**Journal of Medical Laboratory Science, 2020; 30 (3): 136-150**
**http://jomls.org ; info@jomls.org** **Oshim *et al***
**http://doi.org/10.5281/zenodo.4050381**

# Gut Microbiota Compositions and Modulation of Bacterial Metabolic Functional Genes in Type -2 Diabetes Mellitus Individuals at Nnewi, Anambra State, Nigeria.

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**ABSTRACT**

**Background**: There is paucity of information on the gut microbiota compositions of Type 2 Diabetes Mellitus (T2DM) subjects in South East of Nigeria, with Next-Generation Sequencing technology (NGS). Gut microbiota dysbiosis has been associated with metabolic disorders, such as obesity, insulin resistance and T2DM. The objectives of this study are to determine gut microbiome compositions in T2DM individuals and healthy controls and to predict bacterial metabolic functional gene variations. **Methods**: Stool samples were collected from 110 confirmed T2DM and 10 non-T2DM subjects and bacterial DNA extracted. The V4 regions of bacterial 16S rRNA were amplified and sequenced using Illumina NextSeq 500 platform. The raw pair-end FASTQ sequence files were imported into EzBioCloud pipeline and Illumina BaseSpace for taxonomic identification. **Results**: The species richness or alpha diversity were lower in T2DM subjects when compared with the healthy controls. Similar trend was observed in phylogenetic diversity and Shannon index. *Firmicutes* (63.21% vs 64.89%) were the most abundant in the gut of both T2DM and healthy controls, followed by *Bacteroidetes* (18.46% vs 22.35%), *Proteobacteria* (12.77% vs 6.93%), and *Cyanobacteria* (2.24% vs 1.71%). There was significant lower relative abundance of *Bifidobacterium pseudolongum*, *Romboutsia timonensis*, *Blautia coccoides* and others as a marker in T2D patients than healthy controls. The relative abundance of *Bacteriodes vulgatus*, *Prevotella copri* were significantly higher ($p < 0.05$) among T2DM subjects than Non-T2DM subjects. *Lactobacillus mucosae*, *L. salivarius* and *Lactococcus lactis* were significantly higher in T2DM subjects. Over 156 metabolic functional genes were significantly downregulated in T2DM subjects, and bacterial gene ortholog K20890-xylan alpha-glucuronosyltransferase involved in the metabolism of non-digestible fibre was turned off in T2DM patients. **Conclusion**: The high abundance of *Prevotella copri* and low abundance of *Akkermansia muciniphila* in T2DM represents intestinal pathobionts signatures associated with T2DM development. Butyrate and propionate-producing bacteria found in lower abundance in T2DM offer support for dietary or probiotics intervention.

**Keywords**: Type 2-diabetes mellitus, healthy controls, gut, microbiota, *Prevotella*, *Akkermansia*, *Lactobacillus*, *Clostridium*, metabolic functions,

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**Author's contributions**: This study was carried out and approved in collaboration between all the authors who take responsibility for it's accuracy and integrity. KCA designed the study; KCA, IOO sourced for funding; KCA wrote the protocol; IOO and KCA contributed in literature search; IOO, KCA did statistical analysis; IOO and KCA contributed in discussions; IOO drafted the manuscript; KCA and NRA supervised the study; KCA wrote the final manuscript; KCA, IOO and NRA proofread the final version for publication.

**Received**: 06/16, 2020; **Accepted**: 08/07, 2020; **Published**: 09/25, 2020.

**Citation**: Oshim IO, Agbakoba NR, Anukam KC. Gut Microbiota Compositions and Modulation of Bacterial Metabolic Functional Genes in Type -2 Diabetes Mellitus Individuals at Nnewi, Anambra State, Nigeria **J Med Lab Sci, 2020; 30 (3): 136-150**

## INTRODUCTION

Type-2 diabetes (T2DM) characterized by insulin resistance, glucose intolerance, fat deposition, dyslipidemia, and systemic inflammation (**1**) is a non-communicable disease that is increasingly becoming a global burden with a projection as the 7th leading cause of death by the year 2030 (**2**). According to the International Diabetes Federation (**3**), by 2045, over 700 million will be affected with type 2 diabetes, which accounts for over 90% of all diabetes cases globally. Due to westernization of Nigerian diet, a large number of individuals are having T2DM, estimated between 8–10% of the population (**4**). The gut microbiota performs important functions especially in the metabolism of non digestible carbohydrates into short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate (**5**). There is a strong association of SCFAs with G protein coupled-receptor (GPR) GPR43 and GPR41 in the improvement of plasma levels of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), for maintenance of glucose homeostasis and reduction of appetite (**6**). Studies on animal models have reported that butyrate activates the expression of genes involved in intestinal gluconeogenesis through cAMP-dependent mechanism, whereas propionate, which is a substrate for gluconeogenesis, promotes intestinal gluconeogenic gene expression through a gut–brain neural circuit linking GPR41(**7**). In the last decade, studies with 16S rRNA sequencing and quantitative real time polymerase chain reaction (qPCR) are consistently reporting a relationship between the composition of the intestinal microbiota and diabetes (**8, 9**). Most of the studies concentrated in the Western hemisphere, for example in European individuals with T2DM, a depletion in Firmicutes was observed when compared with the healthy population (**10**). Among the Asians, several studies have reported variations in microbiota compositions in T2DM patients (**11, 12**). In North America, gut microbiota changes exist among Opioid users (**13**), while few studies on Type 1Diabetes have been link with changes in gut microbiome in Africans (**14**). Recently, in Nigeria a study demonstrated the relative abundance of *Bifidobacteriaceae*, *Collinsella*, and *Ruminococcus* in the gut of the elderly population with T2DM (**15**). Another study in Nigeria revealed that *Clostridiaceae*, and *Peptostreptococcaceae* were significantly lower in T2DM patients in urban Nigeria than controls without T2DM (**16**). Some intervention studies examining the impact of dietary fiber consumption on insulin resistance and prevention of type 2 diabetes have documented relative positive results (**17**). For example, branch-chain amino acid-producing species such as *Prevotella copri* and *Bacteroides vulgaris* have been found to impact positively on the host serum metabolome and insulin sensitivity (**18**, **19**). There are no robust data on the microbial signatures associated with T2DM in Nigeria. The objectives of this study are two folds; First, to determine the bacteriome compositions in T2DM adult individuals and healthy controls and second, to predict bacterial metabolic functional genes in the gut of adult individuals with type 2 diabetes and healthy controls residing at Nnewi, South East Nigeria.

