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# Fermented sorghum improves type 2 diabetes remission by modulating gut microbiota and their related metabolites in high fat diet-streptozotocin induced diabetic mice

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ABSTRACT

Microbial metabolites play key roles in the pathogenesis of insulin resistance and type 2 diabetes (T2D). A preliminary study on the impact of fermented sorghum (FS) on T2D and its modulation of gut microbiota and their metabolites was assessed using 16S rRNA gene sequencing and metabolomics. FS supplementation ameliorated hyperglycemia, insulin resistance and reversed the abundance of opportunistic pathogenic bacteria (such as *Oscillibcater*, *Acetatifactor* and *Acetivibrio*) positively associated with T2D. FS promoted the growth of beneficial bacteria (*Muribaculum*, *Parabacteroides and Phocaeicola*) positively correlated with fecal butyrate and propionate inversely associated with T2D. FS decreased the serum concentration of microbial metabolites (p-cresol sulfate, indolelactate, indoxyl sulfate, indole-3-aldehyde). FS increased the levels of phenylpropionate, phenyl sulfate, valine, bile acids (taurocholic acid, ursodeoxycholic acid and cholic acid) inversely associated with T2D. The beneficial effect of fermented sorghum on T2D remission could be attributed to modulation of gut microbiota and its related-metabolites.

## 1. Introduction

In 2021, over 10.5% of the adult population worldwide (537 million people) were estimated to be living with diabetes and this prevalence has been projected to reach 783.2 million by 2045 according to the 10th edition IDF Diabetes Atlas report. Diabetes related health and socio-economic burden are increasing globally with enormous impact on quality of life (Sun et al., 2022). Diabetes, a growing global endemic is a chronic disease characterized by hyperglycemia resulting from impairment in insulin secretion, action or both caused by pancreatic β-cell dysfunction and insulin resistance in multiple organs (Chatterjee, Khunti, & Davies, 2017).

The gut microbiota has been recently purported to play critical role in health and diseases. The structure and function of the microbial community has been implicated in the development of T2D. Disruptions in the composition of the gut microbiota have been associated with T2D. A metagenome-wide association study found a link between T2D and gut microbial dysbiosis, with an increase in several opportunistic pathogens and a decrease in butyrate-producing bacteria (Qin et al., 2012). In another recent study, diabetic patients exhibited significantly lower proportions of butyrate-producing bacteria such as *Bifidobacterium* and *Akkermansia* and higher abundance of *Dorea* (Li et al., 2020). The gut microbiota is involved in several functions related to host physiology, metabolism, intestinal barrier integrity and immune processes (Nicholson et al., 2012). Most importantly, microbial metabolites including those synthesized de novo by gut microbes, produced from dietary components and host metabolites biochemically transformed by bacteria serve as key signaling molecules that contribute to host health via host-microbiome and also microbiome-microbiome interaction (Postler & Ghosh, 2017). For example, increased levels of serum p-cresol, a

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bacterial tyrosine derivative converted by host to p-cresyl sulfate contribute to the development of insulin resistance and T2D (Koppe et al., 2013). In addition, other studies have found increased levels of branched-chain amino acids (BCAAs) in the serum of insulin resistant and T2D patients (Pedersen et al., 2016). Indoxylsulfate, an indole derivative produced as a host-microbial co-metabolite is a uremic toxin known to induce insulin resistance (Lau, Savoj, Nakata, & Vaziri, 2018). However, high levels of indole propionate, a bacterial tryptophan metabolism derivative was reported in people with low T2D risk (De Mello et al., 2017).

Diet is emerging as one of the key driving factors that impacts the structure and function of the gut microbiota. Dietary intervention has proven effective in the re-shaping of a perturbed gut microbiota and the production of beneficial microbial metabolites. Non-digestible carbohydrates in dietary fiber (whole grains, resistance starch to mention a few) are accessible and fermented by gut microbes to produce short-chain fatty acids (SCFAs) and other metabolites with healthy gut barrier maintenance and immune system improvement (Wastyk et al., 2021). The consumption of fermented foods have been linked with improvements in health markers related to decreased risk of weight gain, diabetes, cardiovascular diseases and cancers. A recent human studies found that fermented food consumption increased microbiota diversity and decreased inflammatory markers whilst high-fiber diet resulted in an increase in microbiome-encoded glycan-degrading carbohydrates active enzymes (CAZymes) (Wastyk et al., 2021). Sorghum is globally ranked fifth among cereal grains and is cultivated in semi-arid areas of Asia, Australia, Africa, North and South America. Besides its drought and heat tolerant attributes, sorghum grains possess diverse components with health beneficial properties (Awika, Rose, & Simsek, 2018; Girard & Awika, 2018). The development of antidiabetic functional materials or foods with no adverse side effects compared with synthetic drugs, capable of re-shaping a perturbed gut microbiota could be an effective strategy for improving glycemic control and managing diabetes. A previous randomized, single-blind, crossover study found lower incremental areas under the glycemic curve (iAUC) resulting in a lower postprandial glycemic response after consumption of sorghum drinks 30 min before glucose solution ingestion compared to non-sorghum drink (Anunciação et al., 2018). Similarly, compared to whole wheat flour muffin, grain sorghum muffin consumption by healthy male subjects in randomized-crossover study resulted in a significantly lower iAUC glycemic response (Poquette, Gu, & Lee, 2014). Furthermore, our previous studies demonstrated anti-diabetic and anti-oxidative properties of raw and fermented sorghum extracts (Ofosu, Elahi, Daliri, Han, & Oh, 2022; Ofosu et al., 2021). However, the impact of fermented sorghum (FS) on gut microbiota and their metabolites in the remission of T2D still remains unclear. Thus, the aim of this study was to investigate the effect of fermented sorghum supplementation on gut microbiota structure and function, their related-metabolites in the treatment of T2D using omics profiling.

## 2. Materials and methods

### 2.1. Chemical and reagents

Streptozotocin (STZ), citrate buffer solution, formalin, human insulin, metformin hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Insulin mouse ELISA kit Invitrogen was purchased from ThermoFisher Scientific (Waltham, MA, USA). HbA1c mouse ELISA kit was purchased from BioVison Inc. (Waltham, MA, USA). All other analytical grade reagents were purchased from South Korea.

### 2.2. Preparation of fermented sorghum

Brown sorghum M20-WB was obtained from Korean Rural Development Administration (KRDA) and fermented with *Pediococcus acidilactici* OHFR1 according to our previous study (Ofosu et al., 2022). Briefly, seed culture was prepared using 2% (w/v) sorghum M20-WB and autoclaved at 121 °C for 15 mins. After cooling, 2 ml *Pediococcus acidilactici* OHFR1 ($2 \times 10^8$ cfu/ml) was added and incubated at 37 °C with a shaking speed of 180 rpm for 24 h. Small scale fermentation (3L) was performed using a 5 L Fermenter Biostar, Korea. Sorghum M20-WB powder was mixed with distilled water (10%, w/v), autoclaved at 121 °C for 15 mins and then cooled. After, 20 ml seed culture ($2 \times 10^8$ cfu/ml) was added and incubated at 37 °C with a shaking speed of 180 rpm for 72 h. Fermented sorghum was then freeze dried and stored at −20 °C until further use. The composition of fermented sorghum has been reported in our previous study (Ofosu et al., 2022). Briefly, fermented sorghum were found to be high in catechin, 3,4-dihydroxybenzaldehyde, epicatechin, glutamate, leucine, valine, pyroglutamic acid, glycyl-glycine, suberic acid, isobutyryl carnitine and propionylcarnitine.

### 2.3. Animals and experimental design

The T2D animal protocol previously described by Ren et al. (2021) was used with some modification and approved by the Institutional Animal Care and Use Committee (IACUC) of Kangwon National University, South Korea (Registration no. KW-210531-3) and then conducted in compliance to UK/EU/US Animal Research Reporting In Vivo Experiment (ARRIVE) guidelines. Four-week old male C57BL/6 mice were purchased from Nara-Biotechnology Inc. (Seoul, Korea) and maintained in an animal facility with access to food and water ad libitum under 12 h light-dark cycle at a temperature and relative humidity of 22 ± 2 °C and 55 ± 5%, respectively. After one-week acclimatization period, the thirty-five (35) mice were randomly divided into two groups; normal control (10 mice) and high-fat fed (25 mice). Mice in the normal control group were fed standard chow diet (10% calories from fat) whilst those in high-fat fed group were fed high-fat diet (HFD) (60% calorie from fat, D12492) throughout the experimental period. In the fifth week, HFD fed mice were induced to be diabetic by injecting streptozotocin (STZ) freshly prepared in 0.1 M citrate buffer (pH 4.5) at a dose of 40 mg/kg/bw intraperitoneally for five consecutive days (Han et al., 2019). Mice in the control group were intraperitoneally injected with 4 ml/kg/bw 0.1 M citrate buffer (pH 4.5). After 6 h fast (without food), blood glucose levels were measured using a BeneCheck plus multi-monitoring device seven, ten and fourteen days after the last STZ injection. Type 2 diabetes (T2D) mice model was considered successfully developed when fasting blood glucose (FBG) levels was more than 11.1 mmol/L in the seventh week. Intervention studies began in the eighth week. T2D mice were randomly divided into five groups (5 mice per group); diabetes control (DC), diabetic mice treated with 100 mg/kg/bw fermented sorghum (DC+LDFS), diabetic mice treated with 500 mg/kg/bw fermented sorghum (DC+MDFS), diabetic mice treated with 1000 mg/kg/bw fermented sorghum (DC+HDFS) and diabetic mice treated with 100 mg/kg/bw metformin (DC+Met). Mice in the normal control group were divided into two groups (5 mice per group); normal control (NC) and normal control treated with 500 mg/kg/bw FS. Mice in NC and DC were treated with 5 ml/kg/bw normal saline (PBS) by oral gavage once daily at the same feeding frequency of other treatment groups for 8 weeks. Body weight, food intake and FBG were measured once a week. Fecal samples were collected at week 1, week 5, week 7 and on the last day of the experiment and frozen at −80 °C for microbiota analysis. At the end of the intervention, mice were fasted for 6 h and euthanized using diethyl ether. Blood samples were collected and centrifuged at 3000 r/min for 10 min. The serum, liver tissues and colon were collected and stored at −80 °C prior to further analysis.

