Low-doses of Sucralose Alters Fecal Microbiota in High-fat Diet Induced Obese Rats

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Research

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Abstract

Background: Artificial sweeteners (AS) are widely used as sugar substitutes to reduce calorie intake. However, high doses of AS induced glucose tolerance by modulating gut microbiota. The objective of this study was to investigate the effects of lower doses of sucralose on fecal microbiota in obesity.

Methods: Eight weeks after high-fat diet, the male Sprague Dawley rats were randomly divided into four groups (n=24) and administrated by a daily gavage of 2ml normal saline (CON), 0.54mM sucralose (N054), 0.78mM sucralose (N078) and 324mM sucrose (S324) respectively. After four weeks, fecal samples were obtained and used in 16S ribosomal RNA gene sequencing.

Results: The richness and diversity of fecal microbiota did not change by these sucralose and sucrose dosage. Both 0.54mM (0.43mg) and 0.78mM (0.62mg) tended to reduce the beneficial bacteria, *Lactobacillaceae* and *Akkermansiaceae.* The relative abundance of family *Acidaminoccaceae* and its genus *Phascolarctobacteriam* were increased with 0.54mM sucralose. In functional prediction, 0.54mM sucralose increased profiles of carbohydrate metabolism, whereas 0.78 mM sucralose enhanced that of amino acids metabolism.

Conclusions: The lower doses of sucralose altered the compositions and the metabolic functions of fecal microbiota. The benefits of sucralose and its recommended dose for obese patients should be reassessed comprehensively.

Introduction

Obesity has emerged as a major public health challenge affecting over 650 million adults worldwide and increased the risks of type 2 diabetes, cardiovascular diseases, and even certain cancers[1]. It is widely accepted that table sugars contributed to the weight gain and thereby risks for metabolic disorders[2, 3]. In order to reduce energy density, artificial sweeteners (AS) which provide intensive sweet taste without extra calorie, are widely used as sugar substitutes.

The US Food and Drug Administration (FDA) approved the acceptable daily intake (ADI) levels of 6 kinds of AS including saccharin, aspartame, acesulfame potassium (Ace-K), sucralose, neotame, and advantame[4]. However, the effects of AS on glucose homeostasis remain controversial. Some studies demonstrated the benefits of AS exposure[5] whereas others showed AS were associated with the incidence of obesity and type 2 diabetes[6–8].

The plausible mechanisms underlying the metabolic effects of AS are not fully understood. Given that most AS pass through the gastrointestinal trat without being absorbed or digested, they may directly alter the gut microbiota which play crucial roles in the pathogenesis of metabolic diseases[9, 10]. Suez et al. reported that saccharin in ADI dose (5mg/kg body weight) induced glucose intolerance by modulating intestinal microbiota in mice and human group[11]. The transplantation of saccharin-exposed feces also induced glucose intolerance in germ-free mice[11]. Another, it was indicated that administration of

Splenda (1.1% sucralose, 1.1–11 mg/kg sucralose) reduced beneficial fecal bacteria and elevated fecal pH, intestinal p-glycoprotein and cytochrome p-450 in rats[12]. It should be noticed that the doses of AS in most studies were far beyond normal levels of daily consumption (ADI of sucralose 5 mg/kg). Evidence also suggested that chronic consumption of low-dose aspartame also resulted in hyperglycemia possibly via short-chain fatty acids (SCFAs) produced by gut microbiota[13].

Sucralose is derived from sucrose in which three hydrogen-oxygen groups of sucrose are replaced by three chlorine atoms. In this process the sweetness of sucralose is dramatically intensified to about 600 times of sucrose[14]. About 85% of sucralose is excreted without being absorbed or digested in the gastrointestinal tract[14]. Previous studies showed that a single dose of sucralose had no effects on blood glucose in health subjects[15] and patients with type 2 diabetes[16]. However, sucralose exerted strong bacteriostatic effects *in vitro* and altered the structures of microbial communities in normal rodents[17]. It is still unclear whether sucralose particularly in routine dose can modulate the gut microbiota compared with natural sugars. We therefore aimed to evaluate the potential effects of different concentrations of sucralose and sucrose on fecal microbiota in a high-fat diet induced obese rat model.