## MATERIALS AND METHOD

### T2DM subjects and controls enrollment

Type 2 diabetes subjects with random blood glucose levels greater than or equal to 126 mg/dL (**3**) were enrolled at Diabetic Clinic, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria from December,2018 to March, 2020. A simple random sampling was used to obtain samples from the subjects with a total of 110

confirmed T2DM subjects, ranging from 20 to 80 years for inclusion in the present study. Ten (10) age-matched, healthy controls, without T2DM familial history were also enrolled. The study was performed with the approval of Research Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria (Process number :**NAUTH/CS/VOL.11/183/2018/121**).

Subsequent to consent of the subjects, peripheral blood samples were collected for blood glucose levels. Stool samples were also requested for and obtained from the subjects and these were transferred into separate ubiome sample tubes following uBiome sample collection instructions. These samples were stored at ambient temperature before being shipped to uBiome in San Francisco (USA) for processing.

**DNA Extraction, PCR amplification, and sequencing**

DNA was extracted individually from all subjects' stool samples using QiaAMP mini stool kit (Qiagen, Valencia, CA, USA). To assess the composition and diversity of the subjects' gut bacterial communities, we were able to use only 22 T2DM samples out of 110 T2DM samples and 10 non-DM samples with intact and good quantity of DNA to conduct high-throughput sequencing of the V4 region of the 16S rRNA. PCR amplification was performed on this region with primer pair (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT) as previously described (**20**). DNA samples were barcoded with a unique combination of forward and reverse indexes allowing for simultaneous processing of multiple samples. PCR products were pooled, column-purified, and size-selected through microfluidic DNA fractionation to obtain equimolecular concentrations. Consolidated libraries were quantified by quantitative real-time PCR using the Kapa Bio-Rad iCycler qPCR kit on a BioRad MyiQ before loading into the sequencer. Sequencing was performed in a pair-end setup on the Illumina NextSeq 500 instrument rendering 2 x 150 bp pair-end sequences at the uBiome Inc. in San Francisco, USA. The sequencer has a flow cell with four lanes. This means that each sample was read in four different lanes (L001 to L004), and each produced forward (R1) and reverse (R2) reads.

**16S rRNA Metagenomics Sequence Analysis**

The raw pair-end FASTQ sequence files (13 samples representing T2D and 6 healthy controls) were imported into EzBioCloud pipeline for taxonomic identification. 16S rRNA database and Reference Genome Database (RefGD) were the two databases that were used. The EzBioCloud database uses a hierarchical taxonomic system containing 207 phyla, 433 classes, 1019 orders, 2805 families, 11 446 genera, 61 700 species and 387 subspecies. This classification verges basically on the maximum likelihood phylogeny for 16S rRNA gene sequence data, where 97 % similarity cut-off was used for the recognition of phylotypes **(21)**.

The valid reads consist of the number of reads obtained after using a pre-filter to remove low quality reads from raw data produced by the sequencing platform. Reads with short lengths and low Q-values (<30) are removed by the pre-filter, and in the case of paired-end sequencing, unmerged reads are also filtered out. Low quality amplicons (too short, too long, erroneous sequenced, non-specific products created during PCR) were filtered out. Chimeric amplicons and non-target amplicons and that do not match the PCR target taxa. (e.g. reads identified as Archaea or Eukarya) were also removed.

Alpha-diversity was calculated for species richness by Abundance Coverage Estimate (ACE), Chao1 and Jackknife method, while

diversity indexes were calculated by Shannon, Non-parametric Shannon and Simpson index. Principle coordinate analysis (PCoA) with Jensen-Shannon divergence distance metrices were used to evaluate beta diversity for T2D and healthy controls (**22**). Linear discriminant analysis (LDA) effect size (LEfSe) (**23**) was used to identify biologically and statistically significant differences in the OTU relative abundance. Phylogenetics Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to predict the metabolic function of the metagenomes from the 16S rRNA gene dataset (**24**) with reference to Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologs categorizations (**25**).

## RESULTS

In T2DM participants, the species richness or alpha diversity symbolised by ACE, and CHAOI were lower when compared with the healthy controls. Similar trend was observed in diversity indices such as Shannon, Simpson and Phylogenetic diversity **(Figure 1**). Rarefaction curves showing the number of reads for both T2DM and healthy controls are shown in **Figure 2**.