### 2.4. Biochemical analyses

The oral glucose tolerance test (OGTT) was performed in the 8th week of intervention. Briefly, basal FBG level was measured after 6 h fasting period from blood collected from tail. Afterwards, mice were orally given glucose at a dose of 2 g/kg bw after which blood glucose

levels were measured at 30, 60, 90, and 120 min intervals using BeneCheck glucometer. In addition, intraperitoneal insulin tolerance test (IPITT) was performed 4 days prior to the end of the intervention. Baseline FBG levels were measured after 6 h fasting period, after which mice were intraperitoneally injected with human insulin at a dose of 1 IU/kg bw. BeneCheck glucometer was used to measure the glucose levels at 30, 60, 90, and 120 min intervals. The total glucose area under the curve was calculated using Tai's mathematical model (Q. Yuan, Zhan, Chang, Du, & Mao, 2020). Blood serum collected at the end of the experiment were used for the determination of FBG levels. Insulin levels and glycated hemoglobin (HbA1c) were determined using commercial kits (Roche Diagnostics, Germany and Biovision Inc., USA, respectively) according to manufacturer's protocol. HOMA-IR was calculated as follows; HOMA-IR = [fasting blood glucose (mmol/L) × fasting serum insulin (mlU/L)]/22.5 (Ren et al., 2021).

### 2.5. Histopathological observation of liver and colon tissues

Tissue sections of liver and colon (5 μm) were stained with hematoxylin and eosin (H&E) after tissues were fixed in 10% formalin. In addition, glycogen storage in liver was determined by staining with periodic acid-Schiff reagent (PAS). All images were taken using an Olympus CX43RF microscope (Olympus Corporation, Japan).

### 2.6. Sample preparation and metabolomics analysis

Sera samples (200 μl) were mixed with 70% methanol (200 μl) and vortexed for 2 h at room temperature. The mixture was then centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant filtered through a 0.45 μm membrane filter for metabolomics analysis using UHPLC-Q-TOF-MS/MS (AB SCIEX $X500_R$ QTOF). With a resolution of 5000, the negative mode of Q-TOF-MS$\square^2$ was calibrated through 100–1000 mass range. According to our previous protocol, the samples were injected by autosampler and eluted through an Accucore C18 analytical column using a binary mobile phase (water consisting of 0.1% formic acid (A) and methanol (B)). Metabolites were identified by comparing UHPLC-Q-TOF-MS$\square^2$ data with online spectral databases, METLIN and HMDB (Ofosu et al., 2021).

### 2.7. Short chain fatty acids (SCFAs) analysis

For fecal SCFAs analysis, the protocol previously described by Yuan et al. (2020) was used with some modifications (Q. Yuan et al., 2020). Briefly, fecal samples (100 mg) were homogenized in deionized water (5 ml) and acidified with 10% $H_2SO_4$ (v/v). Diethyl ether (5 ml) was then added and vortexed for 10 min after which the mixture was centrifuged for 20 min at 10,000 rpm to obtain supernatant. Supernatant was membrane filtered (0.45 μm pore size) and then used for gas chromatography-mass spectrometer (GCMS) analysis. GCMS (Agilent, USA) equipped with DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. Helium was used as carrier gas at a flow rate of 1 ml/min. The injection and ionization temperatures were maintained at 180 °C and 230 °C, respectively. Acetic acid, propionic acid and butyric acid standards were prepared and quantified in samples.

### 2.8. Gut microbiota analysis

The 16S metagenomic sequencing was performed at Macrogen, Inc. (Seoul, South Korea) using Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2 according to the manufacturer's instruction (Illumina) as described in our previous study (Daliri et al., 2020). In brief, fecal genomic DNA was extracted, subjected to quality control, randomly fragmented, and followed by 5′ and 3′ adapter ligation for sequence library construction. Using polymerase chain reaction (PCR), adapter-ligated fragments were amplified and gel purified. Fragments are then loaded into a flow cell and then clonal clusters generated through bridge amplification. The prepared library was sequenced using Illumina Miseq platform to generate accurate base-by-base sequencing and the raw data converted to FASTQ. Metagenomic KEGG (Kyoto Encyclopaedia of Genes and Genomes) functional profiles was predicted with MicFunPred (https://micfunpred.microdm.net.in/).

### 2.9. Statistical analysis

Data were analyzed with Graphpad Prism 8.0. (Graphpad Software, San Diego, USA). Results were presented as mean ± standard deviation (SD) of at least triplicate analyses determined using one-way analysis of variance (ANOVA) followed by Tukey's test at $p < 0.05$ significance level. False discovery rate (FDR) at $p < 0.01$ were considered statistically significant and used for volcano plots. Spearman's correlation analysis were performed among parameters at $^*p < 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ significance level with OriginPro 2022 (OriginLab, Northampton, Massachusetts, USA).

## 3. Results

### 3.1. Fermented sorghum improves glucose homeostasis, insulin resistance and reduces serum diabetic biomarkers in T2D

To assess the effect of fermented sorghum on glycemic control and glucose homeostasis, the fasting blood glucose (FBG) levels, oral glucose tolerance test (OGTT), insulin tolerance test (ITT) were performed in HFD-STZ induced T2D mice. During the 8 weeks of intervention, FBG levels were significantly high in diabetic control (DC) group compared to normal control (NC) groups ($p < 0.05$). Nonetheless, the FBG levels of NC and NC+HDFS groups were not significantly different (Fig. 1A and B). Compared to DC group, mid (DC+MDFS) and high-dose (DC+HDFS) fermented sorghum (FS) supplemented group showed significantly lower fasting glucose similar to the metformin group (Fig. 1A and B). After the intervention period, the mid-dose, high-dose FS and metformin treatment group exhibited 37.0%, 27.3% and 30.1% decrease in fasting glucose concentration, respectively, compared to DC mice (Fig. 1B). The low-dose FS treated mice showed no significant reduction in FBG levels (1.1% decrease in fasting glucose compared to DC mice). This results corroborates the findings of another study which found blood glucose-lowering effects of a fermented sorghum diet in alloxan-induced diabetic rat (Olawole, Okundigie, Rotimi, Okwumabua, & Afolabi, 2018).

In OGTT test, glucose peak increased sharply after 30 min of glucose consumption in all groups except normal control groups. FS treated mice showed significantly improved glucose tolerance at different time points (30, 60, 90 and 120 min) as shown by lower glucose levels compared with DC mice (Fig. 1C). The total glucose area under curve (AUC) of FS treated mice showed an improvement in glycemic control compared with DC mice. Mid and high-dose FS treated mice showed a better oral glucose tolerance compared with DC mice (Fig. 1D). Thus, FS improves postprandial hyperglycemia in T2D mice. Blood glucose levels significantly declined for all treatment groups after intraperitoneal insulin injection compared to DC group. Diabetic mice showed resistance to insulin after the 2 h period. However, insulin sensitivity was enhanced in treatment groups. The total glucose area under curve (AUC) of FS treated mice was significantly lower compared to DC mice (Fig. 1E and F). These findings are consistent with recent studies which reported an improvement in insulin sensitivity and glucose tolerance after supplementation with dry heated whole sorghum flour in rats fed high-fat high-fructose (HFHF) diet (Martinez et al., 2021). Overall, NC and NC+HDFS groups were not significantly different, thus, revealing the potential of fermented sorghum to maintain glucose homeostasis. Therefore, our results suggest that fermented sorghum ameliorated insulin resistance in HFD-STZ induced T2D mice.

Fasting serum insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) are important indicators used to assess insulin resistance, a risk factor for the development and progression of

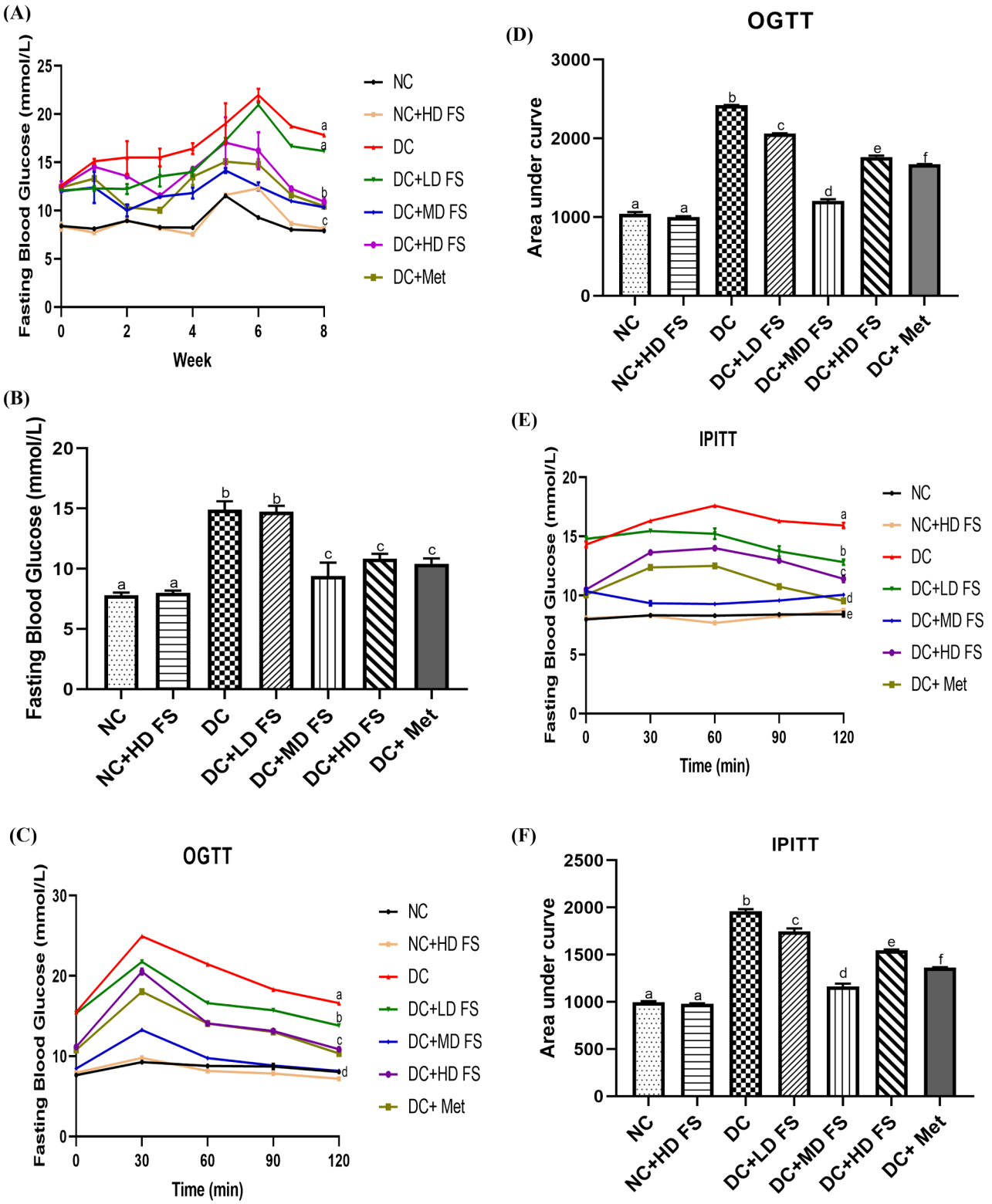


**Fig. 1.** Effect of fermented sorghum (FS) intervention on (A) Weekly fasting blood glucose; (B) Fasting blood glucose at 8 weeks; (C) Glucose tolerance test (OGTT) performed at 8th week of intervention; (D) OGTT glucose area under the curve (AUC); (E) Intraperitoneal insulin tolerance test (IPITT) performed at 8th week of intervention; (F) IPITT glucose area under the curve (AUC); (G) Serum fasting insulin levels; (H) HOMA-IR; (I) Glycated hemoglobin in T2D mice. **NC:** Normal control+saline; **NC+HD FS:** Normal control+1000 mg/kg/day fermented sorghum; **DC:** Diabetic control+saline; **DC+LD FS:** Diabetic+100 mg/kg/day fermented sorghum; **DC+MD FS:** Diabetic+500 mg/kg/day fermented sorghum; **DC+HD FS:** Diabetic+1000 mg/kg/day fermented sorghum; **DC+Met:** Diabetic+100 mg/kg/day metformin. Different letters indicate significant difference at $p < 0.05$. (n = 5 mice per group).

