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Effect of tea polysaccharides on faecal microbiota and their short-chain fatty acid metabolic products

[1,2]Wu, Z., [1]Lei, Z. Y., [1]Zeng, W. Z. and [1]*Yang, J. F.

[1]College of Horticulture, Fujian Agriculture and Forestry University / Provincial Key Laboratory of Tea Science in Universities, No. 15 Shangxiadian Street, Cangshang District, Fuzhou 350002, Fujian, People's Republic of China
[2]College of Pharmacy, Fujian University of Traditional Chinese Medicine, No. 1 Qiuyang Street, Minhou District, Fuzhou 350122, Fujian, People's Republic of China

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Abstract

To explore the effect of tea polysaccharides on the gut microbiota and their short-chain fatty acid (SCFA) metabolic products, we used the faecal microbiota to simulate the gut microbiota *in vitro*, and cultured them to obtain a preculture solution. Ultrapure water, tea polysaccharides, and glucose were added to the precultured solution for anaerobic fermentation. Samples of each group were harvested at 0, 6, 12, and 24th hour during fermentation to test the contents of the SCFAs. In addition, high-throughput 16S rRNA sequencing was performed to analyse the microbiota in the fermentation medium. Results showed that the faecal microbiota used tea polysaccharides to generate SCFAs. When compared with the fermentation group with the addition of ultrapure water, the group with the addition of tea polysaccharides increased the relative abundance of Firmicutes, and decreased the relative abundance of Bacteroidetes at the phylum level. The relative abundances of *Butyricimonas*, *Roseburia*, *Eubacterium rectale* group, *Ruminococcus* 1, *Lachnospira*, and *Parasutterella* increased significantly at the genus level. Based on the LEfSe analysis of key microbiota at the genus level, significant differences between the groups were observed. It was clear that tea polysaccharides selectively enriched the microbiota to produce SCFAs, and the correlation between the SCFAs and faecal microbiota was confirmed.

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Introduction

Tea is made from the leaves of *Camellia sinensis* L., and has been one of the most popular drinks in the world due to its favourable flavour and health-promoting bioactivities. Tea polyphenols are the most famous biological active components of tea which are thought to contribute to the health benefits of tea (Narotzki *et al*., 2013). Tea polysaccharides (TPS) are another biological active component of tea, and have received increasing attention because of their non-toxicity, good biocompatibility, and excellent biological activities (Wu *et al*., 2018).

The toxicity of TPS has been evaluated using mice, and the results showed that no side effects were observed in mice after oral administration of TPS (Chen *et al*., 2007). Modern pharmacological research has shown that TPS have a wide range of physiological activities such as antioxidation (Yuan *et al*., 2015), hypolipidaemic (Xu *et al*., 2015), antidiabetic (Xu *et al*., 2012), and antitumor (Cheng *et al*., 2018). Therefore, TPS is regarded as a promising natural product.

As a high molecular polysaccharide, sugars and uronic acids are abundant in TPS. As a polysaccharide-containing structure of uronic acids, tea polysaccharides were less efficiently absorbed by the small intestinal mucosa due to the electrostatic repulsive force between the negatively charged TPS which contain a carboxylic acid group, and the negatively charged small intestinal mucosa. This in turn reduces its bioavailability (Mao *et al*., 2021).

The human gut microbiota encode a significant proportion of genomes to the degradation of complex carbohydrates (Abdessamad *et al*., 2013). Microorganisms regulate physiological and pathogenic processes in human body by producing various carbohydrate-active enzymes (CAZy) to degrade and modify complex carbohydrates, and generate signal molecules for further utilisation in

*Corresponding author.
Email: wzrenwz@163.com

human cells (Zhou *et al*., 2019). It was found that in Bacteroidetes, these systems are organised as polysaccharide utilisation loci (PULs), which are strictly regulated co-localised gene clusters that encode enzyme and protein ensembles required for the saccharification of complex carbohydrates (Grondin *et al*., 2017).