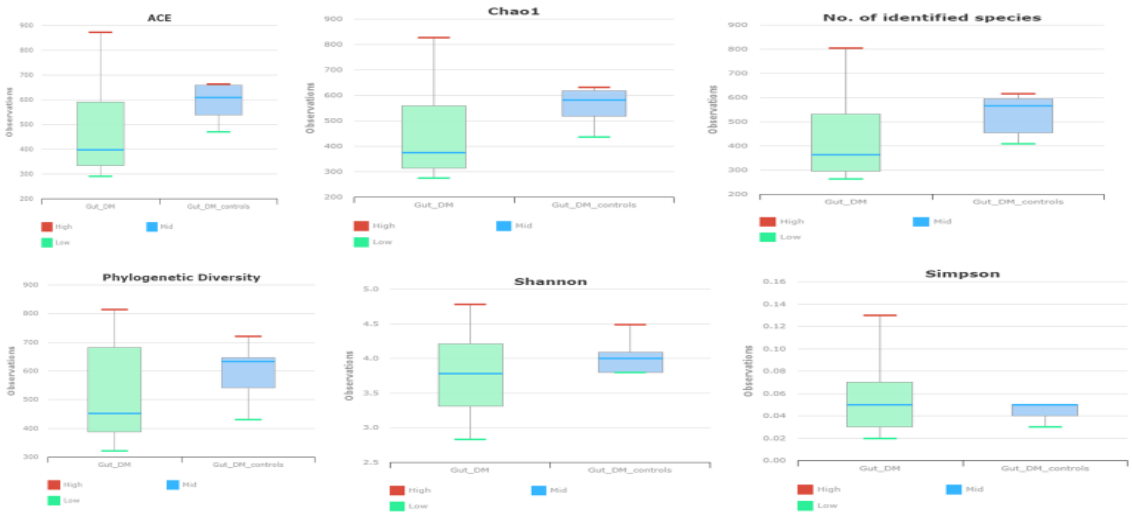


**Figure 1: Alpha diversity indices in T2DM and healthy controls**.

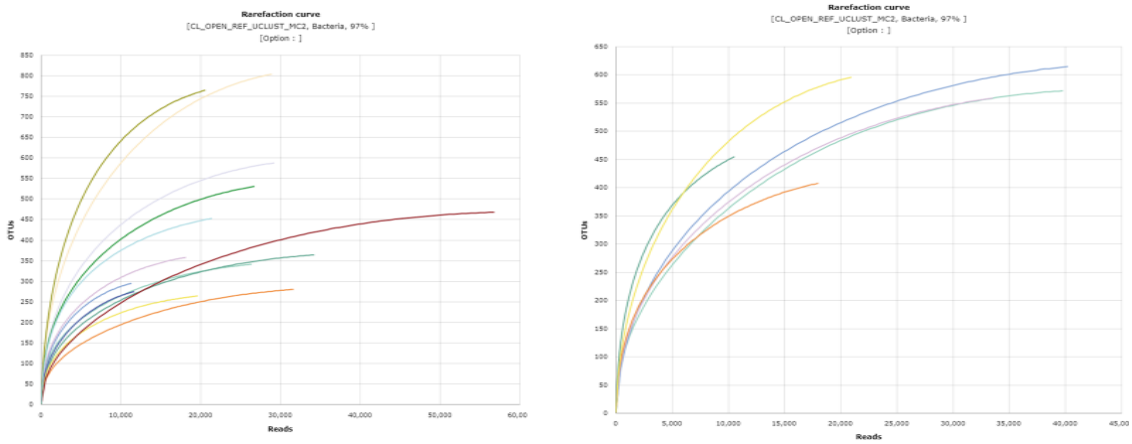


**Figure 2: Rarefaction curves, showing the number of sequence reads in T2DM subjects and healthy controls.**

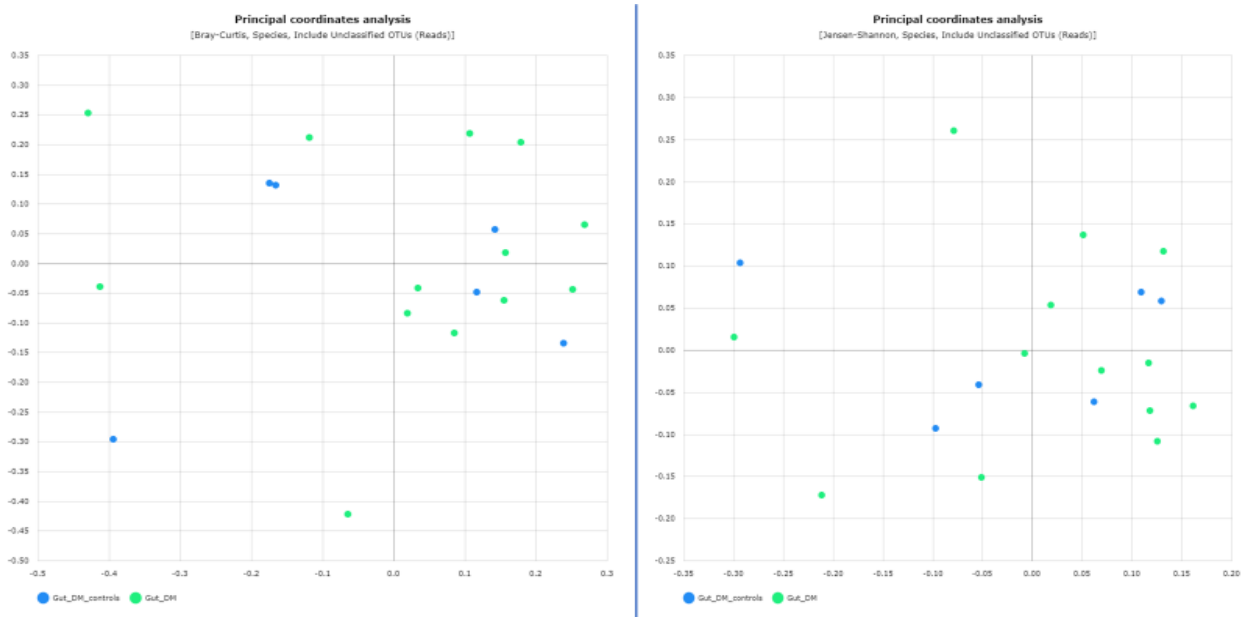


**Figure 3: Principal Coordinate Analysis (PCoA) showing clustering of taxonomic species**

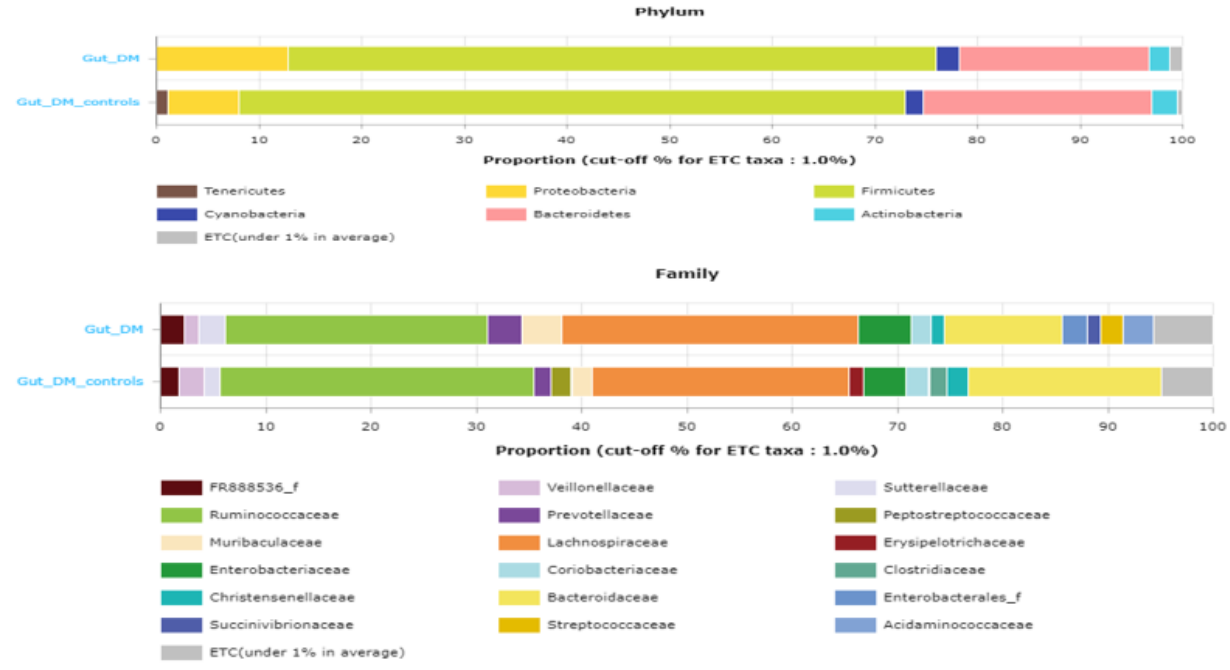


**Figure 4: Taxonomic representation at phyla and Family level categories in T2DM and healthy controls.**

Beta- diversity was determined by Principal Coordinate analysis, using Jensen-Shannon and Bray-curtis dissimilarity metrices which did not show distinct clustering of all the OTUs in T2D and healthy controls as presented in **Figure 3**.

Taxonomic representation at phyla level shows that *Firmicutes* (63.21% vs 64.89%) were the most abundant in the gut of both T2DM and healthy controls, followed by *Bacteroidetes* (18.46% vs 22.35%), *Proteobacteria* (12.77% vs 6.93%), *Cyanobacteria* (2.24% vs 1.71%), *Actinobacteria* (2.02% vs 2.53%) and *Tenericutes* (0% vs 1.12%) respectively.