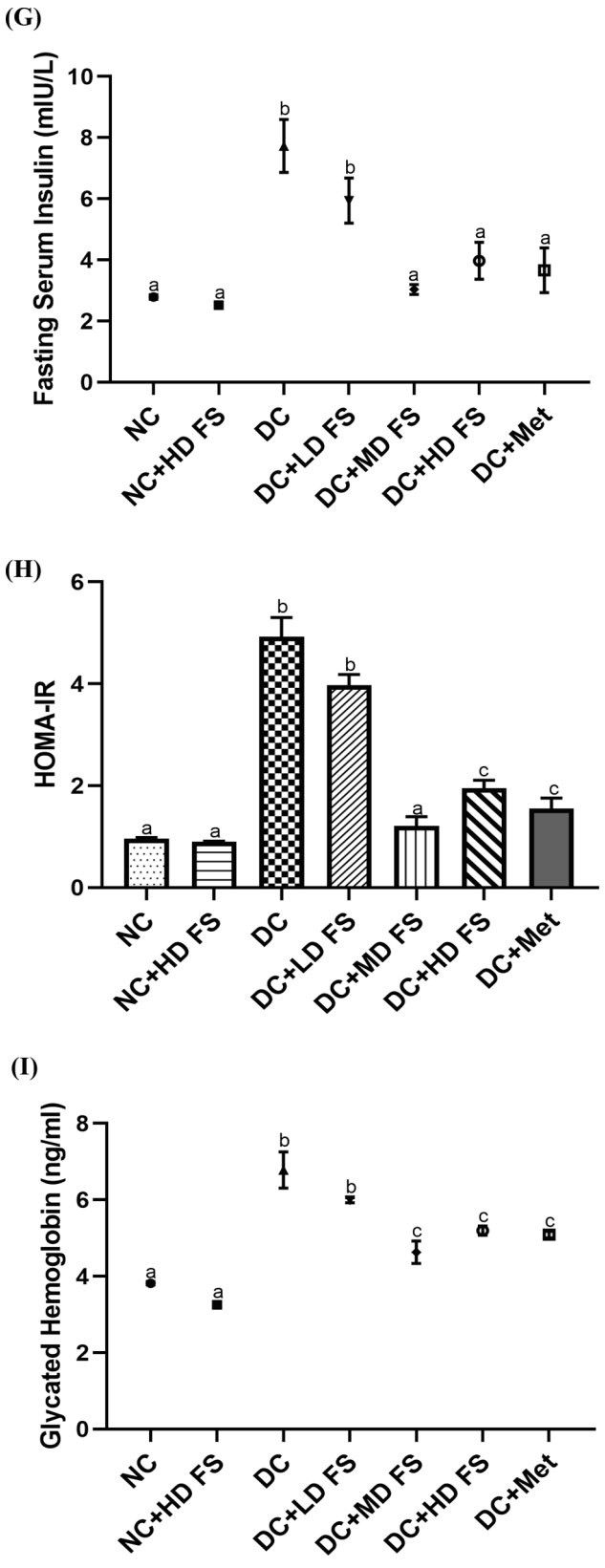


**Fig. 1.** (*continued*).

T2D. DC mice showed evidently higher serum insulin levels and HOMA-IR index compared with NC mice group. Furthermore, there was no significant difference between NC and NC+HDFS groups (Fig. 1G and H). However, after the intervention period, FS treated mice (mid and high-dose) showed a significantly reduced serum insulin levels and HOMA-IR index, similar to the metformin treated group compared to DC group (Fig. 1G and H). This result is consistent with findings from a randomized-crossover study involving healthy male subjects fed grain

sorghum muffin. Grain sorghum muffin consumption significantly reduced iAUC insulin concentration compared to whole wheat flour muffin (Poquette et al., 2014). Furthermore, glycated hemoglobin (HbA1c) which measures the average blood glucose during the past 2–3 months, is currently used to assess long-term glycaemic control and diabetes incidence risk. DC and LDFS mice showed significantly higher HbA1c levels compared to NC, NCFS, FS treated mice (mid and high-dose) and metformin treated groups (Fig. 1I). These results revealed that fermented sorghum could improve hyperglycemia and insulin sensitivity in T2D.

### 3.2. Impact of fermented sorghum on gut microbiota composition in T2D

The onset and progression of T2D has been associated with the disturbance of the gut microbiota (Qin et al., 2012). The gut microbiota composition at week 7 before intervention was examined using 16S rRNA sequencing method. The relative abundance of the phylum *Firmicutes* were significantly increased during early stages of T2D (69.43%) compared to NC (44.80%) and HFD fed (48.33%) mice. However, the phylum *Bacteroidetes* were significantly reduced in HFD fed (27.44%) and T2D (25.25%) mice compared to NC (49.40%) group. The phylum *Verrucomicrobia* was significantly enriched in HFD fed mice (22.72%) compared to NC (0.02%) and T2D mice (0%) (data not shown). Given that mid dose fermented sorghum supplementation (DC+MDFS) demonstrated better efficacy in improving glycemic control and ameliorating insulin resistance in HFD-STZ induced T2D, it was chosen to assess the impact of fermented sorghum on modulation of gut microbiota composition at the end of the intervention period. At the phylum level, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* were the most dominant bacteria in the five groups. The relative abundances of *Bacteroidetes*, *Firmicutes* and *Proteobacteria* in NC group was 67.68%, 29.32% and 1.84%, respectively. In contrast, T2D increased the relative abundance of *Firmicutes* (60.10%) and *Proteobacteria* (7.26%) but decreased the abundance of *Bacteroidetes* (29.44%) at the phylum level. However, DC+FS group (FS supplementation) significantly decreased the abundance of *Firmicutes* (34.85%) and *Proteobacteria* (1.97%) and increased the abundance of *Bacteroidetes* (56.88%) and *Deferribacteres* (6.01%) compared with DC group (Fig. 2A and B). Metformin treatment also significantly decreased the relative abundance of *Firmicutes* (33.88%) and increased the abundance of *Bacteroidetes* (51.72%) and *Proteobacteria* (11.05%). Moreover, T2D showed a markedly increased *Firmicutes/Bacteroidetes* (F/B) ratio (2.04) compared to NC group (0.43). Nonetheless, the F/B ratio was significantly decreased after FS (0.61) and metformin (0.65) treatment, almost similar to the NC group (Fig. 2C). Alpha diversity indices are used to measure the richness (Chao1) and diversity (Shannon and Inverse Simpson) of microbial community. T2D decreased the Chao1 index and increased Inverse Simpson index compared to the NC group. However, no significant differences in Shannon and Inverse Simpson indices were observed between the NC and DC+FS group (Supplementary Fig. 1A-C). Compared with DC group, DC+FS group decreased the Chao1, Shannon and Inverse Simpson indices. In contrast to DC group, DC+Met group increased Chao1 but showed no significant difference in the Shannon and Inverse Simpson indices (Supplementary Fig. 1A-C).

### 3.3. Fermented sorghum gut microbiota modulation is associated with improvement of glucose and insulin homeostasis

Volcano plot based on log fold change and false discovery rate (FDR) with a cut off value of 2 ($p < 0.01$) was used to distinguish the key bacteria groups between NC, T2D and fermented sorghum treated groups. The significantly changed genera with respect to diabetes were selected based on FDR ($p < 0.01$). Compared to the NC mice, T2D significantly affected the composition of gut bacteria (Supplementary Fig. 4A). T2D exhibited dysbiosis of the gut microbiota characterized by noticeable increase in the relative abundance of *Allobaculum*, *Romboutsia*, *Oscillibacter*, *Acetatifactor*, *Enterocloster*, *Helicobacter*, *Acetivibrio*, *Millionella*, *Roseburia*, *Flintibacter, Lachnoclostridium, Desulfovibrio*, in addition to the decreased abundance of *Muribaculum*, *Parabacteroides*, *Prevotellamassilia*, *Paraprevotella*, *Lactobacillus*, *Prevotella* and *Ligilactobacillus* genera (Fig. 5A). Fermented sorghum treatment reversed 13 of the significantly changed genera induced by T2D including decreased abundance of opportunistic pathogens such as *Allobaculum*, *Romboutsia*, *Oscillibacter*, *Acetatifactor*, *Enterocloster*, *Helicobacter*, *Acetivibrio*, *Millionella*, *Roseburia*, *Flintibacter, Lachnoclostridium*, and an increased abundance of *Muribaculum*, *Phocaeicola* and *Parabacteroides* (Fig. 5B).