Materials And Methods

Animals

Male Sprague Dawley (SD) rats (four weeks old) were fed with sterile food and water under specific pathogen-free (SPF) conditions with 12-hour dark-light cycle, controlled temperature (20°C–23°C), and settled humidity (40%–60%) (Laboratory Animal Resources, Chinese Academy of Sciences). After adapting to the environment for one week, the rats were fed with an *ad libitum* high-fat diet (HFD, 20% carbohydrate, 20% protein and 60% fat) or normal chow diet (68% carbohydrate, 22% protein and 10% fat) correspondingly for 8 weeks. 24 rats from high-fat diet were considered as successful obese models with 20% heavier than that of chow diet. The experiment protocol met the standards of the Institutional Animal Care and Use Committee of SLAC (IACUC) Guide for Care of Laboratory Animals.

Treatment

The 24 obese rats (0.64 ± 0.05 kg)were randomly divided into 4 groups (6 in each group) by random number table: normal saline (control group, CON), 0.54 mM sucralose (N054, Sigma-Aldrich, MO, USA), 0.78 mM sucralose (N078), and 324 mM sucrose (S324, Sigma-Aldrich, MO, USA). Rats were intragastric administrated with 2 ml certain solution at a fixed time every day for 4 weeks which was described in our previous study[18]. The translation doses from rats to human were 0.11 mg/kg (N054), 0.16mg/kg (N078) and 56.20 mg/kg (S324) according to the body surface area[19].

Fecal sample collection

At the end of treatment, fecal samples were collected after 12-hour fasting. Each rat was hold in hands and received abdominal massage until fresh pellets were collected directly in a 1.5 mL sterile freezing tube. The tubes were placed immediately in liquid nitrogen and moved to -80 °C refrigerator.

DNA extraction, PCR amplification and 16S rRNA gene sequencing

DNA extraction, PCR amplification and 16S rRNA Sequencing were performed as before[20]. In short, total genomic DNAs of stool samples were extracted by using the E.Z.N.A. soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). Genes of the 16S rRNA V3-V4 regions were amplified using specific 338F and 806R primers by thermocycler polymerase chain reaction (PCR) system (GeneAmp 9700, ABI, USA). The extracted and purified amplicons were sequenced by using Illumina MiSeq platform (Illumina, San Diego, USA).

Statistical analysis

The data points of all the mice were included within the analysis of this study. Bioinformatic analyses were performed by the Majorbio I-Sanger Cloud Platform (https://cloud.majorbio.com/) and SPSS Statistics V.23 software (IBM). Alpha diversity indices were applied to analyze the richness and diversity of samples, including Sobs, ACE, Chao1, Shannon and Simpson. Beta diversity analysis including unsupervised principal coordinates analysis (PCoA) and supervised partial least squares-discriminant analysis (PLS-DA) were performed to explore the similarities or dissimilarities of each sample. Permutational multivariate ANOVA (PERMANOVA) was calculated based on Bray Curtis.

Differences in the relative abundance of taxa among groups were analyzed using the Kruskal-Wallis rank-sum test with Scheffe post hoc analysis. Correlation network according to Spearman correlation analysis was used to determine the interactions of bacterial community. The linear discriminant analysis (LDA) effect size (LEfSe) algorithm differentiated microbial features for biomarker discovery. Only taxa with absolute LDA (log10) scores > 2.0 and an alpha value of 0.05 were discussed in this study. Metabolic functions were predicted by using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt).

Results

Characteristics of bacterial diversity and clustering

In the analyses of alpha diversity, neither sucralose nor sucrose altered the community richness (Sobs, ACE, Chao1 index) or diversity (Shannon, Simpson index) of fecal microbiota (Table S1). In beta diversity, the PCoA plot revealed that most samples treated by 0.78 mM sucralose clustered in a distinct group compared with CON, N054 and S324 groups (PERMANOVA, P = 0.001 and P adjust = 0.001). It was also confirmed by the supervised PLS-DA on OTU level that each of the four groups showed a specific cluster (COMP1 9.04% and COMP2 6.09%), suggesting that they had different bacterial structures (Figure 1).

Alterations of core microbial composition induced by sucralose and sucrose

On phylum level, 0.54 mM sucralose increased the relative abundance of *Firmicutes* but decrease that of *Bacteroidetes* whereas 0.78 mM sucralose had the opposite effects (Figure 2A, 2B). The ratio of Firmicutes to *Bacteroidetes* in N054 was higher than that in N078 ($P = 0.029$, Figure S1). No difference was shown in the ratio of *Bacteroidetes* to *Proteobacteria*. Notably, both 0.54 mM and 0.78 mM sucralose reduced the relative abundance of *Verrucomicrobia*.