At the family taxonomic category, *Lachnospiraceae* (28.12% vs 24.43%), *Prevotellaceae* (3.34% vs 1.64%) were higher in relative abundance in T2DM patients than healthy control, while *Ruminococcaceae* (24.84% vs 29.88%), *Bacteroidaceae* (11.20% vs 18.35%) and *Christensenellaceae* (1.27% vs 2.00%) were in lower in T2DM than healthy controls as shown in **Figure 4**. At the genera taxonomic categories, Bacteroides (14.99% vs 23.81%) occurred as the most relative abundant genera in T2DM patients in contrast to *Faecalibacterium* (12.24% vs (15.80%). Other genera were as follows; *Escherichia* (5.38% vs 5.85%), *Blautia* (4.95% vs 5.35%), *Ruminococcus*_g2 (3.97% vs 4.48%), *Roseburia* (3.83% vs 4.40%), *Streptococcus* (2.56% vs 0.37%), *Subdoligranulum* (2.56% vs 1.30%), *Phascolarctobacterium* (2.40% vs 0.26%), *Parasutterella* (2.35% vs 0.55%), *Enterobacterales*_g (2.26% vs 0.49%), *Oscillibacter* (2.02% vs 2.32%), *Prevotella* (1.71% vs 0.69%), *Akkermansia* (0.16% vs 0.37%) and other shown in **Figure 5.**

