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Characterization of 16S rRNA of the gut microbiome in long-tailed macaque (*Macaca fascicularis*) with spontaneous type 2 diabetes mellitus

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Abstract. *Ramadhanty PW, Saepuloh U, Suparto IH, Darusman HS. 2023. Characterization of 16S rRNA of the gut microbiome in long-tailed macaque (*Macaca fascicularis*) with spontaneous type 2 diabetes mellitus*. *Biodiversitas 24: 6191-6199.* The gut microbiota in the body is very complex and varied. It is known to influence human health. Imbalance (dysbiosis) of the gut microbiota can lead to type 2 diabetes mellitus (T2DM) and become a new feature in the development of the disease. Several human studies have compared the bacterial community in T2DM and non-T2DM patients. Until now, research on gut microbiota associated with T2DM in non-human primate animal models is still rare. T2DM can be developed by long-tailed macaque with clinical features similar to humans, which provides important information about the relationship between gut microbiota and T2DM. This study aims to characterize full-length 16S rRNA genes to obtain gut microbiota profiles in long-tailed macaques with and without spontaneous T2DM. The characterization was carried out using a metagenomic approach targeted at the full-length 16S rRNA gene with Nanopore technology (GridION) as one of the third-generation sequencing. The sample used was rectal swabs from adult male long-tailed macaques in the spontaneous T2DM group (n=3) and non-T2DM group (n=3). An increase in the alpha diversity, Firmicutes phylum, Oscillibacter genus, Oscillibacter valericigenes, and Oscillibacter ruminantium species, as well as a decrease in the Proteobacteria phylum were found in the T2DM group. The Firmicutes/Bacteroidetes ratio was also increased in the T2DM group compared to the non-T2DM group, which indicates dysbiosis. Based on these results, the metagenomic marker of the gut microbiome T2DM was obtained in long-tailed macaque primates, similar to humans with T2DM.

Keywords: 16S rRNA, dysbiosis, gut microbiome, long-tailed macaque, T2DM

INTRODUCTION

The microbiome is a microbial community in a specific environment, including all the molecules it produces (Berg et al. 2020). The human microbiome consists of trillions of microbes in complex systems that vary in composition at every site of the body. The gut is one of the most abundant sites for microbiota, dominated by bacteria (Muñoz-Garach et al. 2016; Hou et al. 2022). Bacterial dominance in the gut comes from the Firmicutes and Bacteroidetes phyla, as well as several minority phyla like Proteobacteria and Actinobacteria, forming patterns of gut microbiota communities that are different for each individual (Hou et al. 2022). Gut microbiota is widely acknowledged to affect human health. They are involved in a variety of normal functions of the human body. One of them is to help the metabolism of undigested nutrients and foods, producing various metabolites that can influence the metabolism of various organs (Rastelli et al. 2018). Changes in gut microbiota community patterns lead to gut microbiota imbalance (dysbiosis) and have been associated with various diseases, including type 2 diabetes mellitus.

Type 2 diabetes mellitus (T2DM) is a metabolic disease due to impaired insulin secretion and/or insulin action, which causes hyperglycemia (Decroli 2019). T2DM is one of the four priority non-communicable diseases that should be treated immediately. The number of cases and prevalence continue to increase due to its potential to cause many complications in the body and increase the risk of premature death (WHO 2016). The International Diabetes Federation (IDF) reports that about 537 million adults between the ages of 20 and 79 are living with diabetes, and more than 90% of them have T2DM (IDF 2021). This number is predicted to increase to 643 million by 2030 if handled improperly. T2DM occurs due to genetic susceptibility, environmental factors, or their interactions (Musso et al. 2011). The diversity and abundance of the gut microbiota also influence the development of T2DM. Dysbiosis in the gut microbiota can cause metabolic dysregulation, like increased insulin resistance and inflammation, leading to T2DM (Sikalidis and Maykish

2020; Arora et al. 2021). Human studies characterizing and comparing the gut microbiome between healthy and T2DM groups have confirmed that dysbiosis occurs in T2DM patients and gut microbiota dysbiosis is considered to be a novel feature in the pathogenesis of T2DM (Larsen et al. 2010; Arora et al. 2021).

