biomarker characteristics of diabetics with periodontitis, Mol. Oral. Microbiol. 40 (2025) 37–49, https://doi.org/10.1111/OMI.12485.

[17] M. Gu, J. Ge, Q. Pan, N. Hu, F. Hua, Salivary microbiome variations in type 2 diabetes mellitus patients with different stages of periodontitis, BMC. Oral. Health 24 (2024) 1–11, https://doi.org/10.1186/S12903-024-05135-3/TABLES/2.

[18] C.A. Selway, E.D. Jensen, A.S. Pena, G. Smart, L.S. Weyrich, Type 1 diabetes, periodontal health, and a familial history of hyperlipidaemia is associated with oral microbiota in children: a cross-sectional study, BMC. Oral. Health 23 (2023), https://doi.org/10.1186/S12903-022-02625-0.

[19] M. Moskovitz, M. Nassar, N. Moriel, A. Cher, S. Faibis, D. Ram, D. Zangen, M. Yassour, D. Steinberg, Characterization of the oral microbiome among children with type 1 diabetes compared with healthy children, Front. Microbiol. 12 (2021) 756808, https://doi.org/10.3389/fmicb.2021.756808.

[20] I. Abola, D. Gudra, M. Ustinova, D. Fridmanis, D.E. Emulina, I. Skadins, A. Brinkmane, U. Lauga-Tunina, L. Gailite, M. Auzenbaha, Oral microbiome traits of type 1 diabetes and phenylketonuria patients in Latvia, Microorganisms 11 (2023) 1–19, https://doi.org/10.3390/microorganisms11061471.

[21] M. Carelli, A. Maguolo, C. Zusi, F. Olivieri, F. Emiliani, G. De Grandi, I. Unali, N. Zerman, C. Signoretto, C. Maffeis, Oral microbiota in children and adolescents with type 1 diabetes mellitus: novel insights into the pathogenesis of dental and periodontal disease, Microorganisms 11 (2023), https://doi.org/10.3390/MICROORGANISMS11030668.

[22] X. Yuan, J. Wu, R. Chen, Z. Chen, Z. Su, J. Ni, M. Zhang, C. Sun, F. Zhang, Y. Liu, J. He, L. Zhang, F. Luo, R. Wang, Characterization of the oral microbiome of children with type 1 diabetes in the acute and chronic phases, J. Oral. Microbiol. 14 (2022) 2094048, https://doi.org/10.1080/20002297.2022.2094048.

[23] M. Chan-yeung, H. Dimich-ward, Invited Review Series : tobacco And Lung Health; Respiratory health effects of exposure to environmental tobacco smoke, Respirology 8 (2003) 131–139. http://onlinelibrary.wiley.com/doi/10.1046/j.1440-1843.2003.00453.x/pdf.

[24] I.L.C. Chapple, B.L. Mealey, T.E. Van Dyke, P.M. Bartold, H. Dommisch, P. Eickholz, M.L. Geisinger, R.J. Genco, M. Glogauer, M. Goldstein, T.J. Griffin, P. Holmstrup, G.K. Johnson, Y. Kapila, N.P. Lang, J. Meyle, S. Murakami, J. Plemons, G.A. Romito, L. Shapira, D.N. Tatakis, W. Teughels, L. Trombelli, C. Walter, G. Wimmer, P. Xenoudi, H. Yoshie, Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions, J. Periodontol. 89 (2018) S74–S84, https://doi.org/10.1002/JPER.17-0719.

[25] C. Lundtorp-Olsen, C. Enevold, S. Twetman, D. Belstrøm, Probiotics do not alter the long-term stability of the supragingival microbiota in healthy subjects: a randomized controlled trial, Pathogens 10 (4) (2021) 391, https://doi.org/10.3390/pathogens10040391.

[26] C. Lundtorp-Olsen, C. Enevold, CA.Juel Jensen, S.N. Stofberg, S. Twetman, D. Belstrøm, Impact of probiotics on the salivary microbiota and salivary levels of inflammation-related proteins during short-term sugar stress: a randomized controlled trial, Pathogens 10 (4) (2021) 392, https://doi.org/10.3390/pathogens10040392.

[27] J.G. Caporaso, C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J.A. Gilbert, G. Smith, R. Knight, Nature.Com, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms, in: C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S.M. Owens, J. Betley (Eds.), L Fraser…The ISME Journal, L Fraser…The ISME Journal, 6, nature.Com, 2012, pp. 1621–1624, https://doi.org/10.1038/ismej.2012.8, 2012•.

[28] I.F. Escapa, T. Chen, Y. Huang, P. Gajare, F.E. Dewhirst, K.P. Lemon, New insights into human nostril microbiome from the expanded human oral microbiome database (eHOMD): a resource for the microbiome of the human aerodigestive