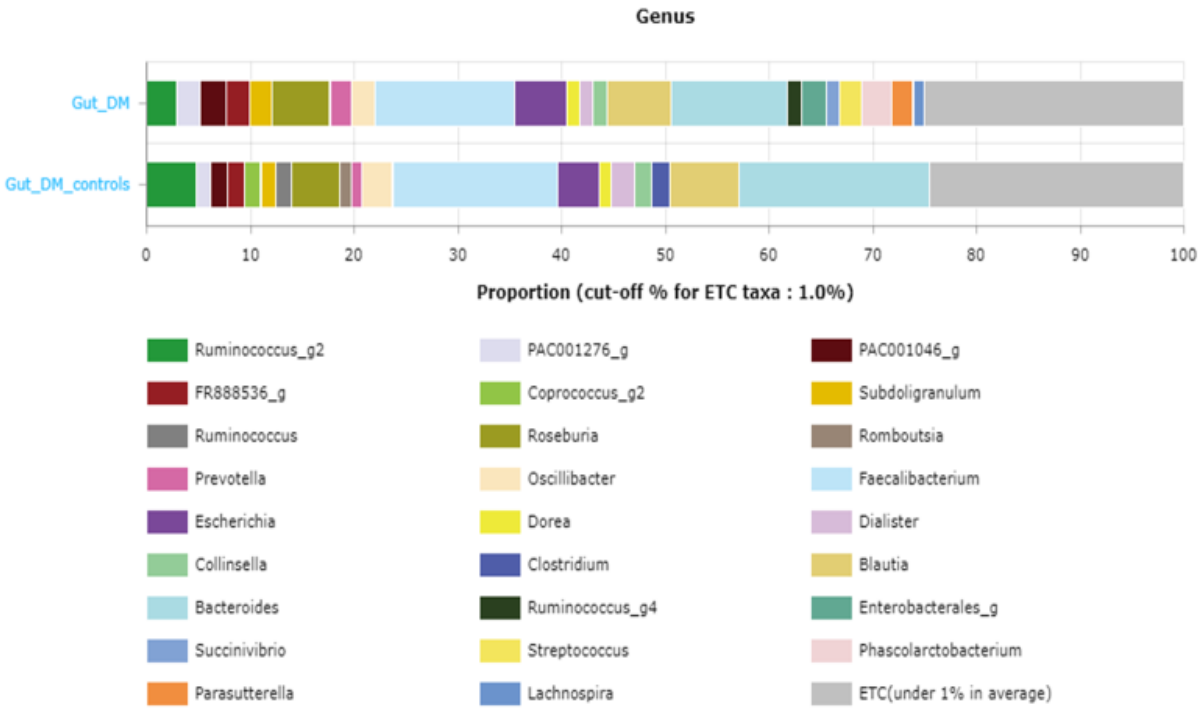

**Figure 5: Genera taxonomic categories showing proportion of relative abundance at 1% cut-off.**

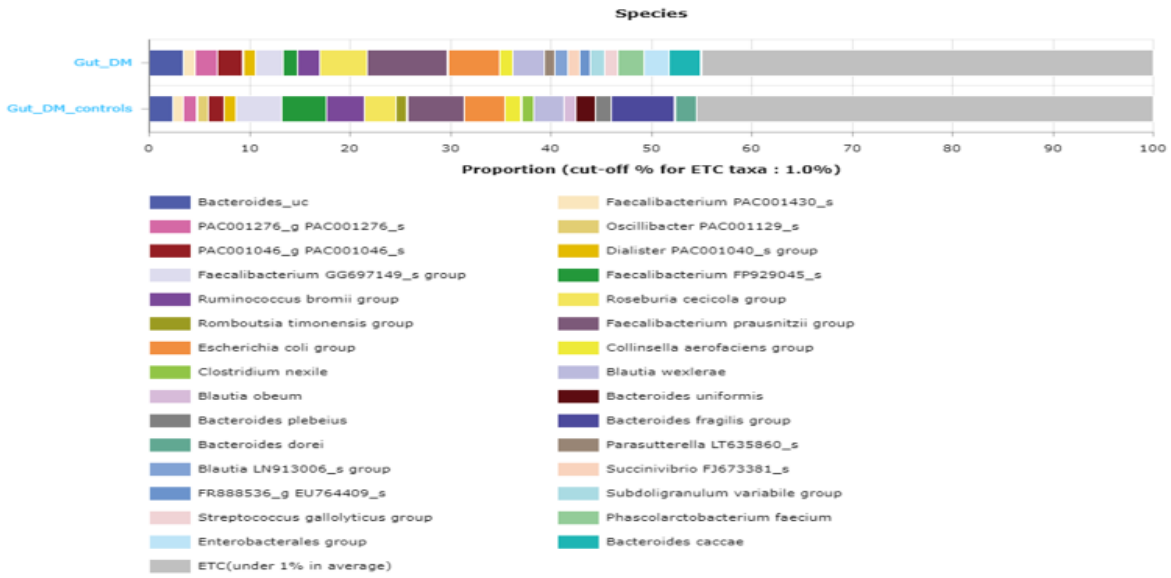


**Figure 6: Species taxonomic categories showing proportion of relative abundance at 1% cut-off.**

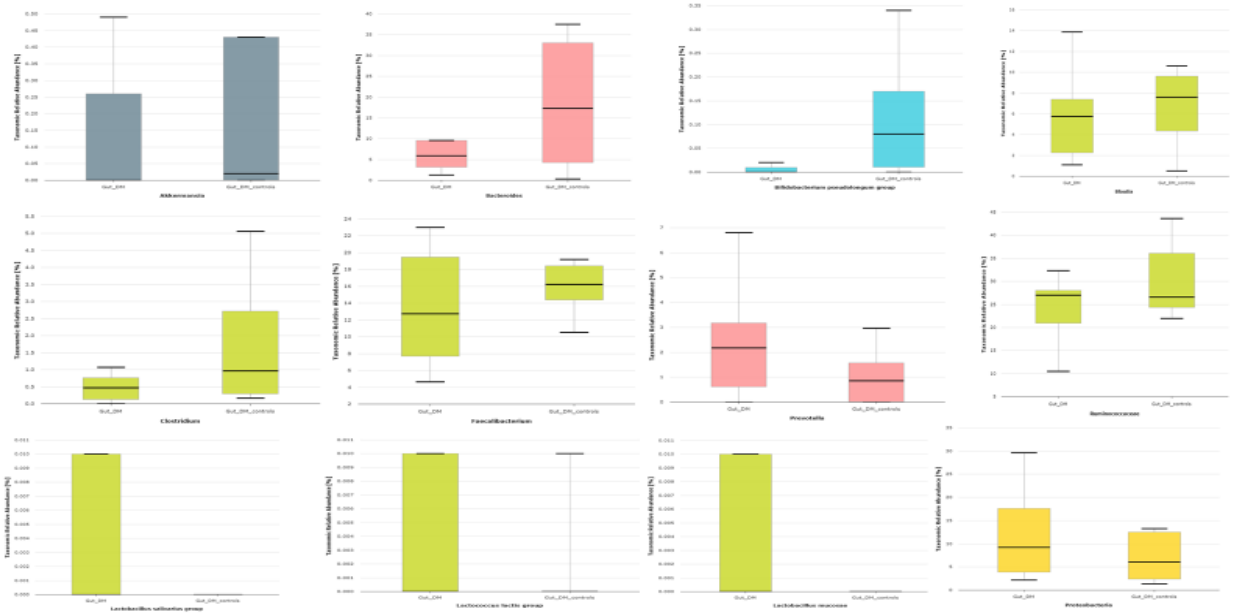

**Figure 7: Differences in the relative abundance of some selected taxa**

At the species taxonomic level, *Faecalibacterium prausnitzii* group (7.92% vs 5.55%) were higher in abundance in T2DM than healthy controls, followed by *Escherichia coli* group (5.00% vs 4.08%), *Roseburia cecicola* group (4.66% vs 3.10%), *Bacteroides*_uc (3.30% vs 2.34%), *Bacteroides caccae* (3.14% vs 0%), *Blautia wexlerae* (3.08% vs 3.05%), *Faecalibacterium* GG697149_s group (2.68% vs 4.55%), *Phascolarctobacterium faecium* (2.66% vs 0%),*Enterobacterale*s group (2.46% vs 0%) and presented in **Figure 6.**

The differences in the relative abundance of some selected taxa are shown in **Figure 7**. Surprisingly, two species of Lactobacillus (*Lactobacillus mucosae*, *Lactobacillus salivarius*) and *Lactococcus lactis* occurred in higher relative abundance in T2DM than in healthy controls. Taxonomic biomarkers with Linear discriminant analysis (LDA) effect size presented in **Table 1**, showing significant relative abundance of *Bifidobacterium pseudolongum*, *Romboutsia timonensis*, *Blautia coccoides* and others as a marker in healthy controls unlike in T2D patients.

Bacterial metabolic functional genes as revealed by PICRUSt shows that 249 gene orthologs were significantly expressed, of which 156 functional genes were significantly downregulated in T2DM subjects. Among the gene orthologs, 10 bacterial functional genes were significantly turned off in T2D Msubjects, which includes **xylan alpha-glucuronosyltransferase** (K20890), polypyrimidine tract-binding protein 2 (K14948), integrin alpha E (K06524), cyclic AMP-responsive element-binding protein 5 (K09047) and solute carrier family 35, member E3 (K15285) as shown in **Table 2**. Glycolysis (Embden-Meyerhof pathway- **ko00010**, **ko01100**, **ko01200**)-the conversion of glucose to pyruvate was also significantly (*P*= 0.0225) down-regulated in T2DM subjects.

**Table 1:** Taxonomic biomarkers with Linear discriminant analysis (LDA) effect size

| Taxon name | Taxon rank | Taxonomy | LDA effect size | p-value | p-value (FDR) | DM | Controls |
|---|---|---|---|---|---|---|---|
| Romboutsia | Genus | Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae | 3.70878 | 0.00134 | 0.00134 | 0.08522 | 1.10786 |