A targeted metagenomics approach may be a method used to characterize the gut microbiome and detect dysbiosis. DNA sequence analysis targeting the 16S rRNA gene is the most commonly used and widely accepted gold standard in identifying of gut microbes to characterize the diversity and abundance of microbiota communities (Bharti and Grimm 2021). Most of the gut microbiome studies in T2DM patients targeted short variable regions of the 16S rRNA gene (e.g., V3-V4 or V4), leading to limited taxonomic resolution and also yielding different data when analysis is performed on other variable regions (Santos et al. 2020; Curry et al. 2021). Ahmad et al. (2019) sequenced the v3-v4 region, which showed that the gut microbiome profile in T2DM patients was marked by an increase in the abundance of the *Firmicutes*, *Proteobacteria*, and *Prevotella*, accompanied by a decrease in the Bacteroidetes phyla. A different pattern is obtained if sequencing is performed in another region. Li et al. (2020) showed an increase in Firmicutes, *Dorea*, and *Fusobacterium* abundance and a decrease in Bacteroidetes. Therefore, the full-length 16s rRNA genes was performed and highlighted in this study to obtain a better taxonomic resolution. Sequencing was performed using the third generation sequencing (TGS) with the nanopore sequencing technique by Oxford Nanopore Technologies (ONT). It can sequence all variable regions of the 16S rRNA gene (V1-V9), enabling taxonomic ranking at the species level.

The gut microbiome has been shown to play a role in the pathogenesis of T2DM. Characterization of the gut microbiome in primate animal models is necessary to prevent, diagnose, and treat T2DM. The long-tailed macaque (*Macaca fascicularis*) has long been used as an animal model and is highly relevant for representing the human microbiome due to the similarity of their gut microbiome profiles (Li et al. 2018; Sawaswong et al. 2021). In T2DM research, long-tailed macaques with spontaneous T2DM are valuable animal models due to the difficulty and time it takes to develop diabetes. Clinical features of diabetes in non-human primates include obesity, insulin resistance, dyslipidemia, and pancreatic pathology similar to humans (Wagner et al. 2006). Long-tailed macaques are commonly used in extensive research to determine the development, characteristics, and other diseases caused by diabetes. The study of the microbiome is very useful for understanding primate health, evolution, behavior, and conservation (Harwood et al. 2012). Moreover, the characteristics of primate animal models in T2DM studies through microbiome studies can help determine strategies for modulating the gut microbiota through non-invasive treatment, both to provide efficient primate models and for the treatment and prevention of T2DM. It is necessary to explore animal model's microbiome profiles before using them in biomedical studies. However, information on the gut microbiome of long-tailed macaque primates is still limited. Therefore, this uses nanopore sequencing technology to characterize the gut microbiome of long-tailed macaques in the spontaneous T2DM and non-T2DM groups based on the full-length 16S rRNA gene.

MATERIALS AND METHODS

Time and place

This research was conducted from June to November 2022. Samples of *M. fascicularis* were collected from Primate Research Center (PRC), IPB University, Bogor, Indonesia. Laboratory analysis was conducted at the Biotechnology Laboratory of PRC. Sequencing and bioinformatics analysis were carried out by PT. Genetika Science, Jakarta, Indonesia.

Procedures

Sample collection

The experimental animals in this study were 6 adult male long-tailed macaques (14-16 years old) obtained from captivity at the PRC IPB University. Macaques are kept in a colony under equal treatment and environmental conditions. None of the macaques were given a dietary intervention to become diabetic.

The grouping of T2DM and non-T2DM macaques was based on the results of routine clinical examinations. The criteria for T2DM macaques were based on fasting plasma glucose (FPG) ≥126 mg/dL and hemoglobin A1c (HbA1c) levels ≥6.5%. The T2DM macaques group (n=3) showed an average FPG and HbA1c of 412.67 mg/dL and 11.43%, respectively. While in the non-T2DM group (n=3), it was 60 mg/dL and 4.37%.

Samples were collected using a rectal swab to obtain fresh stool samples. All procedures on animals have received approval from the Institutional Animal Care and Use Committee (IACUC) Number P.02-19-IR.

DNA extraction

The DNA was extracted from each rectal swab sample using the Zymo Quick-DNA Miniprep Plus Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. The concentration and purity of DNA were measured using a Nanodrop™ One (Thermo Scientific) spectrophotometer and a Qubit fluorometer (ThermoFisher).

PCR amplification and sequencing

Amplification targeting 1484 bp full-length 16S rRNA gene region for bacteria was carried out using specific primers of 27F (5'-AGAGTTTGATCMTGGTCCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Matsuo et al. 2021). The PCR reaction consisted of 1 µL of each 10 pmol μL^{-1} of forward and reverse primer; 12.5 µL GoTaq Green Master Mix (Promega, Madison, WI, USA); 5.5 µL nuclease-free water, and 5 µL (100-250 ng/µL) template DNA samples. PCR was performed under the following conditions: pre-denaturation (94°C for 3 minutes), 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C

for 1 minute, followed by post-extension at 72°C for 7 minutes and then ended at 4°C for 2 minutes. The PCR products were run using 2% agarose gel electrophoresis stained with ethidium bromide, and 1 kb DNA Ladder (Vivantis) was used as a marker. The electrophoresis was observed using the Gel Doc 2000 (Biorad), and the result was read with the Quantity One program (Biorad). The presence of the 1484 bp-sized DNA fragment in the electrophoretic agarose gel indicated the presence of PCR products.