Tea polysaccharides (Chen *et al*., 2018) from Fuzhuan brick tea could not been digested by the digestive system, but it could be degraded and utilised by gut microbiota. It also promoted the proliferation of beneficial bacteria such as *Lactobacillus* and *Akkermansia*, thus significantly increasing the concentration of short-chain fatty acids (SCFAs). SCFAs are suggested to play a crucial role in reinforcing gut integrity, and inhibiting gut inflammation by mechanisms such as T_{reg} cell induction (Gill *et al*., 2018). It has also been found that one of the effects of SCFAs produced by gut microbiota is to activate receptors such as FFAR2 and FFAR3 (Brown *et al*., 2003; Le Poul *et al*., 2003), which in turn promote the secreted expression of GLP-1 and PYY (Tolhurst *et al*., 2012). Moreover, the gut microbiota is considered to play a crucial role in promoting host health, and is now considered to be an essential organ (Rook *et al*., 2017). Studies have shown that intestinal microbial imbalance might be a key environmental factor for a variety of complex diseases.

In our study, healthy human faecal microbiota were used as the material to simulate TPS fermentation by gut microbiota *in vitro*. High-throughput 16S rRNA sequencing was implemented to analyse the microbiota in the fermentation medium. In addition, the contents of SCFAs in the fermentation medium were tested by gas chromatography (GC). The relationship between the changes in the microbiota in the fermentation medium and the metabolism of SCFAs was studied.

Materials and methods

Materials

Wuyi rock tea was purchased from Lapsang Tea Industry Co., Ltd. (Nanping, China); chromatographically pure SCFAs (acetic, propionic, *n*-butyric, *i*-butyric, *n*-valeric, and *i*-valeric acid) were purchased from Shanghai Maclean Biotechnology Co., Ltd. (Shanghai, China); bile salts, L-cysteine hydrochloride monohydrate ($C_3H_7NO_2S{\cdot}HCl{\cdot}H_2O$), and Tween 80 were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China); Omega DNA Kit was purchased from Omega Bio-Tek (Georgia, USA); tryptone, yeast extract, glucose, maltose, NaCl, KCl, $NaHCO_3$, $MgSO_4{\cdot}H_2O$, KH_2PO_4, K_2HPO_4, $CaCl_2$, and $FeSO_4{\cdot}7H_2O$ were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of tea polysaccharides

Tea polysaccharides were prepared from Wuyi rock tea by water extraction and alcohol precipitation, following our previously reported method (Lei *et al*., 2019). Briefly, 50 g tea was extracted with 500 mL of 80% ethanol solution for 24 h to remove some small molecules such as polyphenols and pigments. After filtration, the tea residue was air-dried and extracted three times with distilled water (1:30, w/v) at 70°C for 0.5 h. The extracts were combined and centrifuged, precipitated by addition of quadruple volumes of absolute ethanol, and kept at 4°C overnight. After centrifugation (3,000 *g*, 15 min), the precipitates were collected, dissolved with distilled water, and proteins removed by Sevag method. The final precipitates were dissolved in distilled water, dialysed for 48 h with distilled water, and lyophilised to obtain the crude TPS.

As shown in our published paper (Lei *et al*., 2019), the TPS was mainly composed of neutral sugars and uronic acid. The infrared spectrum analysis showed that TPS contained carbohydrates and proteins.

Faecal preparation and preculture faecal microbiota

Faecal samples were supplied by healthy adult volunteers (two males and one female, aged 22 to 27 years) who had no history of bowel disease, and had not taken antibiotics for the past six months; 30 g of faeces were taken from each volunteer and mixed well. Then, 60 g was taken for preculture. The medium formulation was prepared according to Fernando *et al.* (2004) with slight modifications: 500 mL of preculture medium contained 5 g of tryptone, 2.5 g of yeast extract, 5 g of NaCl, 2.5 g of glucose, and 3 g of maltose. The medium was sterilised (GI80TW, Zealway Instrument Inc., Xiamen, China) at 121°C for 15 min. Fresh faeces (60 g) was then added to 500 mL of preculture medium, immediately loaded into an anaerobic bag (Haibo Biotechnology Co., Ltd., Qingdao, China), and cultured in an incubator (GNP-9160 Shanghai Jing Hong Laboratory Instrument Co., Ltd., Shanghai, China) at

37°C overnight to obtain preculture solution. The solution was filtered through sterilised gauze (eight layers) to remove large particles, and transferred to anaerobic bags to obtain the precultured faecal microbiota. The above procedures were carried out in a super clean bench (SW-CJ-IFD, Suzhou Purification Equipment Co., Ltd., Suzhou, China).