| Romboutsia timonensis group | Species | Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae: Romboutsia | 3.70406 | 0.00134 | 0.00134 | 0.08333 | 1.09492 |
| Terrisporobacter | Genus | Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae | 2.58057 | 0.00301 | 0.00302 | 0.14737 | 0.22329 |
| Terrisporobacter petrolearius | Species | Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae: Terrisporobacter | 2.57937 | 0.00301 | 0.00302 | 0.14297 | 0.21869 |
| Peptostreptococcaceae | Family | Firmicutes: Clostridia: Clostridiales | 3.85616 | 0.00501 | 0.00503 | 0.47369 | 1.90963 |
| Intestinibacter | Genus | Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae | 2.35367 | 0.01738 | 0.01754 | 0.23153 | 0.27648 |
| Intestinibacter bartlettii | Species | Firmicutes: Clostridia: Clostridiales: Peptostreptococcaceae: Intestinibacter | 2.34708 | 0.01738 | 0.01755 | 0.22938 | 0.27365 |
| Enterococcus faecium group | Species | Firmicutes: Bacilli: Lactobacillales: Enterococcaceae: Enterococcus | 2.06095 | 0.01912 | 0.01932 | 0.02270 | 0.00000 |
| Turicibacter | Genus | Firmicutes: Erysipelotrichi: Erysipelotrichales: Erysipelotrichaceae | 2.83007 | 0.02272 | 0.02297 | 0.07491 | 0.20992 |
| Turicibacter sanguinis | Species | Firmicutes: Erysipelotrichi: Erysipelotrichales: Erysipelotrichaceae: Turicibacter | 2.78133 | 0.02272 | 0.02299 | 0.07279 | 0.19344 |
| Sutterella wadsworthensis | Species | Proteobacteria: Betaproteobacteria: Burkholderiales: Sutterellaceae: Sutterella | 3.54075 | 0.02688 | 0.02724 | 0.00072 | 0.69518 |
| Ruminococcaceae_uc | Genus | Firmicutes: Clostridia: Clostridiales: Ruminococcaceae | 3.06023 | 0.02833 | 0.02875 | 0.18259 | 0.41211 |
| Blautia coccoides group | Species | Firmicutes: Clostridia: Clostridiales: Lachnospiraceae: Blautia | 2.30454 | 0.03223 | 0.03274 | 0.04319 | 0.00318 |
| Fenollaria | Genus | Firmicutes: Tissierellia: Tissierellales: Peptoniphilaceae | 2.07920 | 0.03246 | 0.03309 | 0.00000 | 0.02372 |
| Fenollaria massiliensis group | Species | Firmicutes: Tissierellia: Tissierellales: Peptoniphilaceae: Fenollaria | 2.07920 | 0.03246 | 0.03312 | 0.00000 | 0.02372 |
| Bifidobacterium pseudolongum gr | Species | Actinobacteria: Actinobacteria_c: Bifidobacteriales: Bifidobacteriaceae: Bifidobacterium | 2.72765 | 0.04136 | 0.04281 | 0.00724 | 0.11371 |
| Clostridium_uc | Species | Firmicutes: Clostridia: Clostridiales: Clostridiaceae: Clostridium | 2.54508 | 0.04183 | 0.04332 | 0.03827 | 0.10806 |
| Fusobacterium nucleatum group | Species | Fusobacteria: Fusobacteria_c: Fusobacteriales: Fusobacteriaceae: Fusobacterium | 2.00263 | 0.04914 | 0.05097 | 0.00085 | 0.02007 |