The purified DNA was then sent to PT. Genetika Science Jakarta (a private company that provides sequencing services) for sequencing process and analysis. Library preparations were conducted using commercial kits manufactured by Oxford Nanopore Technology (ONT). Nanopore sequencing was performed using MinKNOW software version 22.05.7 on an R9.4 flow cell with a GridION sequencer (ONT).

Bioinformatics program

The sequencing results (FAST5 data) were converted into FASTQ files (basecalling) and were performed using Guppy v6.1.5 with the high-accuracy model (Wick et al. 2019). Obtained FASTQ files were excised from the barcode and attached adapter sequences to obtain the original sample sequence. Furthermore, the quality of FASTQ files was visualized using Nanoplot 1.40.0, and quality filtering was performed using NanoFilt 2.8.0 (de Coster et al. 2018; Nygaard et al. 2020). Filtered reads were classified using a Centrifuge classifier (Kim et al. 2016). The bacteria and Archaea index was built using the NCBI 16S RefSeq database (https://ftp.nbci.nlm.nih.gov/refseq/TargetedLoci). Downstream analysis and visualizations were performed using Pavian (https://github.com/fbreitwieser/pavian), Krona Tools (https://github.com/marbl/Krona), and Rstudio using R version 4.2.0 (https://www.R-project.org/).

Data analysis

The richness of biodiversity in a sample was monitored using alpha and beta diversity. Alpha diversity was calculated based on Obeserved-species, Chao1, Shannon, InvSimpson, ACE, and Fisher. Community diversity was evaluated using the Shannon and InvSimpson index, while the community abundance/richness index were calculated using Chao1, ACE, and Fisher index. Beta diversity estimates relative abundance to understand whether differences in the microbiota composition of the groups being compared are significant. Rstudio was used to perform the ANOSIM, and a Linear Discriminant analysis effect size (LefSe) was performed to characterize the different bacterial community structures in the two groups. This algorithm was used to screen for bacteria significantly differing between the two groups, created using an LDA threshold score ≥2; the result is also shown in a cladogram. A statistical t-test was performed to compare differences between groups. Statistical significance was defined by a P-value <0.05; statistical analysis was performed with Ms. Excel 2019 software.

RESULTS AND DISCUSSION

The electrophoresis result and data quality information

The full-length 16S rRNA gene of rectal swab samples of *M. fascicularis* with spontaneous type 2 diabetic and non-diabetic was successfully amplified, resulting in a specific band of about 1,484 bp (Figure 1).

Nanopore sequencing was performed, the results were filtered for quality readings (Table 1). The base-calling process obtained 81,197,674 bases in the T2DM group and 60,883,287 bases in the non-T2DM group. Overall, 47,453 reads were generated from the T2DM group and 38,578 reads from the non-T2DM group. The average read length of the T2DM group was 1,575 nucleotides (nt) and 1,578 nt for the non-T2DM group. All sequenced samples had good readability, with mean read quality for the T2DM and non-T2DM groups of 11.87 and 11.8, respectively.

Table 1. NanoPlot's statistical result for the filtered FASTQ files generated from all samples in T2DM and non-T2DM groups

Statistics	T2DM	Non-T2DM
Total number of reads (reads)	47,453	38,579
Mean read length (nt)	1,575	1,578
Mean read quality	11.87	11.8
Total bases (bp)	81,197,674	60,883,287

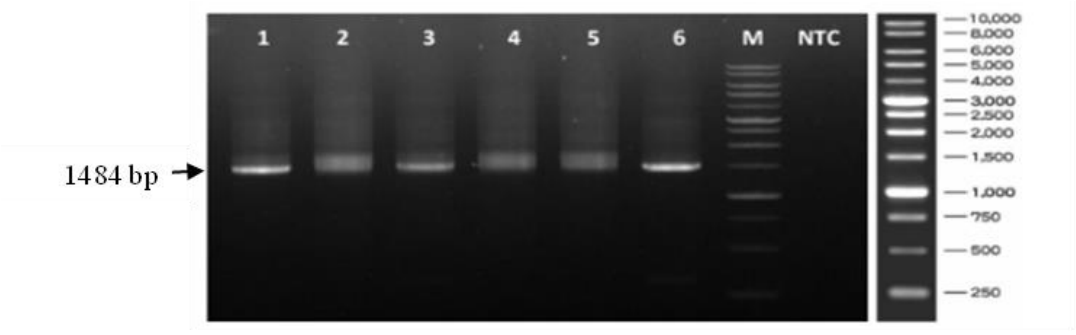

Figure 1. PCR amplification of full-length 16S rRNA gene from T2DM and non-T2DM groups of long-tailed macaque samples. 1-3 were T2DM samples, 4-6 were non-T2DM samples, M was DNA ladder, and NTC was non-template control