tract, MSystems 3 (2018), https://doi.org/10.1128/MSYSTEMS.00187-18/SUPPL_FILE/SYS006182299ST7.XLSX.
[29] N. Segata, J. Izard, L. Waldron, D. Gevers, L. Miropolsky, W.S. Garrett, C. Huttenhower, Segata-LEfSe-gb-2011, Genome. Biol. 12 (2011) R60.
[30] D. Dakovic, M.D. Pavlovic, Periodontal disease in children and adolescents with type 1 diabetes in Serbia, J. Periodontol. 79 (2008) 987–992, https://doi.org/10.1902/JOP.2008.070549.
[31] G. Aren, E. Sepet, D. Özdemır, N. Dınççağ, B. Güvener, E. Firatli, Periodontal Health, Salivary status, and metabolic control in children with type 1 diabetes mellitus, J. Periodontol. 74 (2003) 1789–1795, https://doi.org/10.1902/JOP.2003.74.12.1789;REQUESTEDJOURNAL:JOURNAL:19433670;WGROUP:STRING:PUBLICATION.
[32] I. Dicembrini, L. Serni, M. Monami, M. Caliri, L. Barbato, F. Cairo, E. Mannucci, Type 1 diabetes and periodontitis: prevalence and periodontal destruction—A systematic review, Acta. Diabetol. 57 (2020) 1405–1412, https://doi.org/10.1007/s00592-020-01531-7.
[33] E. Lalla, B. Cheng, S. Lal, S. Kaplan, B. Softness, E. Greenberg, R.S. Goland, I. B. Lamster, Diabetes mellitus promotes periodontal destruction in children, J. Clin. Periodontol. 34 (2007) 294–298, https://doi.org/10.1111/j.1600-051X.2007.01054.x.
[34] P.F. de Groot, C. Belzer, Ö. Aydin, E. Levin, J.H. Levels, S. Aalvink, F. Boot, F. Holleman, D.H. van Raalte, T.P. Scheithauer, S. Simsek, F.G. Schaap, S.W.M. O. Damink, B.O. Roep, J.B. Hoekstra, W.M. de Vos, M. Nieuwdorp, Distinct fecal and oral microbiota composition in human type 1 diabetes, an observational study, PLoS. One 12 (2017) e0188475, https://doi.org/10.1371/JOURNAL.PONE.0188475.
[35] H.A. Alhadainy, A.H. Abdel-karim, A.F. Fouad, Prevalence of fusobacterium species in endodontic infections detected with molecular methods: systematic review and meta-analysis, J. Endod. 49 (2023) 1249–1261, https://doi.org/10.1016/j.joen.2023.08.009.
[36] C.A. Brennan, W.S. Garrett, Fusobacterium nucleatum — Symbiont, opportunist and oncobacterium, Nat. Rev. Microbiol 17 (2018) 156–166, https://doi.org/10.1038/s41579-018-0129-6, 2018 17:3.
[37] E. Könönen, D. Fteita, U.K. Gursoy, M. Gursoy, Prevotella species as oral residents and infectious agents with potential impact on systemic conditions, J. Oral. Microbiol. 14 (2022), https://doi.org/10.1080/20002297.2022.2079814.
[38] A. Jolivet-Gougeon, M. Bonnaure-Mallet, Screening for prevalence and abundance of Capnocytophaga spp by analyzing NGS data: a scoping review, Oral. Dis. 27 (2021) 1621–1630, https://doi.org/10.1111/odi.13573.
[39] M. Ferreira, D. João, G. Alessandra, Microbiological, lipid and immunological profiles in children with gingivitis and type 1 diabetes mellitus, J. Appl. Oral. Sci. 25 (2017) 217–226.
[40] C. Lu, Q. Zhao, J. Deng, K. Chen, X. Jiang, F. Ma, S. Ma, Z. Li, Salivary microbiome profile of diabetes and periodontitis in a chinese population, Front. Cell. Infect. Microbiol. 12 (2022) 1–10, https://doi.org/10.3389/fcimb.2022.933833.
[41] K. Mahalakshmi, P. Arangannal, Santoshkumari, Frequency of putative periodontal pathogens among type 1 diabetes mellitus: a case-control study, BMC. Res. Note. 12 (2019) 328, https://doi.org/10.1186/s13104-019-4364-3.
[42] E.R.K. Eribe, I. Olsen, Leptotrichia species in human infections II, J. Oral. Microbiol. 9 (2017), https://doi.org/10.1080/20002297.2017.1368848.
[43] K. Krishnan, T. Chen, B.J. Paster, A practical guide to the oral microbiome and its relation to health and disease, Oral. Dis. 23 (2017) 276–286, https://doi.org/10.1111/odi.12509.
[44] S.K. Sonnenschein, J. Meyle, Local inflammatory reactions in patients with diabetes and periodontitis, Periodontol. 2000 69 (2015) 221–254, https://doi.org/10.1111/PRD.12089;PAGE:STRING:ARTICLE/CHAPTER.
[45] C. Aral, Ö. Nalbantoğlu, B. Nur, M. Altunsoy, H.M. Akgül, F.A. Akalın, et al., Metabolic control and periodontal treatment decreases elevated oxidative stress in the early phases of type 1 diabetes onset, Arch. Oral. Biol. 82 (2017) 115–121, https://doi.org/10.1016/j.archoralbio.2017.05.012.
[46] G.E. Salvi, L.M. Franco, T.M. Braun, A. Lee, G. Rutger Persson, N.P. Lang, W. V. Giannobile, Pro-inflammatory biomarkers during experimental gingivitis in patients with type 1 diabetes mellitus: a proof-of-concept study, J. Clin. Periodontol. 37 (2010) 9–16, https://doi.org/10.1111/J.1600-051X.2009.01500.X.
[47] K.Q. De Andrade, C.L.C. Almeida-Da-Silva, R. Coutinho-Silva, Immunological pathways triggered by porphyromonas gingivalis and fusobacterium nucleatum: therapeutic possibilities? Mediat. Inflamm. 2019 (2019) 7241312 https://doi.org/10.1155/2019/7241312.