To describe the alterations of bacterial communities, the relative abundance of families was detected (Figure 2C, 2D). The beneficial bacteria, *Lactobacillaceae* and *Akkermansiaceae* tended to be lower in both 0.54- and 0.78-mM sucralose, compared to control and sucrose group (Figure 2D). These concentrations of sucralose supplementation increased the relative abundance of *Barnesiellaceae*, whereas they decreased that of *Streptococcaceae*. Sucralose and sucrose consistently upregulated *Christensenellaceae* and downregulated *Micrococcaceae* as well as *Eubacteriaceae* ($P < 0.01$). There were some inconsistent effects of the different concentrations of sucralose. 0.54 mM sucralose significantly reduced the relative abundance of *Muribaculaceae* but increased that of *Acidaminococcaceae*. The LEfSe analysis showed that genus *Phascolarctobacterium*, belonged to the family *Acidaminococcaceae* was enriched in N054 group. Family *Muribaculaceae* (S24-7) was considered as the biomarker of N078 group (Figure 3).

In the network of significantly interacting families in N054 group (Figure S2), it was noticed that *Akkermansiaceae* was positively correlated with *Christensenellaceae*, *Veillonellaceae* and *norank Gastranaerophilales* whereas it showed negative correlation with *Acidaminococcaceae*. The most abundant family *Muribaculaceae* had a positive interaction with *Bifidobacteriaceae* and negative interactions with *Deferribacteraceae* and *Burkholderiaceae*.

Effects of predicted metabolic functions of fecal microbiota

PICRUSt and LEfSe were used to determine the changes in predicted functional composition (Figure 4). At KEGG level 3, ABC transporters (also known as ATP-binding cassette transporters) and the Carbohydrate metabolism were enhanced by N054 group. The exposure of 0.78 mM sucralose increased the functional profiles related to metabolism including Amino acid related enzymes, Energy metabolism, Alanine, aspartate and glutamate metabolism, Pantothenate and CoA biosynthesis, and Vitamin B6 metabolism. The biosynthesis of fatty acid was related to the 324mM sucrose intervention.

Discussion

AS consumption at high doses (ADI dosage) might impair glucose homeostasis by interfering with gut microbiota. In current study, we demonstrated 4-week lower doses of sucralose (0.54 mM and 0.78 mM) altered the compositions and metabolic functions of fecal microbiota in obese rats.

The richness and diversity of fecal microbiota were not changed by the sucralose and sucrose dosages. Previous *in vitro* studies found that sucralose exerted bacteriostatic effects in a dose-dependent manner via inhibiting the invertase and sucrose permease of bacteria[12]. However, sucralose did not reduce the

overall richness and diversity of intestinal bacteria *in vivo* which was consistent with our results[17]. It was probably due to the wide variety of microorganisms and their complex interactions with each other and their hosts in gastrointestinal tract[21].

Beta diversity was used to explore the differences and similarities of microbial compositions among samples. 0.54 mM (~ 0.43 mg daily) and 0.78 mM (~ 0.62 mg daily) sucralose groups had different clusters. It indicates that even the lower doses of sucralose significantly altered the structures of fecal microbiota. Few studies investigated the impacts of sucralose on beta diversity. There was a study found that neotame changed the beta diversity after 4 weeks intervention on CD-1 mice[22].

Firmicutes and *Bacteroidetes* were the two most abundant phyla, accounting for over 90% of the gut microbiota[23]. We observed that 0.54 mM increased the relative abundance of *Firmicutes* and decrease that of *Bacteroidetes,* while 0.78 mM sucralose exerted the opposite effects. Notably, it was reported that sucralose did not alter the levels of *Firmicutes* nor *Bacteroidetes* either after short-term intervention in healthy adults (780mg/d, 7 days)[24] or after long-term treatment in normal mice (1.5 and 15 mg/kg body weight, 8 weeks)[25]. Nevertheless, when sucralose was supplemented with HFD simultaneously, there were obvious changes of increased *Firmicutes* and decreased *Bacteroidetes*. It suggested that sucralose in combination with HFD could alter the predominant phyla[17]. A recent study also highlighted the intake of sucralose with carbohydrate impaired insulin sensitivity and glucose metabolism[26]. Given the widely use of AS in patients with obesity, the relation between AS and HFD should be considered.