**FDR: False Discovery Rate**

**Table 2:** Bacterial metabolic functional genes that were down-regulated

| Gene Ortholog | Definition | p-value | p-value (FDR) | T2DM | Controls |
|---|---|---|---|---|---|
| K08077 | UDP-sugar diphosphatase | 0.004632537 | 0.004637342 | 1.29709E-05 | 0.000104628 |
| K17214 | inositol transport system permease protein | 0.00500696 | 0.005012554 | 0.000850761 | 0.000384328 |
| K21401 | menaquinone-9 beta-reductase | 0.00500696 | 0.005013354 | 5.99575E-05 | 0.000167784 |
| K05672 | ATP-binding cassette, subfamily C (CFTR/MRP), member 12 | 0.006316474 | 0.006326055 | 9.60627E-05 | 0.000335219 |
| K21350 | sucrose 6(F)-phosphate phosphorylase | 0.006316474 | 0.00632656 | 2.76177E-05 | 0.00011805 |
| **K20890** | **xylan alpha-glucuronosyltransferase** | 0.007094394 | 0.007109696 | **0** | 3.68453E-06 |
| K14948 | polypyrimidine tract-binding protein 2 | 0.007094394 | 0.007110264 | **0** | 3.68453E-06 |
| K06524 | integrin alpha E | 0.007094394 | 0.007110832 | **0** | 4.30664E-06 |
| K11194 | PTS system, fructose-specific IIA component | 0.008393861 | 0.008417345 | 5.54972E-05 | 0.000356526 |
| K19421 | polysaccharide biosynthesis protein EpsC | 0.010975803 | 0.011017079 | 0.008288215 | 0.011771776 |
| K19428 | sugar transferase EpsL | 0.014058605 | 0.014123905 | 0.000431359 | 0.00339907 |
| K14258 | facilitated trehalose transporter | 0.014058605 | 0.014128431 | 3.22685E-05 | 7.26313E-05 |
| K14032 | nuclear receptor subfamily 2 group F member 3 | 0.032459283 | 0.032910507 | **0** | 2.97128E-06 |
| K15285 | solute carrier family 35, member E3 | 0.032459283 | 0.032913168 | **0** | 7.41493E-06 |
| K16131 | microcystin synthetase protein McyB | 0.032459283 | 0.032915828 | **0** | 1.14756E-05 |
| K02132 | F-type H+-transporting ATPase subunit alpha | 0.032459283 | 0.032918489 | **0** | 1.43261E-06 |
| K05282 | gibberellin-44 dioxygenase | 0.032459283 | 0.03292115 | **0** | 6.70356E-06 |
| K09047 | cyclic AMP-responsive element-binding protein 5 | 0.032459283 | 0.032923812 | **0** | 4.61927E-06 |
| K15059 | 2-aminophenol/2-amino-5-chlorophenol 1,6-dioxygenase subunit beta | 0.032459283 | 0.032926474 | **0** | 6.70356E-06 |

**FDR= False Discovery Rate**

## DISCUSSIONS

In this study, we determined the gut microbiota of individuals diagnosed with T2DM and healthy controls at Nnewi, Anambra State, Nigeria, for the purpose of understanding what type of bacterial communities dominate the tested cohorts in the population. The species richness represented as alpha-diversity, was lower in T2DM subjects. This finding corroborates previous studies in T2DM subjects whereby alpha-diversity is often decreased (**26**, **27**). However, a recent study in Nigerian subjects with T2DM that reside in urban communities have the gut microbiome profiles with increased alpha-diversity (**16**). Our study revealed that the dominant microbiota of the phyla taxonomy in the gut of the T2DM participants include; *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Verrucomicrobia*, and *Actinobacteria*. The proportion of *Bacteroidetes* and *Proteobacteria* were higher in T2DM patients, which is consistent in a previous study that found higher abundance of *Proteobacteria* in the gut microbiota of elderly T2DM patients (**15**).