Figure 2. Venn diagram based on OTUs showing the number of bacterial OTUs shared within and between groups. A. Inter-sample microbiota in the non-T2DM group, B. Inter-sample microbiota in the T2DM group, C. Microbiota between groups

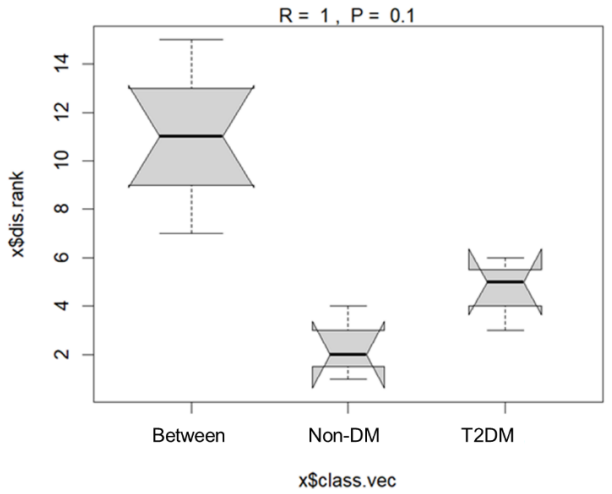

Figure 3. ANOSIM boxplot based on rank (between-group and within-group). The R-value shows the high and low variation of the sample. The P-value represents the confidence degree (p>0.05 not significant)

Alpha diversity

The alpha diversity can reflect a community microbe's richness and diversity in each sample. These values generally have no units and are used for comparing groups of samples. The T2DM group had significantly higher Shannon (5.27 vs 3.95; p=0.007) and InvSimpson (63.81 vs 15.61; p=0.057). Measurements of Chao1 abundance (3,032.36 vs 1,610.9; p=0.013) ACE (3,019.98 vs 1,609.54; p=0.013) and Fisher (392.5 vs 190.2; p=0.002) also show similar results, significantly higher in the T2DM group compared to non-T2DM group. The result of the alpha diversity analysis showed that, overall, the T2DM group had a higher diversity and abundance of microbial communities than the non-T2DM group.

The OTU cluster analysis shows the number of unique OTUs and shared OTUs within and between groups of samples, presented as a Venn diagram (Figure 2).

Among the 3,743 OTUs detected, 1,988 OTUs were present in the T2DM group, and 478 OTUs in the non-T2DM group. Each sample in the group (T2DM and non-T2DM) contains higher shared OTUs than the unique OTUs. There are 751 shared OTUs in the T2DM group and 476 shared OTUs in the non-T2DM group, while between groups has 1,277 shared OTUs, less than the unique OTUs in the T2DM group.

Beta diversity

Beta diversity estimates relative abundance to understand whether differences in the microbiota composition of the groups being compared are significant. Beta diversity is investigated using Analysis of Similarity (ANOSIM) to determine any differences in microbial community structure between the non-T2DM and T2DM groups, as well as to assess the classification of the samples into each group, and the results are presented in the boxplot (Figure 3).

The ANOSIM result, (R=1; p=0.1), showed that variation between groups was considered greater, but the microbial community structure was not significantly different. Further analysis using Linear Discriminant Analysis (LDA) Effect Size (LefSe) was performed to characterize the different bacterial community structures in the two groups. Then the cladogram was created to represent the phylogenetic difference between the two groups (Figure 4).

The result of the LefSe analysis indicates that there were specific enrichment differences between the T2DM group compared to non-T2DM. Furthermore, 107 bacterial taxa had differences in relative abundance in the T2DM and non-T2DM groups, with 79 different microbial taxa in the T2DM group. Dysbiosis of the gut microbiome between phylum to genus level in the T2DM group was observed. The T2DM group has 4 bacteria phyla, 9 classes, 8 ordo, 17 families, 21 genera, and 19 more specific species, in contrast to the non-T2DM group which has 1 phylum, 1 class, 1 ordo, 1 family, 2 genera, and 22 species that are higher specific.