Furthermore, we and Wang et al. [17] presented that sucralose had no effects on phylum *Proteobacteria* level in HFD induced obesity. It was reported that *Proteobacteria* was elevated after the commercial sucralose (Splenda) dosage in a Crohn's disease model (SAMP mice) and the related control (AKR/J mice)[27]. In fact, the higher level of *Proteobacteria* was closely related to inflammation, it was not difficult to detect its increase in the models of immune system dysfunction[28]. Therefore, the effect of sucralose on *Proteobacteria* needs to be further clarified.

Our results highlighted that both 0.54mM and 0.78mM sucralose tended to reduce the relative abundance of beneficial bacteria *Lactobacillaceae* and *Akkermansiaceae* which could improve metabolic symptoms via various mechanisms. Notably, *Lactobacilli* were reduced by 39.1% after a 12-week intervention of Splenda in healthy rats[12]. The reduction of *Lactobacillus* was also confirmed in acesulfame potassium treated mice[29]. *Akkermansia Muciniphila,* a mucin-degrading bacterium, is lower in human or animal models with obesity and type 2 diabetes[30]. Bian et al. observed that the abundance of *Akkermansia* did not change in 3 months supplementation of sucralose but it was increased after further 3 months consumption in health mice[31]. In our study, a four-week administration with sucralose reduced *Akkermansiaceae* of obese rats. Whether the reduction remains in the long-term of low doses of AS in obesity need further investigation.

In our study, the family *Acidaminoccaceae* was significantly negative associated with *Akkermansiaceae* in the network analysis. The genus *Phascolarctobacteriam*, belonging to family *Acidaminoccaceae,* was strongly correlated with metabolic dysfunction including weight gain, blood triglyceride, insulin level,

glucose tolerance[32]. We found that *Phascolarctobacteriam* was enriched in the 0.54 mM sucralose group. *Phascolarctobacterium* could ferment carbohydrate and produced short chain fatty acids such as acetate and propionate[33]. It was consistent with our functional prediction that carbohydrate metabolism was enhanced in 0.54 mM sucralose dosage.

In accordance with the changes of bacterial compositions, we provided evidence that sucralose in doses (0.53mM and 0.78 mM) changed functional profiles of fecal microbiota related to the metabolism of carbohydrates and amino acids. Gut-microbial metabolite from nutrients in daily diet was linking to the development of obesity and insulin resistant[34]. Sucralose was showed to alter the metabolism of some amino acids and derivatives in feces in particular the tryptophan and tyrosine[31]. Additionally, Suez et al[11] reported that the consumption of saccharin in ADI dosage increased the pathway genes related to glycosaminoglycan and other glycan. Furthermore, we presented that the dose of sucralose was an important factor when interacting with gut microbiota. Particularly, 0.54 mM sucralose (~2.2% of FDA ADI dosage) enhanced the ATP-binding cassette (ABC) transporters and carbohydrate metabolism, while the exposure of 0.78 mM sucralose (~3.2% of ADI dosage) was more related with the Amino acid metabolism. We previously indicated that 0.78 mM instead of 0.54 mM sucralose lowered the blood glucose of HFD obese rats. It should be noticed that the different effects of these sucralose dosages in gut microbiota might be partly responsible for the distinct metabolism. Thus, AS might have complex effects on fecal microbiota, taste receptors and hormone secretion when they pass through the gastrointestinal tract.

There are some limitations that should be considered. Stable bacterial colonies may form over a long period. The intervention time was relatively short in our study. Moreover, the use of 16S rRNA gene sequencing limited the detection of bacterial taxonomy and functions. Nonetheless, we preliminary observed the changes of compositions and predicted functions caused by sucralose and sucrose. The different strains and the potential mechanisms should be further explored in vitro and in vivo.

In conclusion, our study demonstrated that 4-week dosages of sucralose (0.54 mM and 0.78 mM) changed the compositions and potential functions of fecal microbiota in high fat induced obese rats. Lower doses of sucralose (0.54 mM and 0.78 mM) tended to reduce the beneficial bacteria, *Lactobacillaceae* and *Akkermansiaceae.* Furthermore, 0.54 mM increased the predictive functions of carbohydrates and the consumption of 0.78 mM sucralose was related to amino acids metabolism. The effects of sucralose on energy metabolism varies with dosage and intervention period. Since AS are widely used in our daily life, the ADI needs to be revaluated.