Fermented sorghum supplementation increased fecal SCFAs levels in T2D, specifically butyrate and propionate. However, supplementation

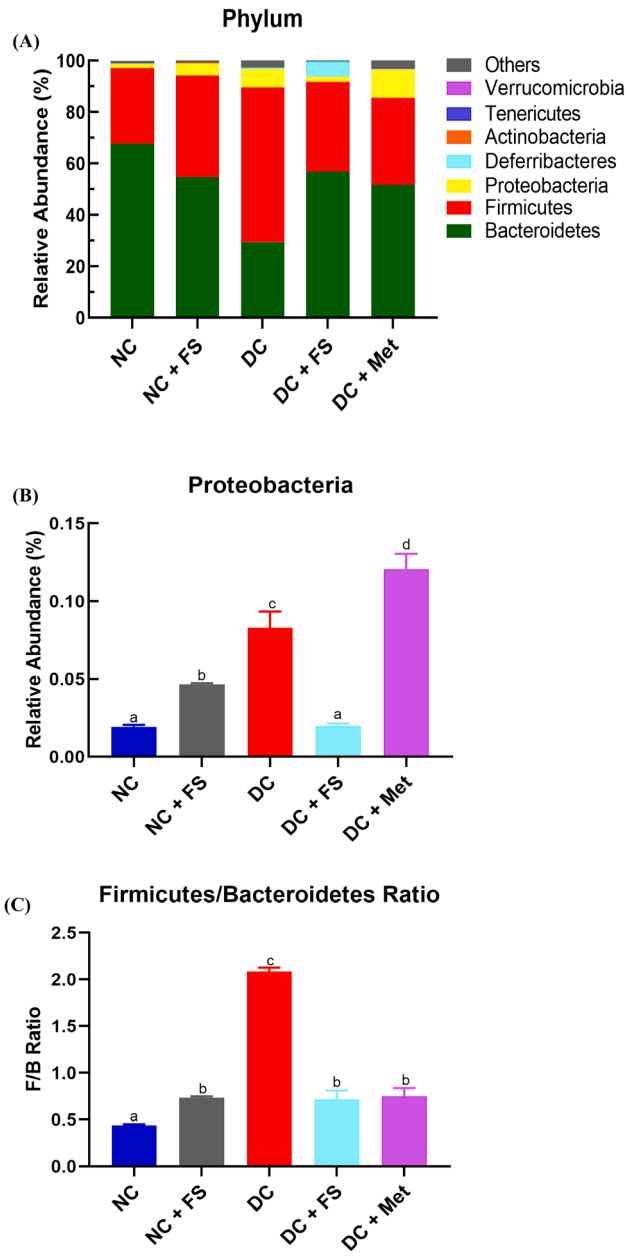


**Fig. 2.** (A) Phylum level composition of gut microbiota in different groups; (B) Relative abundance of *Proteobacteria* in different groups; (C) *Firmicutes/Bacteroidetes* ratio in different groups. **NC:** Normal control+saline; **NC+FS:** Normal control+1000 mg/kg/day fermented sorghum; **DC:** Diabetic control+saline; **DC+FS:** Diabetic+500 mg/kg/day fermented sorghum; **DC+Met:** Diabetic+100 mg/kg/day metformin. Different letters indicate significant difference at $p < 0.05$. ($n = 5$ mice per group).

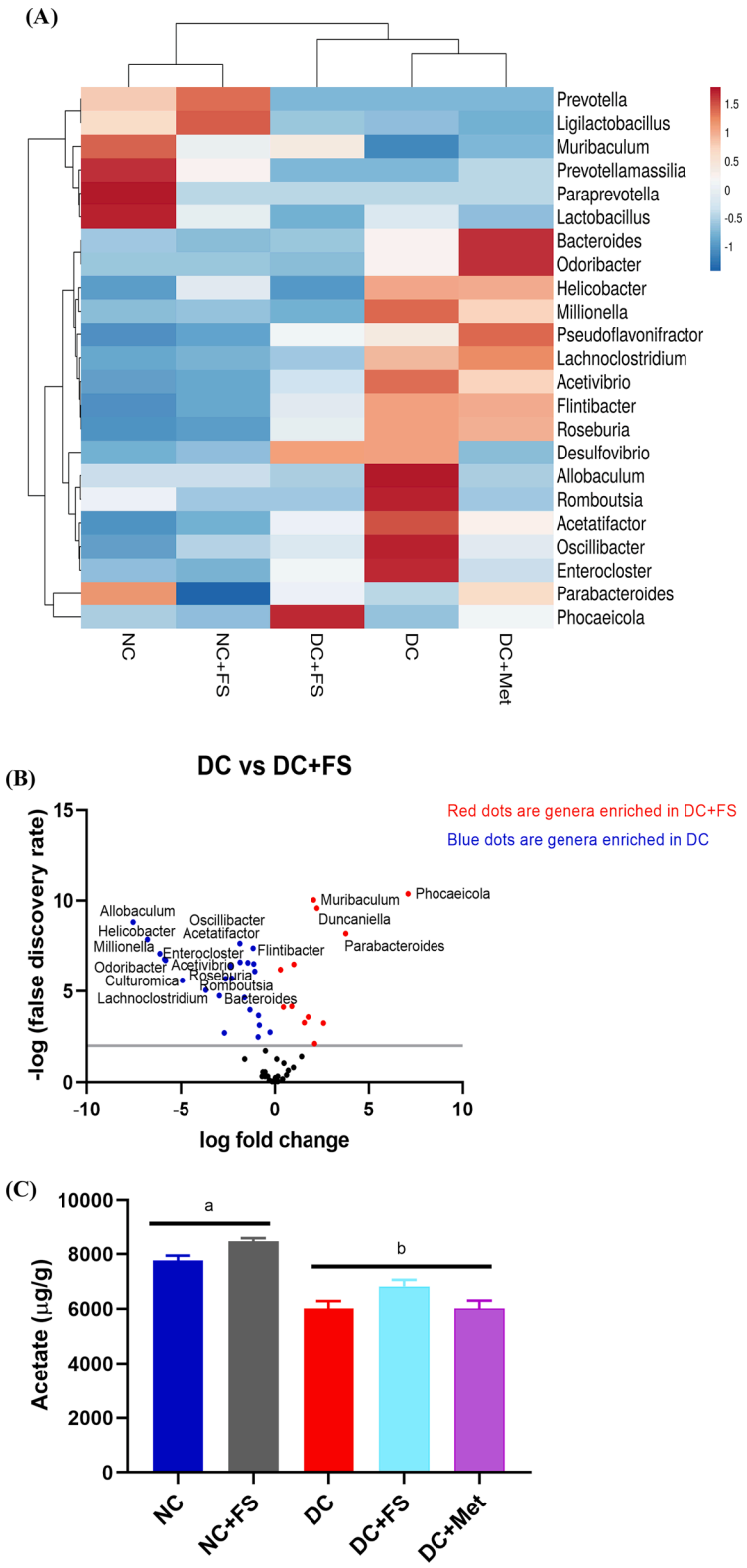


**Fig. 3.** (A) Heatmap of gut microbiota composition in different groups a genus level; (B) Volcano plots showing significantly changed genera using log fold change and false discovery rate at $p < 0.01$ in diabetic control (DC) vs diabetic control treated with fermented sorghum (DC+FS) group. Red color denotes significantly increased genera, whereas blue color denotes significantly reduced genera in DC+FS group; (C) Acetate levels in feces among different groups; (D) Butyrate levels in feces among different groups; (E) Propionate levels in feces among different groups, different letters indicate significant difference at $p < 0.05$, ns: not significant ($p > 0.05$); (F) Heatmap of spearman's correlation between gut microbiota, SCFA's and T2D-related physiological traits at ***$p \leq 0.001$. **NC:** Normal control+saline; **NC+FS:** Normal control+1000 mg/kg/day fermented sorghum; **DC:** Diabetic control+saline; **DC+FS:** Diabetic+500 mg/kg/day fermented sorghum; **DC+Met:** Diabetic+100 mg/kg/day metformin. (n = 5 mice per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

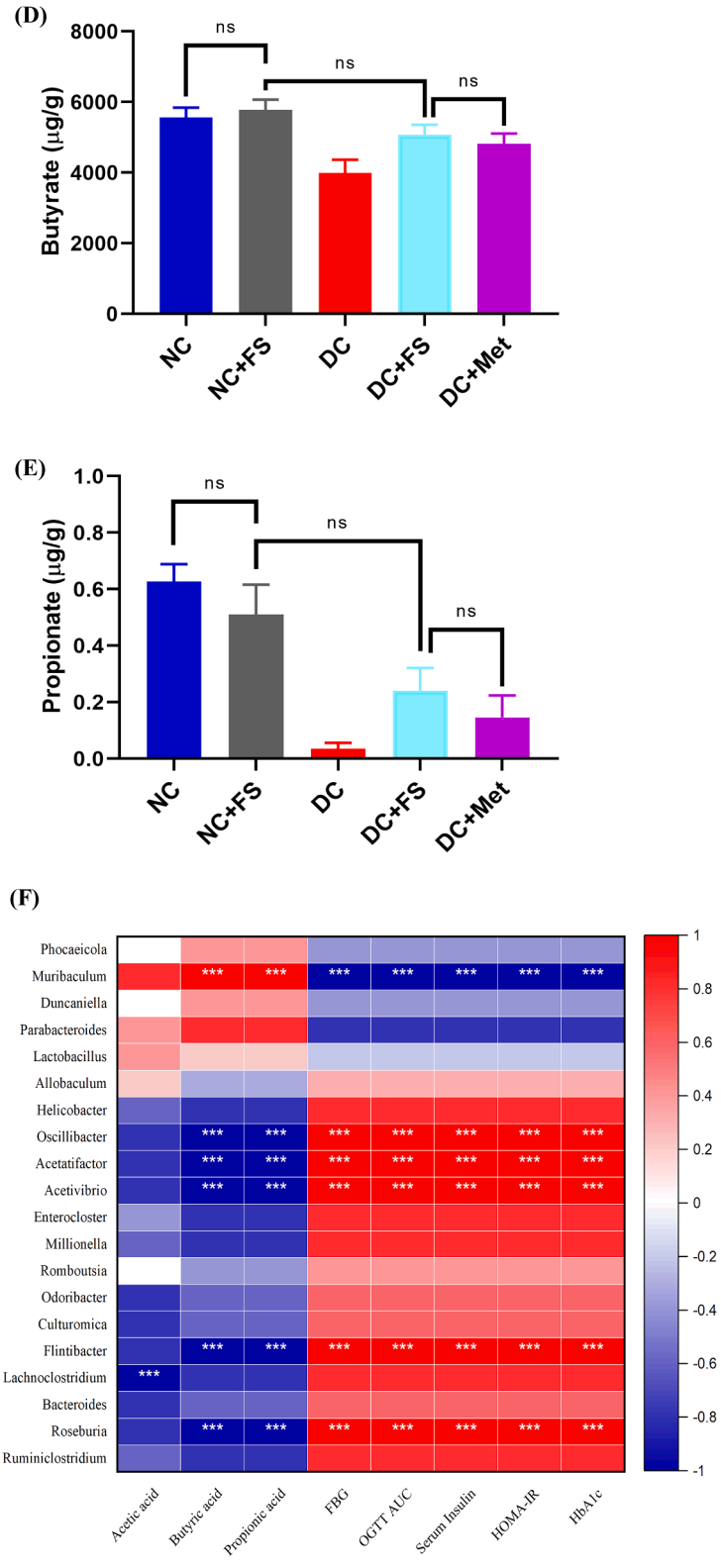


**Fig. 3.** (*continued*).

with fermented sorghum had no significant effect on acetate levels compared to DC group (Fig. 3C-E). Spearman correlation was used to investigate the association between significantly altered gut microbiota in T2D and after treatment, SCFAs and T2D physiological traits. *Muribaculum*, *Parabacteroides,* and *Phocaeicola* were positively correlated with butyrate, propionate and acetate, whereas *Oscillibacter*, *Acetatifactor*, *Acetivibrio*, *Enterocloster*, *Helicobacter, Flintibacter, Lachnoclostridium, Roseburia, Millionella, Ruminiclostridium, Culturomica, Bacteroides and Odoribacter* were negatively correlated to SCFAs. Furthermore, *Muribaculum* and *Parabacteroides* also showed negative correlation with FBG, OGTT AUC, serum insulin, HOMA-IR and HbA1c. Similarly, SCFAs were found to be negatively correlated with FBG,