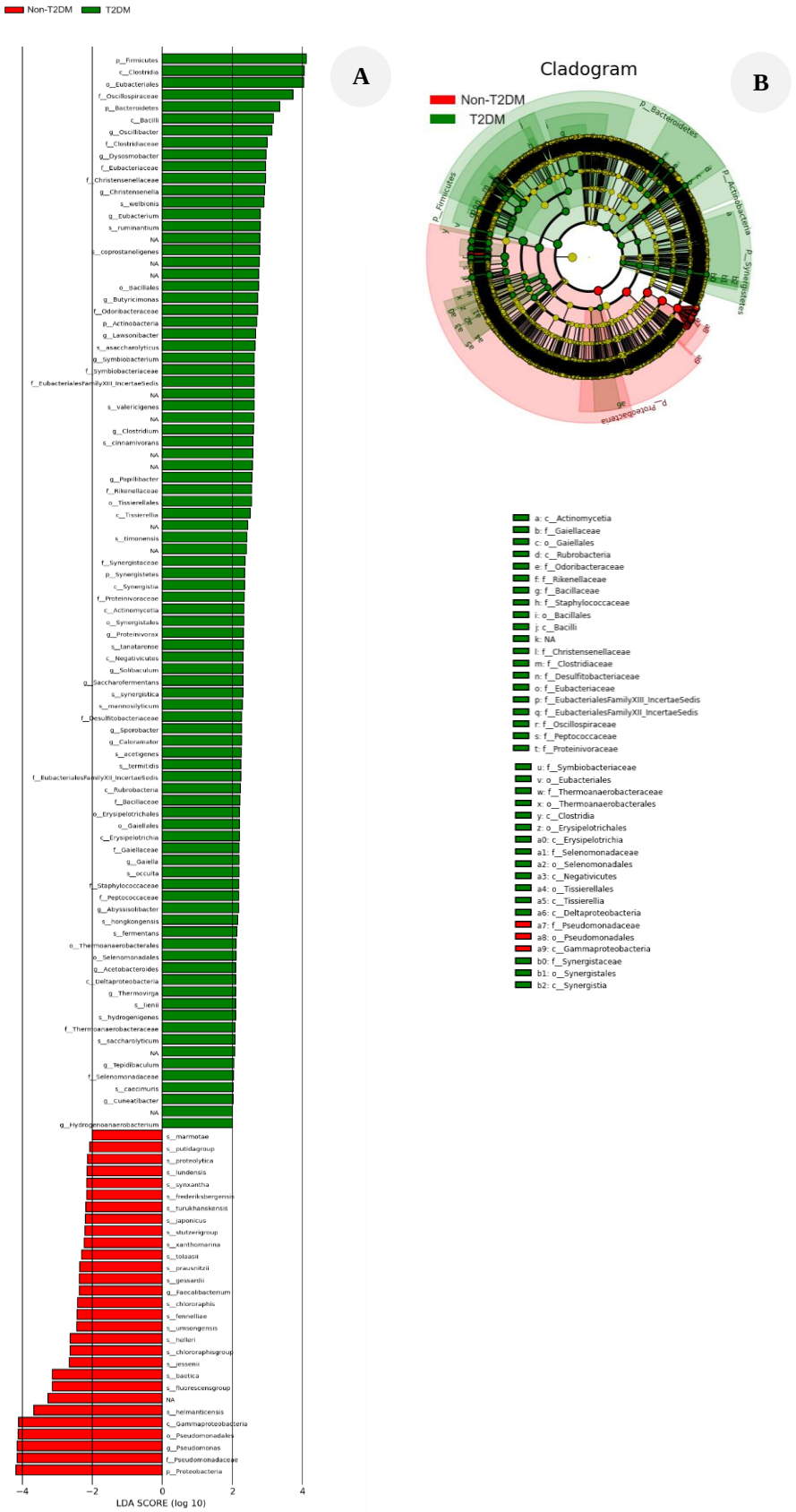

Figure 4. LefSe analysis. A. Histogram of the LDA scores was computed to distinguish important taxonomic differences between the T2DM group (positive score) and non-T2DM group (negative score). The bar represents taxon features with significantly higher expression in the two groups. B. Cladograms were designed to describe the phylogenetic distribution of gut microbiota associated between the two groups. The nodes indicate different groups (green: non-T2DM, red: T2DM, yellow: insignificant), with classification at the phylum level to species shown from the inside to the outside