Abbreviations

AS
Artificial sweeteners
CON
Control

RNA
Ribonucleic acid
US
United States
FDA
Food and Drug Administration
ADI
Acceptable daily intake
Ace-K
Acesulfame potassium
SCFAs
Short-chain fatty acids
SD
Sprague Dawley
SPF
Specific pathogen-free
HFD
High-fat diet
IACUC
Institutional Animal Care and Use Committee
DNA
Deoxyribonucleic acid
PCR
Polymerase chain reaction
PCoA
Principal coordinates analysis
PLS-DA
Partial least squares-discriminant analysis
ANOVA
Analysis of Variance
PERMANOVA
Permutational multivariate ANOVA
LDA
Linear discriminant analysis
LEfSe
Linear discriminant analysis Effect Size
PICRUSt
Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
OUT
Operational taxonomic units

COMP
Component
KEGG
Kyoto Encyclopedia of Genes and Genomes
ATP
Adenosinetriphosphate
ABC
ATP-binding cassette

Declarations

Ethics approval and consent to participate

The protocol of this study was approved by the institutional ethics committee of Institutional Animal Care and Use Committee of Shanghai Laboratory Animal Center, Chinese Academy of Sciences on January 8, 2018.

Consent for publication

Not applicable.

Availability of data and material

The data and material are accessible from the authors upon reasonable request.

Competing interests

The authors declare no conflict of interest in this study.

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Authors' contributions

M.C.Z did the data analysis and prepared the manuscript; J.C wrote the manuscript; M.L.Y collected the samples and did the fecal DNA extraction; C.Q, W.L, Y.C.Q and R.L.F performed the animal experiments; M.Y checked the data analysis; W.L contributed to the study design; J.M is the guarantor of this study to ensure the accuracy and integrity of the data.

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Figures

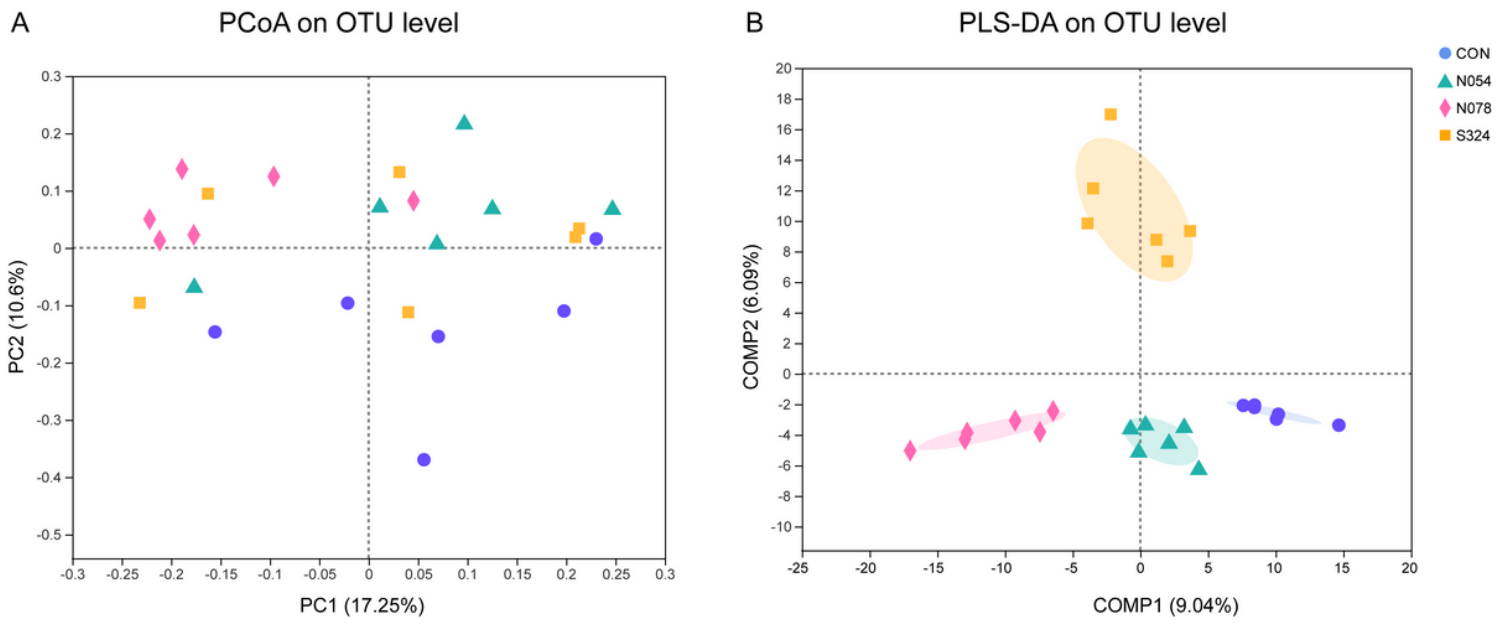

Figure 1

Beta analysis in four groups with unsupervised and classification. (A) Principal Coordinates Analysis (PCoA). Bray-Curtis distances and permutational multivariate analysis of variance (PERMANOVA) was performed. P = 0.001 and P adjust = 0.001. (B) Partial least squares-discriminant analysis (PLS-DA). Each sample was represented by a dot (n=6). CON: control group; N054: 0.54 mM sucralose; N078: 0.78 mM sucralose; S324: 324 mM sucrose.