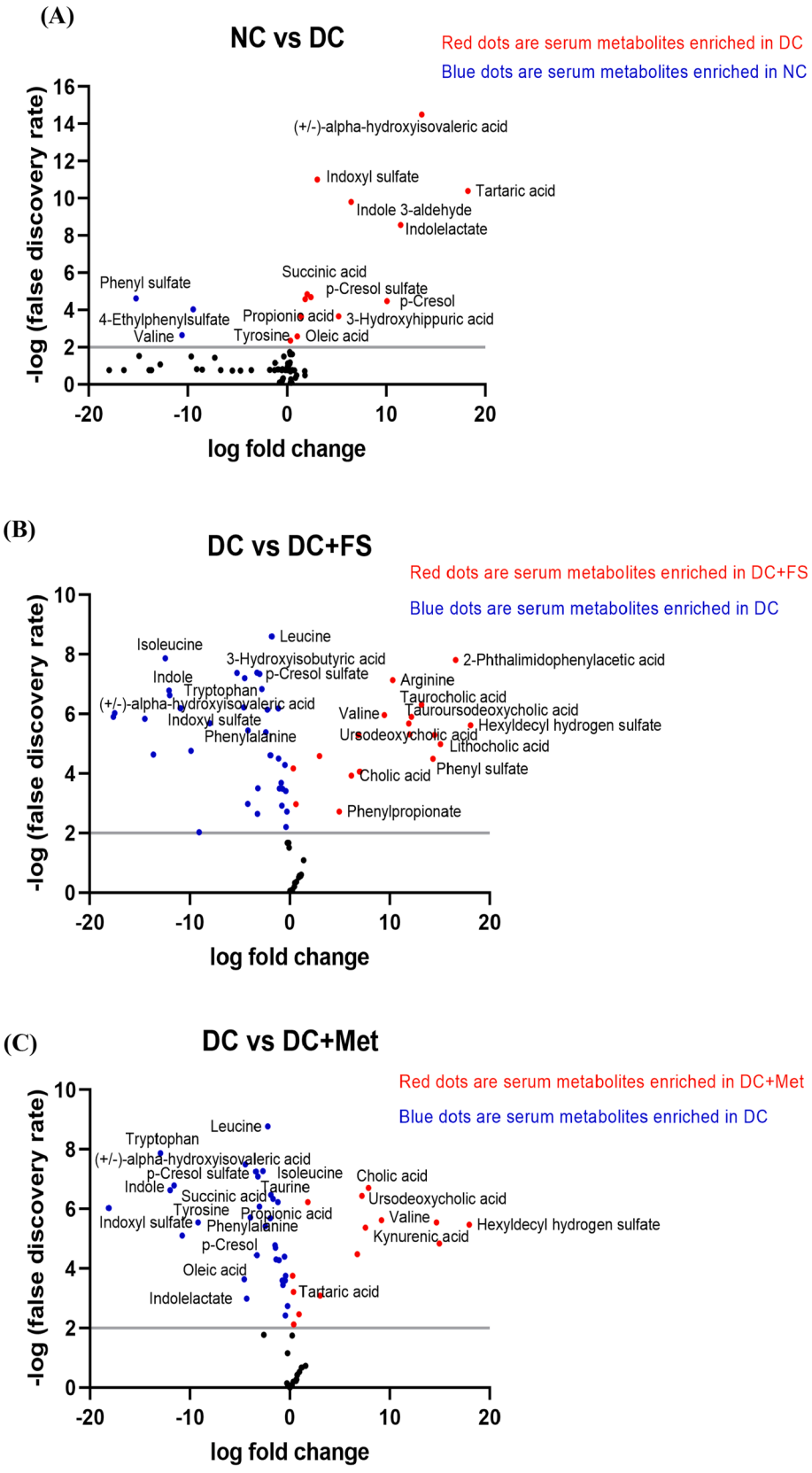


**Fig. 4.** Volcano plots showing significantly changed metabolites using log fold change and false discovery rate at $p < 0.01$ in (A) Normal control (NC) vs diabetic control (DC) group; (B) Diabetic control (DC) vs diabetic control treated with fermented sorghum (DC+FS) group and (C) Diabetic control (DC) vs diabetic control treated with metformin (DC+Met) group. Red color denotes metabolites significantly increased in DC, DC+FS and DC+Met groups; whereas blue color denotes significantly reduced metabolites in same groups. (D) Heatmap of Spearman's correlation between T2D-related physiological traits and significantly changed metabolites, ***$p \leq 0.001$. ($n = 5$ mice per group). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

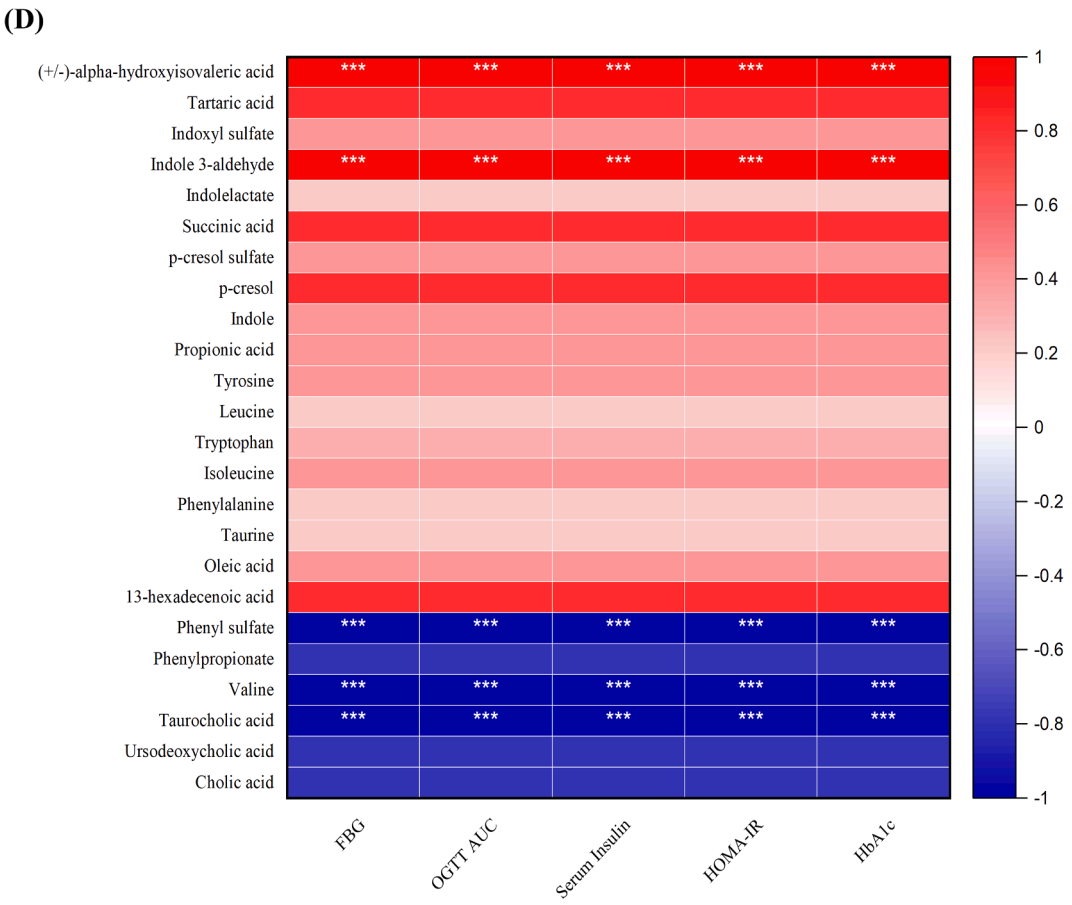


**Fig. 4.** (*continued*).

OGTT AUC, serum insulin, HOMA-IR and HbA1c. However, *Oscillibacter*, *Acetatifactor*, *Acetivibrio*, *Enterocloster*, *Helicobacter, Flintibacter, Lachnoclostridium, Roseburia, Millionella* and *Ruminiclostridium* were positively correlated with FBG, OGTT AUC, serum insulin, HOMA-IR and HbA1c (Fig. 3F).

### *3.4. Metabolites associated with T2D and fermented sorghum supplementation*

Serum metabolomics analysis was performed to further explore the T2D-related signatures mediated by gut microbiota. Metabolites including amino acids (AA) and their derivatives, bile acids (BA), fatty acids and vitamins were identified. Among AAs and their derivatives enriched in T2D compared to NC included isoleucine, leucine, tyrosine, tryptophan, phenylalanine, lysine, taurine and 3-hydroxy-l-proline. In contrast, the concentration of methionine and valine were low in T2D but high in NC (Supplementary Fig. 5A). Compared with NC, several microbial metabolites (tryptophan and tyrosine derivatives) enriched in T2D included indoxyl sulfate, p-cresol, p-cresol sulfate, indolelactate, indole and indole 3-aldehyde. On the contrary, 3-methylindole, 4-ethylphenylsulfate, indole-3-carbinol, phenol, phenylacetic acid, phenyl sulfate and hexyldecyl hydrogen sulfate were enriched in NC (Supplementary Fig. 5B). Compared with NC, fatty acids found to be enriched in T2D included oleic acid, palmitic acid and 13-hexadecenoic acid. On the contrary, linoleic acid, α-linolenic acid and eicosadienoic acid were enriched in NC. Furthermore, taurocholic acid, ursodeoxycholic acid and cholic acid were among the bile acids enriched in NC compared with T2D. Ascorbic acid was found to be enriched in T2D compared with NC (Supplementary Fig. 5C). However, supplementation with fermented sorghum decreased the serum levels of aromatic amino acids (tyrosine, tryptophan, phenylalanine), branch chain amino acids (leucine and isoleucine), taurine, orthinine, lysine, microbial metabolites (indoxyl sulfate, p-cresol, p-cresol sulfate, indolelactate, indole and indole 3-aldehyde), and fatty acids (oleic acid, palmitic acid and 13-hexadecenoic acid) enriched in T2D mice. Moreover, the levels of arginine, valine, phenylpropionate, 2-phthalimidophenylacetic acid, hexyldecyl hydrogen sulfate, phenyl sulfate, bile acids (taurocholic acid, ursodeoxycholic acid, lithocholic acid, cholic acid and tauroursodeoxycholic acid) were increased after fermented sorghum supplementation (Supplementary Fig. 5A-C).