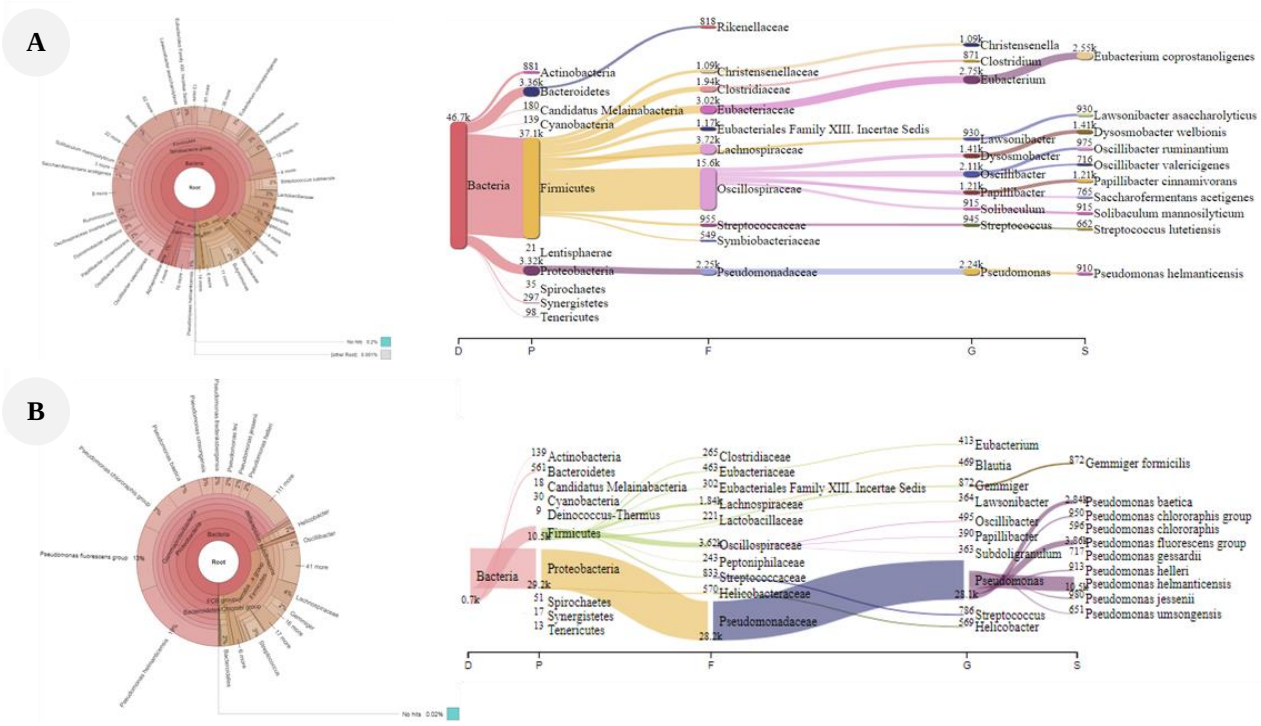

Figure 5. Krona visualization and Sankey diagram representing bacterial diversity among all samples. Krona chart showing the broader and highly detailed taxonomic hierarchy, the relative abundance from phylum to species level can be known. Sankey diagrams indicate the top taxa abundance quantity at each taxonomic level. A. T2DM group, B. Non-T2DM group

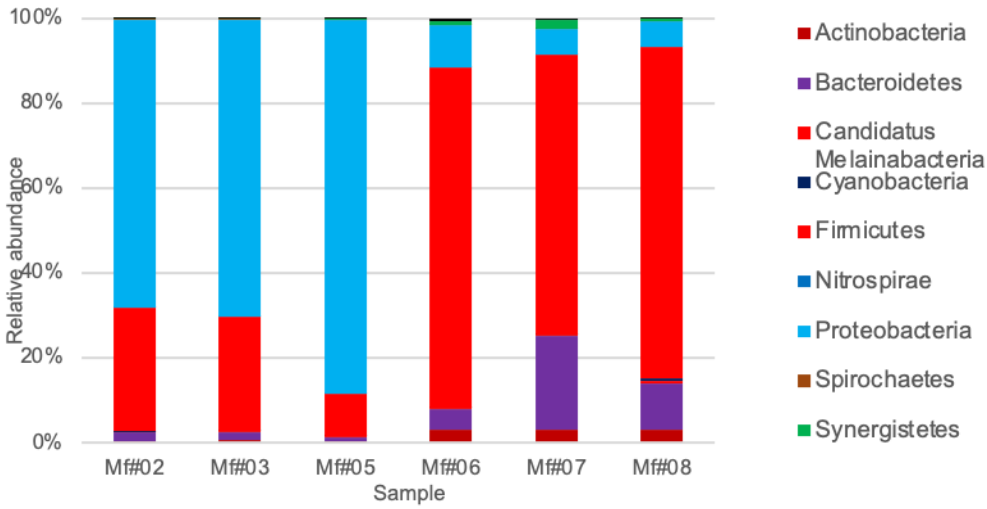

Figure 6. Illustration of the relative abundance of the top phyla of each sample. The sample codes Mf#02, Mf#03 and Mf#05 represent the non-T2DM group, while Mf#06, Mf#07 and Mf#08 represent the T2DM group

Significant differences in genera were observed in the gut microbiota between 2 groups. A particular clade broadly characterizes each group, and the expressed biomarkers differed between the two groups. Overall, the abundance of Firmicutes phyla was higher in the T2DM group than in the non-T2DM group, with a higher decrease in the Proteobacteria phyla in the non-T2DM group. At the family and genus level, *Oscillospiraceae* (*Oscillibacter*) was more abundant in the gut of T2DM group, while *Pseudomonadaceae* (*Pseudomonas*) was more abundant in the gut of non-T2DM group.