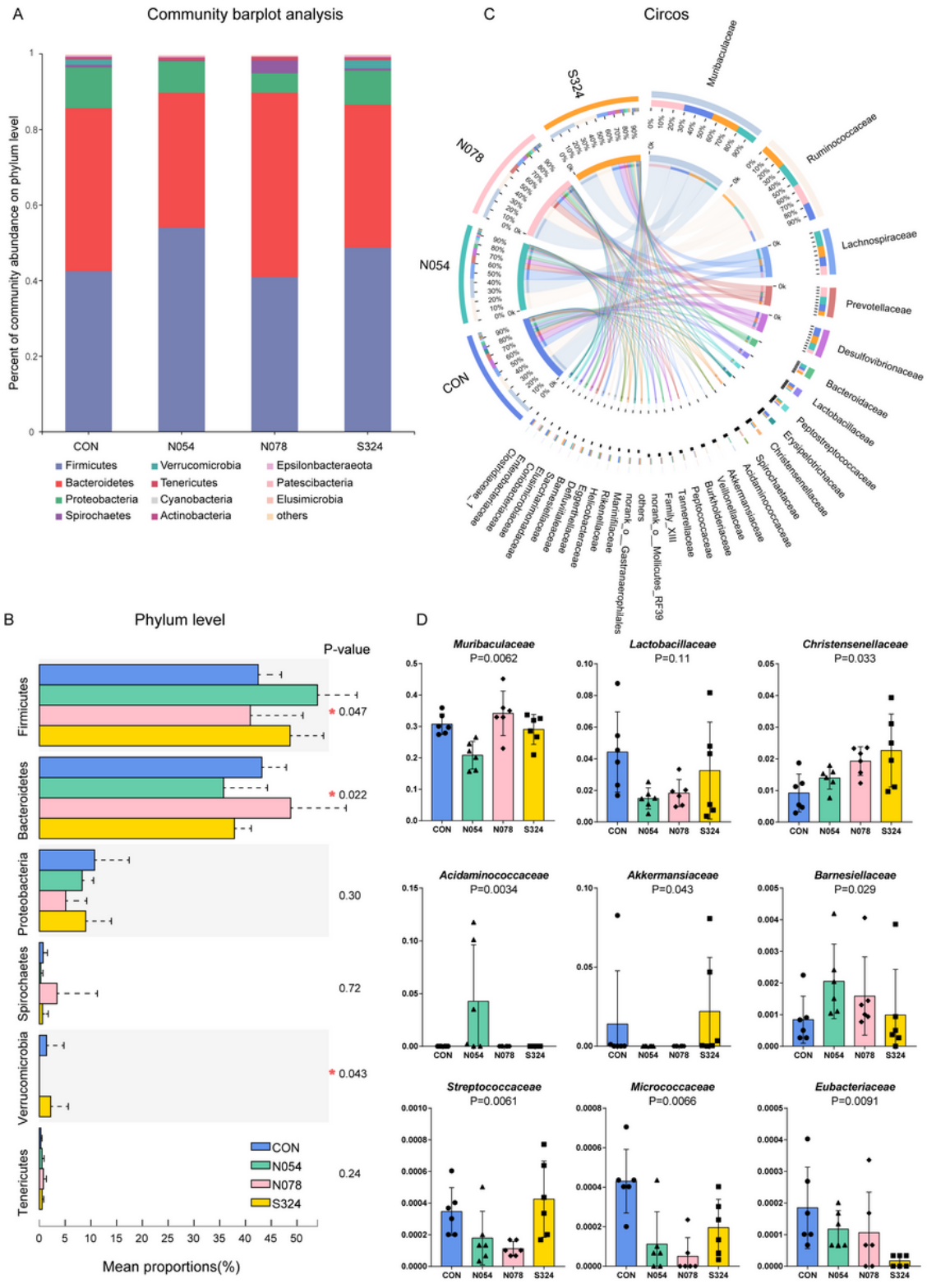

Figure 2

Main bacterial communities of different taxonomies. (A) Community barplot of the domain phyla. (B) Relative abundant of the domain phyla of four groups. (C) Circos plot showing the relationship between microbial families and samples. (D) Relative abundance of core bacterial families. Kruskal-Wallis rank sum test was performed (n=6). * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