Furthermore, log fold change and false discovery rate (FDR) with a cut off value of 2 ($p < 0.01$) was used to select discriminant metabolites between NC, T2D, FS and Met treated groups (Fig. 4A-C). Metabolites with significantly strong prediction for higher T2D incidence or risk included indole 3-aldehyde, (+/-)-alpha-hydroxyisovaleric acid, p-cresol, p-cresol sulfate, indoxyl sulfate, indole, succinic acid, tartaric acid, tyrosine, isoleucine, 13-hexadecenoic acid, oleic acid, and propionic acid. Moreover, metabolomic signatures such as phenyl sulfate, 4-ethylphenylsulfate and valine were significantly decreased in T2D compared with NC mice (Fig. 4A). Fermented sorghum supplementation significantly decreased the levels of indoxyl sulfate, p-cresol, p-cresol sulfate, indolelactate, indole, indole 3-aldehyde, leucine, isoleucine, tyrosine, tryptophan, phenylalanine, (+/-)-alpha-hydroxyisovaleric acid, tartaric acid, propionic acid, succinic acid, oxalosuccinic acid, along with increased levels of arginine, valine, phenylpropionate, phenylsulfate, 2-phthalimidophenylacetic acid, hexyldecyl hydrogen sulfate, taurocholic acid, ursodeoxycholic acid, cholic acid, lithocholic acid, and tauroursodeoxycholic acid (Fig. 4B). Furthermore, phenylsulfate, phenylpropionate, valine, cholic acid, taurocholic acid and ursodeoxycholic acid were found to strongly predict lower incidence or risk of T2D (Fig. 4D).

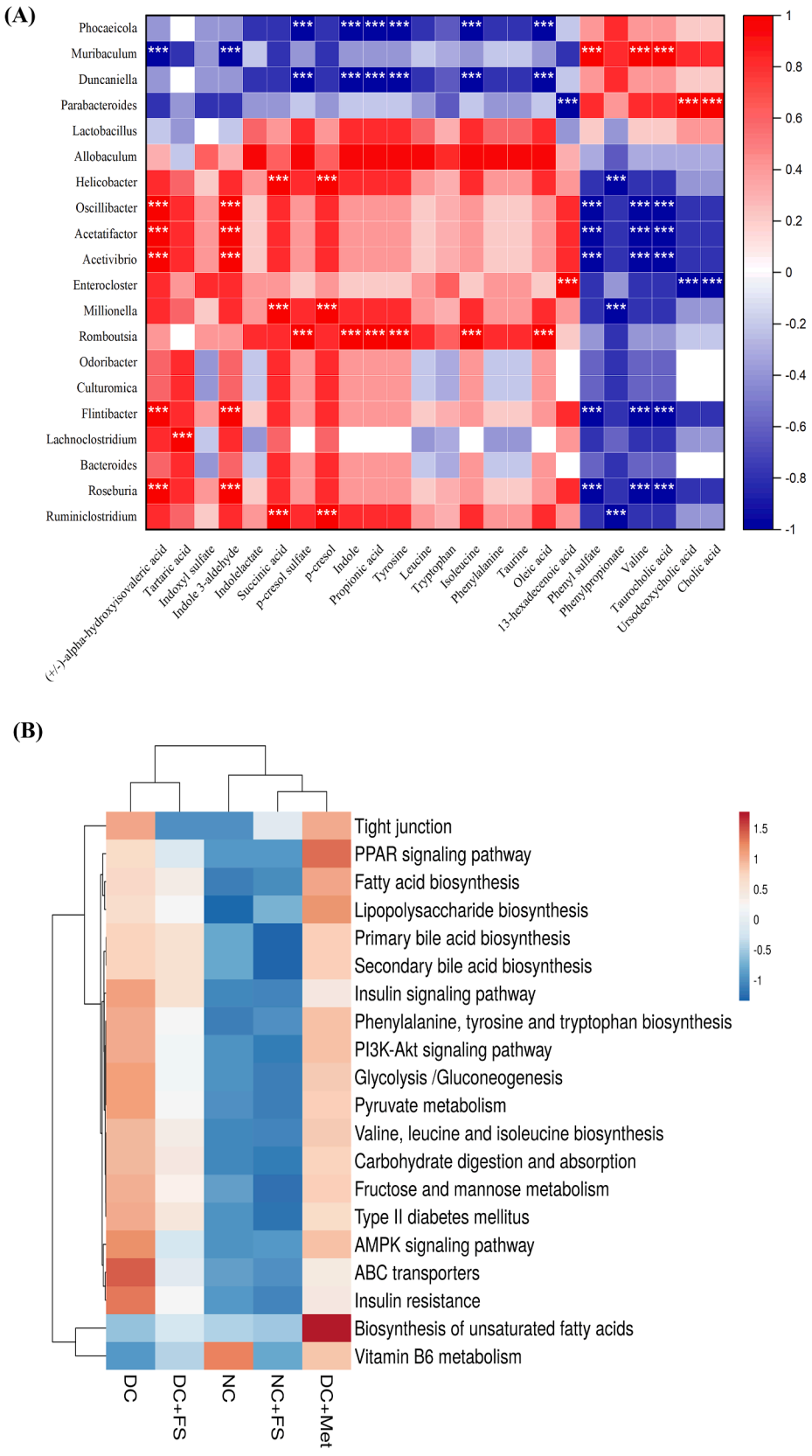


**Fig. 5.** (A) Heatmap of association between gut microbiota and significantly changed serum metabolites with T2D at ***p ≤ 0.001; (B) Heatmap of KEGG functional profile based on bacterial 16S rRNA on the effects of fermented sorghum on potential gut microbiota functions via MicFunPred (http://micfunpred.microdm.net.in/). **NC:** Normal control+saline; **NC+FS:** Normal control+1000 mg/kg/day fermented sorghum; **DC:** Diabetic control+saline; **DC+FS:** Diabetic+500 mg/kg/day fermented sorghum; **DC+Met:** Diabetic+100 mg/kg/day metformin. (n = 5 mice per group).

### 3.5. Association of gut microbes and metabolites with T2D

Spearman's correlation analysis was used to ascertain the relationship between the main differential bacteria genera and metabolites associated with T2D (Fig. 5A). Amongst bacteria genera inversely correlated with metabolites positively associated with T2D (mentioned in Fig. 4D) include *Muribaculum, Duncaniella, Phocaeicola* and *Parabacteroides*. *Muribaculum* was negatively correlated with (+/-)-alpha-

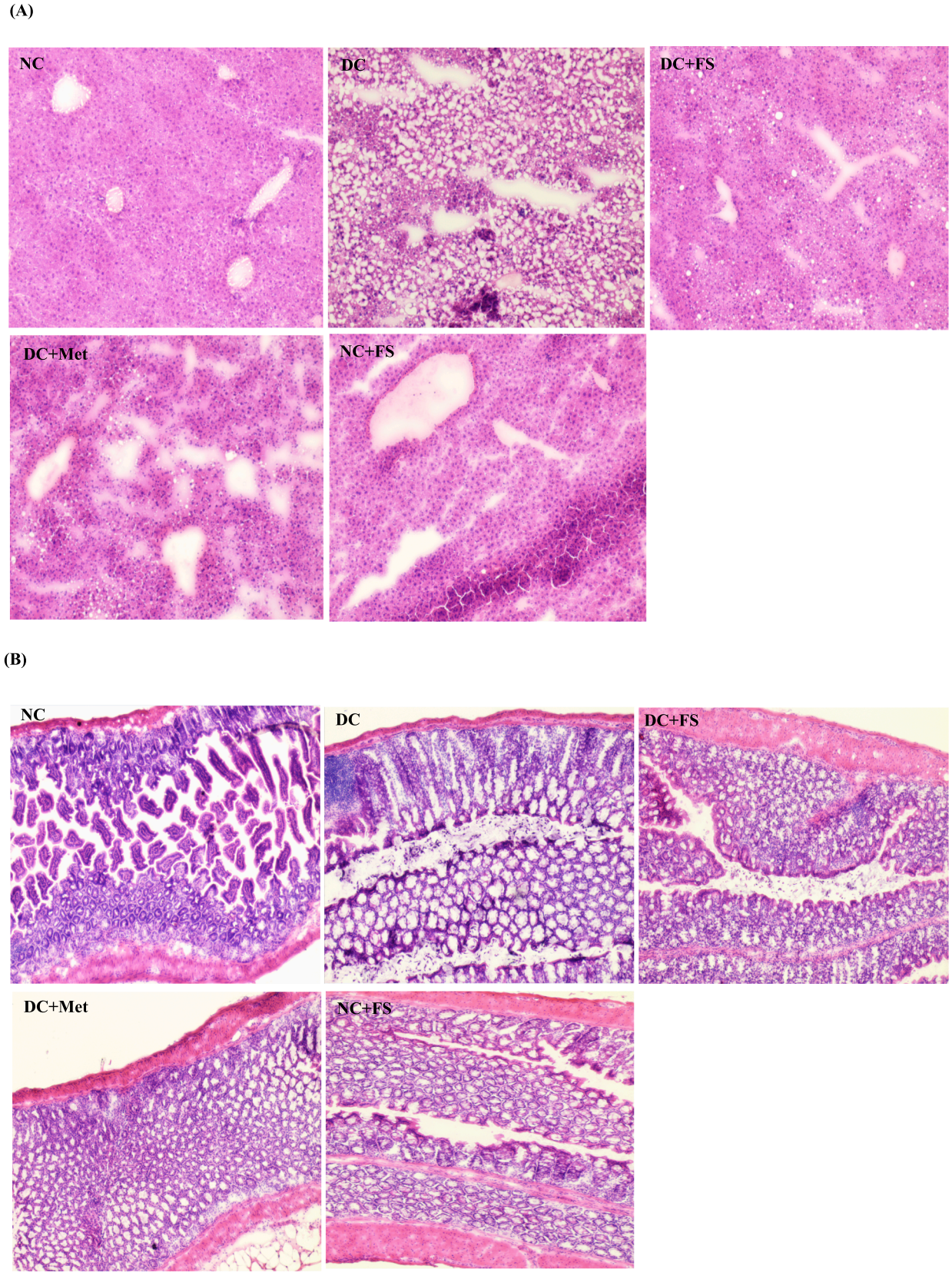


**Fig. 6.** Effect of fermented sorghum on tissue sections in HFD-STZ induced T2D mice. Pathological examination of (A) liver and (B) colon by H&E staining; (C) liver glycogen by PAS staining. Images were captured using 100x magnification. **NC:** Normal control+saline; **NC+FS:** Normal control+1000 mg/kg/day fermented sorghum; **DC:** Diabetic control+saline; **DC+FS:** Diabetic+500 mg/kg/day fermented sorghum; **DC+Met:** Diabetic+100 mg/kg/day metformin. (n = 5 mice per group).