Diversity and composition of microbial communities

The result of metagenomic analysis using nanopore sequencing on rectal swab samples of T2DM and non-T2DM long-tailed macaque provides variations at different

taxonomic levels. Krona visualization and Sankey diagrams were made to show abundance (Figure 5). The top phyla from each sample were selected to form a histogram of the distribution of taxa abundance to make it easier to observe the relative abundance and their proportions. The relative abundance of taxa at the phylum level is illustrated in Figure 6.

Based on Figure 6, the gut microbiota of both groups are dominated by Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria phyla. The abundance of microbiota at the phylum level was in the T2DM group sequentially, namely Firmicutes (74.67%), Bacteroidetes (12,67%), Proteobacteria (7.3%), and Actinobacteria (3%). The non-T2DM group was dominated by Proteobacteria (75.3%), Firmicutes (22%), Bacteroidetes (1.67%), and Actinobacteria (0.3%). The presence of other phyla was abundant, but to a lesser degree and in different proportions for each group, including the phyla Candidatus Melainabacteria, Nitrospirae, Cyanobacteria, Spirochaetes, Synergistetes, and Tenericutes. Differences in dominant taxa are also observed at various taxonomic levels (Figure 5). At the genera level in the T2DM group, *Streptococcus* and *Oscillibacter* were found to be abundant, while in the non-T2DM group, the abundance was high in *Pseudomonas* genera. Bacteria from *Streptococcus lutetiensis*, *Oscillibacter valericigenes*, *Oscillibacter ruminantium*, and *Dysosmobacter welbionis* dominated the T2DM group. Bacteria from *Pseudomonas* genera were the most dominant, with *Pseudomonas helmanticensis* species being the most abundant in the non-T2DM group.

Discussion

This study focused on characterizing full-length 16S rRNA genes from the gut microbiome of long-tailed macaques with spontaneous T2DM. The incidence of spontaneous T2DM in this study was marked by elevated fasting plasma glucose and HbA1c levels above normal. In long-tailed macaques, spontaneous cases of T2DM are rare, even after long-term induction with a diet high in sugar and fat. Alteration of normal long-tailed macaques into T2DM is not always successful (Kleinert et al. 2018), making long-tailed macaques with spontaneous T2DM a precious animal model. This study used long-tailed macaques with spontaneous T2DM and non-diabetic macaques from the same group (controls) to obtain a profile of their gut microbiome. The results showed that the microbiome profiles of the two groups were different. The microbiome profile of the T2DM group represents a profile similar to that of T2DM in humans.

Characterization of the gut microbiota community is essential to measure a particular community's bacterial composition and relative abundance, including alpha (within the community) and beta (between communities) diversity. Alpha diversity analysis of the bacterial community in the T2DM group was significantly more diversified and abundant than in the non-T2DM group. The abundance index (Chao1, ACE, and Fisher), diversity, and evenness index (Shannon and InvSimpson) were the three metric calculations used to calculate alpha diversity. The T2DM group had an average Chao1 index of 2,900 to 3,200 species. This value must be combined with other indices due to its tendency to be higher than the observed number of species. ACE and Fisher index were also found to be greater in the T2DM group. According to these findings, there is a statistically significant difference between the gut microbiota of the T2DM and non-T2DM groups, with the T2DM group having a significantly greater abundance of microbiota. To calculate the relative abundance and evenness of taxa present, the Shannon and InvSimpson indices were used, with community richness being considered (Hagerty et al. 2020). The Shannon index for the T2DM group was 5.27, indicating the high diversity of the bacterial community. If the Shannon index value is 3, bacterial diversity is believed to be high (Yin et al. 2019). However, the InvSimpson value for the non-T2DM group was not significantly different from the T2DM group. The community's species diversity decreases as the InvSimpson values go up (Yin et al. 2019). The T2DM group's lack of microbiota diversity points to the impact of T2DM on monkeys. The T2DM group experienced an increase in alpha diversity based on the overall results of alpha diversity analysis. Results from this study indicate that the T2DM group had a different gut microbiota profile than the non-T2DM group. According to the result of alpha diversity analysis, the bacterial community of the T2DM group was significantly more diversified and abundant than the non-T2DM group. These findings contrast with several previous studies that indicated a significant decrease in gut microbial diversity in human patients with T2DM (Chávez-Carbajal et al. 2020; Li et al. 2020). A lower or less bacterial community is usually associated with an unhealthy state, while a diverse and abundant bacterial community is associated with a healthy condition (Pickard et al. 2017). However, Kumar et al. (2022) reported that high alpha diversity in T2DM patients in the Kenya population indicates the diversity, density, and activity of gut bacteria lead to the signs of T2DM, namely gut microbiota dysbiosis. Increasing or decreasing alpha diversity is a helpful way to detect dysbiosis, but it is not always better to have high alpha diversity. It is dependent on whether the bacteria's abundance is beneficial or pathogenic.