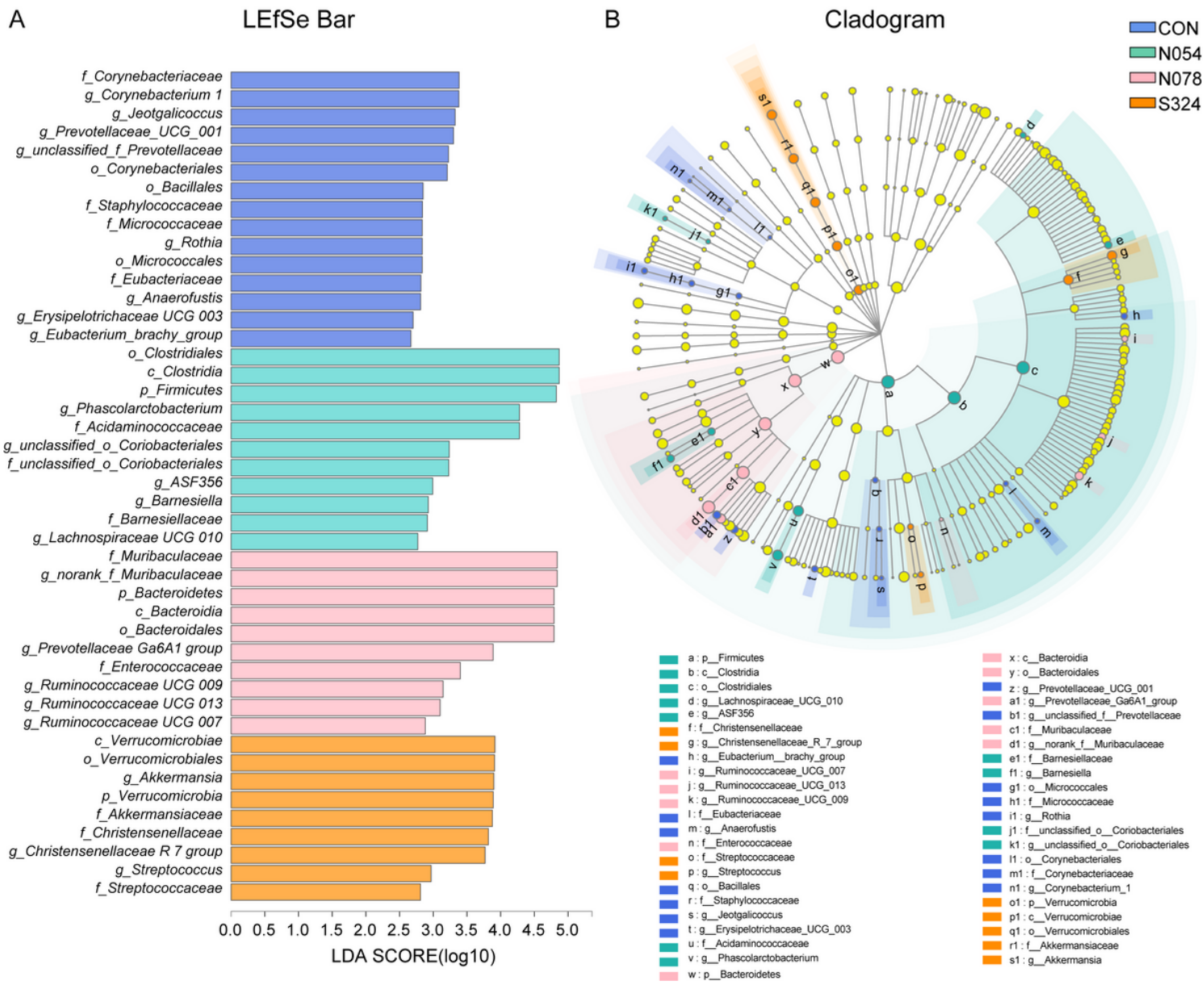

Figure 3

Linear discriminant analysis (LDA) effect size (LEfSe) analysis based on genus level among four groups. (A) LEfSe Bar plot demonstrating the significant bacterial differences. (B) Cladogram indicating the phylogenetic distribution of fecal microbiota with phyla in the outermost and genera in the innermost ring. Multiple comparison strategy was all-against-all (n=6). Only LDA score > 2.0 are shown.