(C)

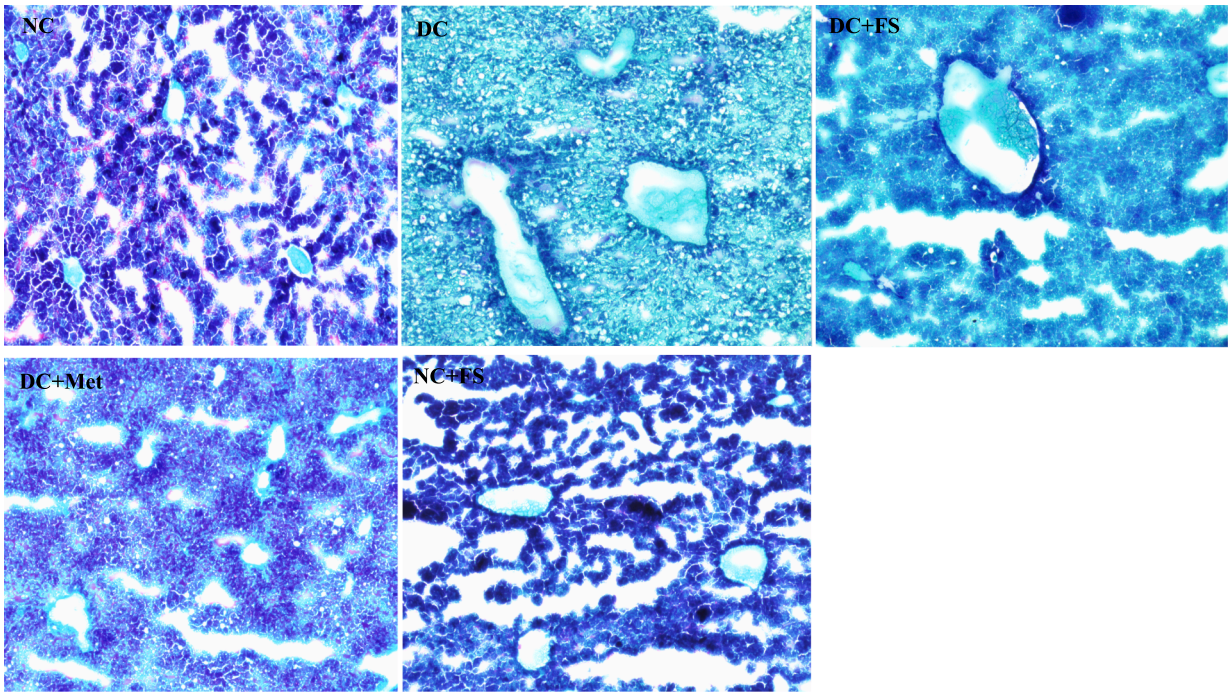


**Fig. 6.** (*continued*).

hydroxyisovaleric acid, indole 3-aldehyde, p-cresol, succinic acid and tartaric acid, whilst *Phocaeicola* and *Duncaniella* was negatively correlated with p-cresol sulfate, indole, propionic acid, tyrosine, isoleucine and oleic acid. The metabolites (+/-)-alpha-hydroxyisovaleric acid, indoxyl sulfate, indole 3-aldehyde and 13-hexadecenoic acid were negatively correlated with *Parabacteroides.* Nevertheless, metabolites inversely associated with T2D such as phenylpropionate, phenylsulfate, valine, taurocholic acid, ursodeoxycholic acid and cholic acid were positively correlated with *Muribaculum, Parabacteroides, Phocaeicola* and *Duncaniella.* On the contrary, *Oscillibacter*, *Allobaculum*, *Helicobacter*, *Acetatifactor*, *Acetivibrio*, *Enterocloster*, *Helicobacter, Romboutsia, Flintibacter, Millionella, Lachnoclostridium, Roseburia* and *Ruminiclostridium* were positively correlated with metabolites positively associated with T2D. For example, *Oscillibacter, Acetatifactor*, *Acetivibrio, Flintibacter* and *Roseburia* were strongly positively correlated with (+/-)-alpha-hydroxyisovaleric acid and indole 3-aldehyde. Likewise, *Helicobacter, Millionella* and *Ruminiclostridium* were strongly positively correlated with succinic acid and p-cresol. Furthermore, p-cresol sulfate, indole, propionic acid, tyrosine, isoleucine and oleic acid were strongly positively correlated with *Romboutsia.* The genera *Enterocloster* was positively correlated with (+/-)-alpha-hydroxyisovaleric acid, indoxyl sulfate, indole 3-aldehyde and 13-hexadecenoic acid, whilst *Lactobacillus* positively correlated with p-cresol sulfate, indole, propionic acid, tyrosine, isoleucine and oleic acid. In addition, the genera *Allobaculum* was positively correlated with indolelactate, p-cresol sulfate, indole, propionic acid, tyrosine, isoleucine, leucine, tryptophan, phenylalanine, taurine and oleic acid. In contrast, *Oscillibacter*, *Allobaculum*, *Helicobacter*, *Acetatifactor*, *Acetivibrio*, *Enterocloster*, *Helicobacter, Romboutsia, Flintibacter, Millionella, Lachnoclostridium, Roseburia* and *Ruminiclostridium* were inversely correlated with phenylpropionate, phenylsulfate, valine, taurocholic acid, ursodeoxycholic acid and cholic acid (Fig. 5A).

Furthermore, MicFunPred was used to predict the KEGG functional profile based on the 16S rRNA bacteria gene in other to investigate the association between metabolites and the perturbed gut microbiota in T2D. Compared with NC and DC+FS groups, the gut microbiota in DC group were more enriched in functions involved in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan), BCAA (leucine and isoleucine), fatty acids and lipopolysaccharides. Moreover, T2D-enriched KEGG orthologues markers included insulin signaling, insulin resistance, epithelial tight junction and pathways involved in PPAR, AMPK and PI3K-Akt (Fig. 5B). This may suggest that the T2D gut environment stimulates defense mechanisms related to the aforementioned processes necessary to prevent the disease. Biosynthesis of phenylalanine, tyrosine and tryptophan, lipopolysaccharides, fatty acids, ABC transporters and pathways involved in PPAR, AMPK, PI3K-Akt, and insulin signaling insulin resistance, tight junction, glycolysis/gluconeogenesis, pyruvate, fructose and mannose metabolism were significantly down-regulated after fermented sorghum supplementation (Fig. 5B).

### 3.6. *Effect of fermented sorghum on liver and colon histopathology*

Liver histopathological examination was performed using H&E and PAS staining to investigate the effect of fermented sorghum on liver morphology and glycogen storage, respectively. Colon tissues were stained with H&E to examine the intestinal epithelium intergrity and morpholgy. Liver H&E staining revealed a normal hepatic lobular morpholgy with hepatocytes arranged in plates or cords in the NC and NC+FS groups (Fig. 6A). On the contrary, DC group showed considerable hepatic steatosis and necrosis characterized by severe fat accumulation and a loss of the hepatic lobular structure indicative of hepatic damage or injury. These abnormalities were ameliorated by fermented sorghum and metformin treatment (Fig. 6A). Our results corroborates recent studies which found liver steatosis reducing effect of dry heated whole sorghum flour in rats fed high-fat high-fructose diet (Martinez et al., 2021). Colon tissues histological analysis revealed significant disruption of epithelial cells characterized by severe goblet cell hyperplasia and inflammatory cell infiltration in the DC group. However, colon epithelial morphology in the NC and NC+FS groups showed a uniform and compact arrangement of villi and crypts. Disorderly crypts arrangement and branching indicative of destruction, inflammation and

leaky intestinal epithelial were observe in DC group. Disrupted intestinal epithelial membranes were improved after fermented sorghum and metformin treatment compared to DC group (Fig. 6B). Taken together, fermented sorghum consumption demonstrated a protective effect against liver and colon damages in T2D. Photomicrographs of PAS stained liver tissues demonstrating hepatic glycogen storage are shown in Fig. 6C. Compared to NC and NC+FS groups, DC group had a decreased hepatic glycogen content. However, fermented sorghum and metformin treatment increased glycogen content in the liver compared to the DC group. Our findings demonstrate the potency of fermented sorghum to improve insulin sensitivity and promote the conversion of glucose to glycogen in the liver.

## 4. Discussion

Recent evidence reveals that the composition and structural functions of the gut microbiome greatly influences host health and wellness. As such the metabolic crosstalk between the gut microbiome and host physiology is receiving increasing attention as novel target in the treatment and remission of T2D and other diseases (Martínez-López et al., 2022). The gut microbiome dysbiosis signatures in T2D have generally lower abundance of *Bacteroidetes* but enriched in *Proteobacteria* and *Firmicutes* at the phyum level (Martínez-López et al., 2022). Our findings showed a similar signature with higher abundance of *Firmicutes* and *Proteobacteria* and a higher (F/B) ratio as previously reported in T2D patients compared to healthy subjects (L. Zhao et al., 2019). However, the supplementation of fermented sorghum significantly decreased the abundance of these phyla in T2D. The pathogenicity of the genus *Helicobacter* and *Desulfovibrio* belonging to the *Proteobacteria* phylum has been reported to induce pro-inflammatory effect which contributes to the development of insulin resistance (Han et al., 2019). At the genus level, increased abundance of opportunistic pathogens such as *Allobaculum, Desulfovibrio* and *Lachnoclostridium* and depletion of butyrate-producing bacteria are some examples of the microbiome signatures observed in T2D (Jia et al., 2017). Fermented sorghum consumption significantly decreased the abundance of opportunistic pathogens such as *Allobaculum*, *Romboutsia*, *Oscillibacter*, *Acetatifactor*, *Enterocloster*, *Helicobacter*, *Acetivibrio*, *Millionella*, *Roseburia*, *Flintibacter, Lachnoclostridium and* promoted the growth and increased abundance of *Muribaculum*, *Phocaeicola* and *Parabacteroides* at the genus level. Our results agrees with previous studies which observed lower abundance of *Muribaculum* in diabetic mice, however, their abundance was significantly increased after mulberry leaf polysaccharide and Apocynum venetum extracts treatment (Y. Yuan et al., 2020; X. Zhao et al., 2022). The genus *Muribaculum* belonging to the phylum *Bacteroidetes* has been positively correlated with healthy outcomes such as reduction of inflammatory markers and improvement of glucose tolerance and dyslipidemia (Y. Yuan et al., 2019). Metabolic signals from *Muribaculum* promotes the integrity of gut barrier and possesses beneficial physiological functions resulting in insulin sensitivity with improved glucose homeostasis (Y. Yuan et al., 2020). The species *Phocaeicola vulgatus* (formerly *Bacteroides vulgatus*) belonging to the genus *Phocaeicola*, a member of the phylum *Bacteroidetes* was decreased in diabetics (Nie et al., 2019). However, *P. vulgatus* was found to be significantly increased in T2D after fermented sorghum treatment. This agrees with a previous study which observed an increased abundance of *P. vulgatus* in diabetic rat after *Plantago asiatica* L. polyscaccharide treatment (Nie et al., 2019). In addition, *P. vulgatus* has been reported to reduce gut permeability via the upregulation of tight junction genes and ameliorates endotoxemia by suppressing systemic inflammation via reduction of lipopolysaccharide (LPS) production (Yoshida et al., 2018). The beneficial effect of increased abundance of *Parabacteroides* (*Bacteroidetes* phylum) in decreasing risk of weight gain, hyperglycemia, inflammation, maintaining gut barrier integrity, improving insulin resistance and antioxidant enzyme activities has been reported in recent studies (Y. Yuan et al., 2019). These results demonstrate the beneficial role of fermented sorghum in T2D remission by improving T2D-induced gut microbiome dysbiosis.