It should be noted that the outer membranes in gram-negative bacteria are lipopolysaccharides (LPS), known as potent stimulators of inflammation, which can potentially induce endotoxaemia in the bloodstream and induce systemic inflammation (**28**).

Firmicutes decreased in T2DM subjects compared to controls. The significant relative abundance of Clostridiaceae and Peptostreptococcaceae may explain the difference as these two taxa occurred in lower abundance. A similar study from Denmark also reported that Firmicutes and Clostridiales were significantly reduced in T2DM subjects (**10**). However, some studies from China presented conflicting results showing an increase in the relative abundance of Firmicutes in T2DM subjects (**11**,**29**).

This study revealed that *Lachnospiraceae* family and *Prevotellaceae* (Genus-*Prevotella*) were enriched in subjects with T2DM than healthy controls, while *Ruminococcaceae* (Genus-*Ruminococcus*), *Bacteroidaceae* (Genus-Bacteroides), and *Christensenellaceae* were reduced in T2DM patients.

It is not surprising to observe the depletion of members of the family *Bifidobacteriaceae* especially *Bifidobacterium pseudolongum* in individuals with T2DM in the study population, as it lends credence to previous studies reporting the deprivation of family Bifidobacteriaceae in T2DM individuals rather than healthy individuals (**30**). Our finding is in contrast to previous studies in some fulani tribe that live a nomadic pastoral lifestyle in Nigeria (**31**), and in rural Bassa and urban settlers (**32**), where members of the family *Bifidobacteriaceae* appears to have been depleted in healthy individuals. This suggests that gut microbiome for individuals with T2DM may vary from regional location probably due to differences in dietary practises and glucose tolerance. A previous study with animal model demonstrated that some members of the *Bifidobacteriaceae* especially *Bifidobacterium pseudolongum* are linked with improved glucose tolerance (**33**).

The depletion of *Ruminococcaceae* in T2DM subjects was not surprising as members of these family in the human gut microbiota are involved in the metabolism of plant fibres or polysaccharides (**34**). Studies also have found that reduction of *Ruminococcus* in the gut are associated with high-fat diet (**35**). The significantly higher levels of some *Lactobacillus* species especially *Lactobacillus mucosae*, *Lactobacillus salivarius* and *Lactococcus lactis* in diabetic subjects compared to controls in this study

raises many questions how these lactic acid bacteria might potentially contribute to inflammatory response in diabetic subjects and at the same time involved in immuno-modulatory processes (**36**). Nevertheless, some studies have previously reported the role of *Lactobacillus/Lactococcus* in relation to type 2 diabetes in mice models (**37**).

Several taxa at the genus and species level were differentially abundant between T2DM and healthy controls. For example, the genus *Clostridium* (*Clostridium nexile)* were significantly decreased in T2DM subjects compared to healthy controls and recent studies have shown that most Clostridium spp. are butyrate producers in the colon (**38**). It has been established that T2DM patients have decreased Short Chain Fatty Acid or butyrate-producing bacteria and recent studies have demonstrated that reduced butyrate production is associated with insulin resistance (**39**).

This study revealed that mucin-degrading spp such as *Akkermansia* were decreased in higher proportion in T2DM subjects compared to healthy controls. A study reported that dietary intervention with functional foods reduces metabolic endotoxaemia and attenuates biochemical abnormalities by modifying faecal microbiota in people with type 2 diabetes (**40**). In the study, there was a decrease in *Prevotella copri* and increases in *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* as found in healthy control in this study.

Metabolic functional genes of the gut microbiota as predicted by 16S rRNA data set revealed down-regulation of important bacterial metabolic pathways in T2DM subjects. For example, the gene ortholog K20890- xylan alpha-glucuronosyltransferase was turned off in T2DM patients, which may have affected the production of SCFA such as butyrate and propionate. It is worth knowing that non-digestible dietary fibre derived from plant material is highly enriched in the lignocellulosic polysaccharides, cellulose and xylan. Genes encoding enzymes involved in xylan degradation have been identified previously in *Bacteroidetes* members from the human colon such as *Bacteroides ovatus* V975 and *Bacteroides xylanisolvens* XB1A (**41**). In this study, *Bacteroides xylanisolvens, Bacteroides uniformis, Bacteriodes dorei, Bifidobacterium gallicum, Ruminococcus bomii, Bacteroides thetaiotaomicron,* were significantly lower ($p<0.05$) among T2DM subject than non-T2DM subject. The reduction of these bacterial species may explain why some important metabolic functional genes were down-regulated in T2DM subjects.

**Conclusion**: This is the first study from the South Eastern part of Nigeria that has provided new insight and relevant information on the composition of the gut microbiota in association with the diabetic state in the tested population. We report that significant reduction in *Ruminococcus* taxa, *Akkermansia muciniphila, Faecalibacterium taxa, Clostridium nexile, Bifidobacterium pseudolongum, Romboutsia timonensis,* and *Blautia coccoides* were identified as taxonomic markers in T2DM subjects unlike in healthy controls. The study also revealed that bacterial metabolic functional genes involved in metabolism of non-digestible dietary fibres were either turned off or down-regulated in T2DM.

**Acknowledgements:**
We wish to thank all the participants who consented to this study.

**Data Availability**
The data used to support the findings of this study are included in the article. The raw data of this study will be made available by the corresponding author on reasonable request.

**Conflicting interests:** Authors declare no conflict of interests.

**Funding source:**
The study was funded by **uBiome Inc**., San Francisco, California, USA, under an academic consortium grant-in-kind award to **Dr. Kingsley C Anukam**. We thank Uzobiogene Genomics, London, Ontario, Canada for all the logistical supports.

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