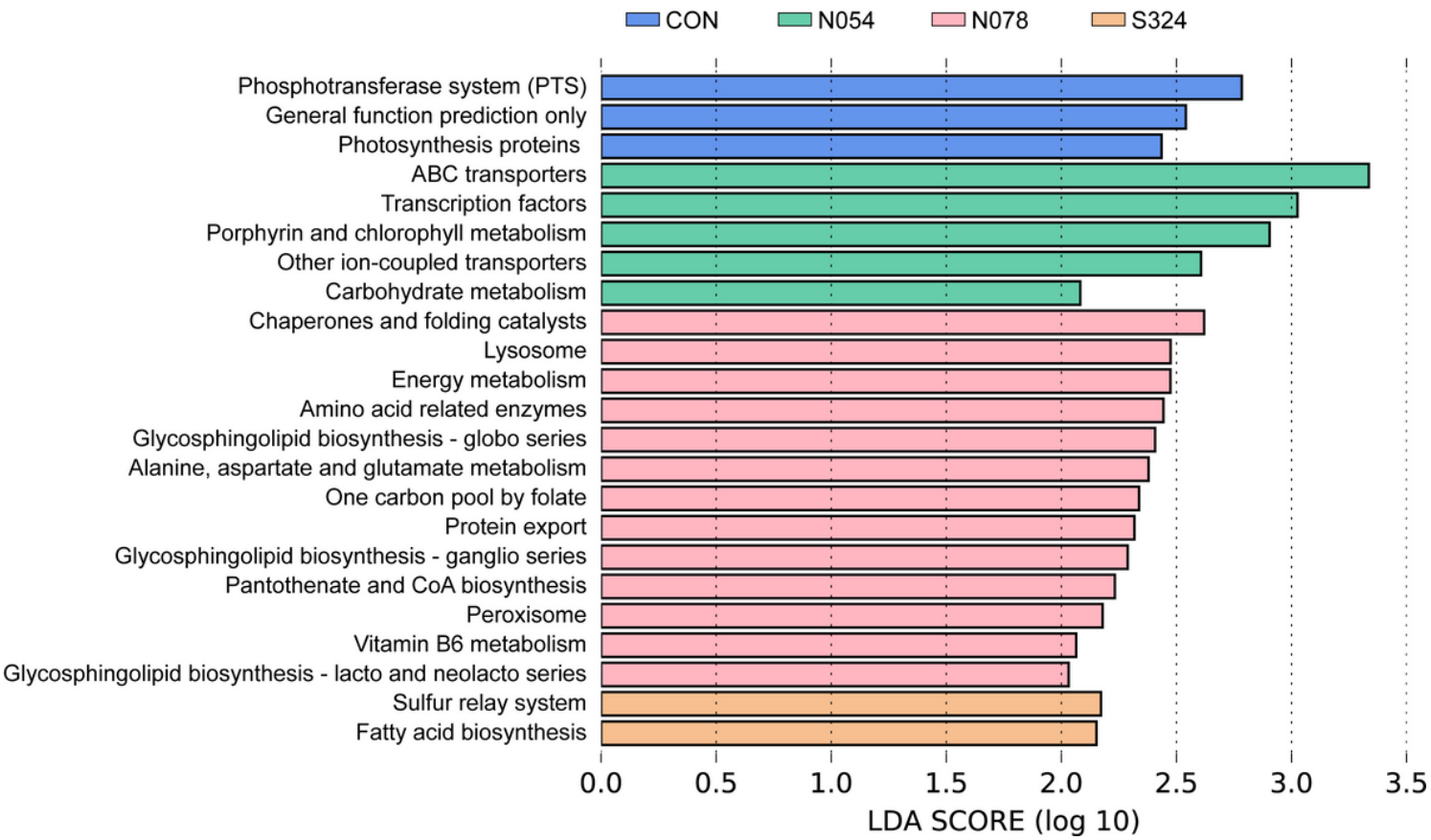

Figure 4

LEfSe analysis on predictive functions of KEGG level 3 identified via PICRUSt. Only a Log LDA > 2.0 was considered as significant difference. KEGG: Kyoto Encyclopedia of Genes and Genomes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.tif
- FigureS2.tif
- Supplementary.docx