As mentioned earlier, the host-microbiome metabolic crosstalk involves key signaling molecules such as SCFAs, bile acids (BA), amino acids and indole derivatives that influences host's metabolism and health (Martínez-López et al., 2022). Microbial fermentation of dietary carbohydrates such as fiber and resistant starch results in the production of SCFAs which have been shown to exert beneficial effects in host glucose metabolism and health (Postler & Ghosh, 2017). In this study, the levels of SCFAs, specifically butyrate and propionate were increased in T2D after fermented sorghum treatment. Potential beneficial genera *Muribaculum*, *Phocaeicola and Parabacteroides* were positively correlated with these SCFAs. However, opportunistic pathogens including *Oscillibacter*, *Acetatifactor*, *Acetivibrio*, *Flintibacter* and *Roseburia* were strongly negatively correlated with butyrate and propionate. Furthermore, SCFAs negatively correlated with diabetes markers (FBG, OGTT AUC, serum insulin, HOMA-IR and HbA1c). Recent evidence supports the role of SCFAs (butyrate and propionate) in the secretion of the incretin hormone, glucagon-like peptide 1 (GLP-1), improving glucose tolerance and insulin sensitivity and appetite control (De Vadder et al., 2014). Moreover, *Muribaculum*, *Phocaeicola* and *Parabacteroides* were inversely correlated with FBG, OGTT AUC, serum insulin, HOMA-IR and HbA1c, whereas, *Oscillibacter*, *Acetatifactor*, *Acetivibrio*, *Enterocloster*, *Helicobacter, Flintibacter, Lachnoclostridium, Roseburia, Millionella* and *Ruminiclostridium* showed positive correlation with these diabetic markers.

Bile acids (BA) are cholesterol-derived signaling molecules produced by host and then biotransformed by intestinal microbiota which facilitates the absorption of dietary fat, certain vitamins, thereby regulating glucose and lipid metabolism and inflammation through Takeda G protein-coupled receptor 5 (TGR5) and nuclear farnesoid X receptor (FXR). Thus, the gut microbiota plays an important role in modulating the BA pool in host (Postler & Ghosh, 2017). Circulating levels of taurocholic acid (TCA), ursodeoxycholic acid (UDCA), cholic acid (CA), lithocholic acid (LCA) and tauroursodeoxycholic acid (TUDCA) were increased after fermented sorghum treatment in T2D. These results were consistent with a recent study which examined the BA profile in T2D and non-diabetic patients. They found lower levels of TCA and CA in T2D patients (Mantovani et al., 2021). In addition, lower serum levels of CA in HFD-fed rats were significantly increased after treatment (Nakade et al., 2021). Another study has reported that increased serum levels of CA in diabetic rats treated with ginseng berry could promote insulin secretion, improve insulin resistance and thus reduce blood glucose by stimulating TGR5 receptor which activates the GLP-1 pathway (Wang et al., 2021). UDCA treatment was found to stimulate GLP-1 secretion, improve insulin sensitivity and reduced HbA1c levels in Japanese T2D patients (Shima et al., 2018). Recent evidence suggests UDCAs role in improving glucose control and could be used as a potential treatment for gestational diabetes mellitus (GDM) (Lovell et al., 2022). Thus, fermented sorghum treatment in T2D could regulate BA metabolism via modulation of the gut microbiota to exert its antidiabetic effects.

On the other hand, BCAAs (valine, leucine and isoleucine) and aromatic amino acids (tyrosine, tryptophan, and phenylalanine) have been strongly associated with insulin resistance and risk of T2D incidence. BCAAs and aromatic amino acids have been reported to impair pancreatic beta cell function and insulin signalling in skeletal muscle, respectively, thereby inhibiting glucose uptake (Yang, Chi, Burkhardt, Guan, & Wolf, 2010). Increased levels of serum BCAAs have been found in people with insulin resistance and higher T2D risk (Pedersen et al., 2016). Circulating levels of BCAAs (leucine and isoleucine) were decreased after fermented sorghum treatment in T2D. *Phocaeicola* was negatively correlated with leucine and isoleucine. In contrast, *Allobaculum*, *Helicobacter*, *Romboutsia, Millionella* and *Ruminiclostridium* were positively associated with leucine and isoleucine. In addition, circulating levels of aromatic amino acids (tyrosine, tryptophan, and phenylalanine) which are sources of microbial metabolites were

decreased after fermented sorghum treatment in T2D. Tryptophan was found to be positively associated with T2D risk (Qi et al., 2022). *Phocaeicola* showed inverse association with tyrosine, tryptophan, and phenylalanine. However, *Allobaculum*, *Helicobacter*, *Romboutsia, Millionella* and *Ruminiclostridium* were positively correlated with tyrosine, tryptophan, and phenylalanine. Recent human studies have reported the association of circulating levels of microbial tryptophan derivatives or metabolites with T2D risk (Qi et al., 2022). Fermented sorghum supplementation decreased the circulating levels of tryptophan metabolites and indole derivatives such as indoxylsulfate, indolelactate, indole and indole 3-aldehyde. Elevated serum levels of indoxylsulfate, a uremic toxin is reported to induce insulin resistance and positively associated with detrimental metabolic health such as vascular diseases and chronic kidney diseases (Lau et al., 2018). Elevated circulating levels of indolelactate has been positively associated with the incidence and risk of T2D (Qi et al., 2022). Furthermore, microbial tyrosine derivatives such as p-cresol and p-cresol sulfate have been positively associated with the development of insulin resistance (Koppe et al., 2013). Our findings revealed that fermented sorghum treatment in T2D decreased the serum levels of p-cresol and p-cresol sulfate. *Helicobacter, Ruminiclostridium, Millionella, Oscillibacter*, *Acetatifactor*, *Acetivibrio*, *Enterocloster*, *Romboutsia* and *Allobaculum* were positively associated with p-cresol and p-cresol sulfate. Higher circulating levels of the microbial metabolite, phenylpropionate has been associated with lower incidence of T2D (Menni et al., 2020). Phenyl sulfate, a gut microbial metabolite derived from tyrosine metabolism was reported to markedly increase in diabetic kidney disease (DKD) and correlated with the progression of albuminuria in a diabetic patient cohort (Kikuchi et al., 2019). However, the present study found lower levels of circulating phenyl sulfate in T2D group but higher levels in healthy and fermented sorghum treatment groups. Our findings revealed higher circulating levels of phenylpropionate, phenyl sulfate, taurocholic acid, ursodeoxycholic acid, cholic acid, arginine and valine after fermented sorghum treatment in T2D. The beneficial effects of arginine in obese, insulin resistant and diabetic people has been recently documented (McNeal, Meininger, Reddy, Wilborn, & Wu, 2016). Results from MicFunPred analysis showed decreased biosynthesis of phenylalanine, tyrosine and tryptophan, lipopolysaccharides, fatty acids, ABC transporters and pathways involved in PPAR, AMPK, PI3K-Akt, and insulin signaling, insulin resistance, tight junction, glycolysis/gluconeogenesis, pyruvate, fructose and mannose metabolism after fermented sorghum treatment. Thus, fermented sorghum supplementation could improve insulin resistance, glucose tolerance and hyperglycemia by decreasing the circulating levels of detrimental metabolites indoxylsulfate, indolelactate, indole, indole 3-aldehyde, p-cresol, p-cresol sulfate, leucine, isoleucine, tyrosine, tryptophan, and phenylalanine, as well as increase phenyl sulfate, phenylpropionate, taurocholic acid, ursodeoxycholic acid, cholic acid and valine predicting gut microbiota inversely associated with T2D risk.

## 5. Conclusion

The findings of this study demonstrate the beneficial role of fermented sorghum in improving glycemic control and ameliorating insulin resistance in T2D via the modulation of the gut microbiota and their related metabolites. Fermented sorghum reversed the abundance of opportunistic pathogens positively associated with T2D and selectively promoted the growth of *Muribaculum*, *Phocaeicola* and *Parabacteroides* positively correlated with fecal SCFA's (butyrate and propionate) and inversely associated with T2D. Furthermore, serum metabolic biomarkers derived from bacteria or modified by bacteria and diet predictive of diabetes risk and microbiome signature and function were identified. Fermented sorghum consumption was associated with lower circulating levels of deleterious metabolites such as p-cresol, p-cresol sulfate, indolelactate, indoxyl sulfate, indole-3-aldehyde, indole, leucine, isoleucine, tyrosine, tryptophan, phenylalanine, succinic acid, oleic acid and 13-hexadecenoic acid positively associated with opportunistic pathogens and T2D. Moreso, the consumption of fermented sorghum was associated with higher circulating levels of favorable metabolites such as phenylpropionate, phenyl sulfate, valine, taurocholic acid (TCA), ursodeoxycholic acid (UDCA) and cholic acid (CA) inversely associated with T2D and positively associated with beneficial bacteria. Therefore, the beneficial role of fermented sorghum in the remission or treatment of T2D was associated with modulation of gut microbiota and its related-metabolites. Despite these promising effect of fermented sorghum on T2D, this study is not without limitations. First and foremost, in other to minimize discrepancies in results, larger number of mice per group are needed in follow up studies involving the final industrial product using fermented functional sorghum materials. Furthermore, clinical studies are required to investigate and confirm the metabolic crosstalk and molecular mechanism between host physiology and gut microbiome in the treatment of T2D with fermented functional sorghum product.

## CRediT authorship contribution statement

**Fred Kwame Ofosu:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Fazle Elahi:** Investigation, Writing – review & editing. **Eric Banan-Mwine Daliri:** Validation, Formal analysis, Writing – review & editing. **Simon Okomo Aloo:** Investigation, Formal analysis. **Ramachandran Chelliah:** Formal analysis, Writing – review & editing. **Sang-Ik Han:** Project administration, Resources, Validation, Writing – review & editing. **Deog-Hwan Oh:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

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*Data availability statement*

The data supporting the findings of this research are available within the article and supplementary materials and could also be accessed at https://data.macrogen.com/~macro3/HiSeq02//20211229/Hn00163262/HN00163262.zip.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105666.

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