The Bray-Curtis distance matrix with 999 permutations was used to test beta diversity, and the result is shown in the ANOSIM plot. Despite the non-significant differences, the beta diversity results showed that the T2DM group had a different gut microbiota structure than the non-T2DM group. Another study on spontaneous T2DM rhesus macaques obtained similar results, the beta diversity in the control group was different from the hyperglycemia group, although not significant (Jiang et al. 2022). Beta diversity has no relation to T2DM status, as shown by the results of beta diversity measurements.

Taxonomic analysis at the phylum level showed a significant increase in Firmicutes and a significant decrease in Proteobacteria in the T2DM group. Human studies have reported similar findings (Bhute et al. 2017; Nuli et al. 2019). Firmicutes play an important role in forming short-chain fatty acids (SCFA) in the form of butyrate, which is important for maintaining intestinal integrity by maintaining optimal glucose-energy levels and reducing glucose synthesis

by the liver (Cao et al. 2019). Excess of Firmicutes is positively correlated with increased body weight, which is a factor in developing T2DM disease (Jandhyala et al. 2015). Firmicutes are also known to positively correlate with plasma glucose through an increasing monosaccharide harvest mechanism from the host intestine, causing increased hepatic triglyceride production and insulin resistance (Zhang et al. 2013). Furthermore, it is known that Proteobacteria has a negative correlation with blood glucose levels (Ahmad et al. 2019). Consistent with the results of this study, Proteobacteria was the dominant phylum in the non-T2DM group with low plasma glucose levels. Proteobacteria are involved in the body's digestion, metabolism, and energy supply. Members of this phylum are linked to cellulose degradation, which contributes to the efficient use of carbon sources and energy accumulation. Proteobacteria are known to be metagenomic markers of dysbiosis (Shin et al. 2015), so decrease in their abundance can be proposed as an indicator of dysbiosis in the T2DM group.

A higher Firmicutes/Bacteroidetes (F/B) ratio was reported to be used as a metagenomic marker of T2DM gut microbiome (Kumar et al. 2022). Increased levels of Firmicutes in the T2DM group in this study resulted in a significantly higher F/B ratio. A higher F/B ratio promotes intestinal uptake and biosynthesis energy, which increases plasma glucose levels (Larsen et al. 2010).

The T2DM group showed an increase in *Oscillibacter* genera and a decrease in *Pseudomonas* genera. No further studies have been conducted on these results. However, hypoglycemic treatment in patients with T2DM has been reported to target DPP-4 inhibitors (dipeptyl peptidase 4) to lower blood glucose levels primarily through inhibition of GLP-1 (glucagon-like peptide) degradation. It is known that DPP-4 inhibitors restore dysbiosis of the gut microbiota by modulating the gut microbiota through an increase in *Lactobacilli* spp. and propionate production as the abundance of *Oscillibacter* decreases (Olivares et al. 2018).

In the case of human T2DM, *Oscillibacter valericigenes* was identified as a microbe that causes insulin resistance because it is a specific microbe that promotes Mmp^{12+} (macrophage metalloelastase) expression, causing insulin resistance by increasing glucose metabolism (Li et al. 2022). *Oscillibacter ruminantium* bacteria were also positively correlated with decreased blood glucose levels and negatively correlated with increased HOMA-β scores in human T2DM patients (Huang et al. 2022). As reported in this study, *O. valericigenes* and *O. ruminantium* increased in the T2DM group. The significant abundance in the T2DM group indicated that these two bacteria could be proposed as specific bacteria for T2DM in primates and could potentially become targets for T2DM prevention and therapy management.

Although research on T2DM and gut microbiome has been carried out, the gut microbiome profile of healthy long-tailed macaques cannot yet be determined because many factors influence differences in microbiome patterns. The exact role of gut microbiota in T2DM is also still being investigated, which is limitation of this study. Investigating the role of gut microbiota may help predict metabolic functions affected by changes in gut microbiota. Nevertheless, this study's characterization of the gut microbiome is a first step to demonstrating the precise role of the T2DM gut microbiota. Characterization of the full-length 16S rRNA gene has a real impact on the resulting data and minimizes bias. Shared microbiota and unique microbiota were successfully obtained which are useful for establishing biomarkers of health and disease. Further studies are strongly recommended to complete the information on the causal relationship between changes in gut microbiota and T2DM.

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