Fermentation of tea polysaccharides by precultured faecal microbiota

The fermentation medium was prepared according to Fisher and Woods (2000) with slight modifications: 1 L of medium contained 4.5 g of NaCl, 4.5 g of KCl, 1.5 g of $NaHCO_3$, 0.69 g of $MgSO_4 \cdot H_2O$, 0.5 g of KH_2PO_4, 0.5 g of K_2HPO_4, 0.5 g of bile salt, 0.8 g of L-cysteine hydrochloride monohydrate, 0.08 g of $CaCl_2$, 0.005 g of $FeSO_4 \cdot 7H_2O$, and 1 mL of Tween 80. The medium was aliquoted into 50 mL Erlenmeyer flasks, sterilised at 121°C for 15 min, and then moved to an anaerobic bag.

The final volume (25 mL) of the fermentation solution was composed of 40% medium (10 mL), 40% precultured faecal microbiota solution (10 mL), and 20% sample (5 mL). This was divided into three groups of *in vitro* fermentation (with three replicates in each group): (i) *in vitro* fermentation for 24 h with the addition of ultrapure water (BLK group), (ii) *in vitro* fermentation for 24 h with the addition of 5 mg/mL TPS (TPS group), and (iii) *in vitro* fermentation for 24 h with the addition of 5 mg/mL glucose (GLU group).

The precultured faecal microbiota solution and the water, TPS, or glucose was added to fermentation solution at the same time. Subsequently, the fermentation solution was placed in anaerobic bags, and cultured at 37°C. The samples were harvested at 0, 6, 12, and 24th hour, respectively, during fermentation, and stored in a -80°C freezer for subsequent analyses.

Determination of SCFAs contents

The samples were centrifuged for 10 min (12,000 rpm). Each supernatant was filtered through 0.22-μm filters to obtain a test sample, and then analysed by GC (7890A, Agilent Technologies, USA). The method used was as follows: a 1 μL of test sample was injected at 240°C and loaded on a GC equipped with an HP-INNOWAX column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, USA). Nitrogen (N_2) was provided as the carrier gas at a flow rate of 20.0 mL/min. The flow rates of air and hydrogen (H_2) were 300 and 30 mL/min, respectively. The initial column temperature was maintained at 100°C for 0.5 min. The temperature was raised to 150°C at a rate of 4°C/min, and then raised to 185°C at a rate of 5°C/min. The FID (Agilent Technologies, USA) temperature was maintained at 240°C. The run time for each analysis was 20.0 min.

Extraction and sequencing of genomic DNA of microbiota

The method of DNA extraction was performed following the manufacturer's instructions of the Omega DNA Kit. The extracted DNA samples were tested by 1% agarose gel electrophoresis and spectrophotometry (260/280 nm optical density ratio) for mass detection. After being tested, the samples were stored at -80°C for subsequent experiments. PCR amplification and high-throughput 16S rRNA sequencing of the V4 region was performed by the Allwegene Tech. Co., Ltd. (Beijing, China). Sequencing and bioinformatics were carried out on the Illumina MiSeq PE250 platform to generate 2 × 250 bp paired end reads. Information about the processing group sequence was clustered into operational taxonomic units (OTUs) for species classification, and the OTU similarity was set to 97%. The Silva database was used to determine the species classification information matching each OTU.

Alpha diversity index can reflect the richness and evenness of faecal flora. In the present work, the Chao1 estimator, Shannon index, Observed species, and PD whole tree were used to analyse the alpha diversity of faecal flora. The Chao1 estimator is a measure of community richness, and primarily concerned with information on the community richness of a sample. The Shannon index comprehensively reflects the richness and evenness of community. The Observed species indicates the number of OTUs detected by sequencing. The PD whole tree index can assess the degree of diversity based on the phylogenetic characteristics of OTU sequence evolutionary tree. The higher the value of alpha diversity index, the higher the community diversity.

Statistical analysis

All experiments were performed in triplicate, and data were presented as mean ± standard deviation (SD). Comparison of multiple samples was conducted by one-way analysis of variance

(ANOVA) with SPSS 19 software (IBM). $p < 0.05$ was considered to be significant.

Results

Contents of SCFAs in fermentation medium

SCFAs are considered to be an important bridge for the physiological effects of dietary fibres (Koh *et al*., 2016), and TPS is the most important dietary fibre in tea. Testing SCFAs in the medium at different time points is conducive to understanding the physiological effect of TPS on the faecal microbiota.

The concentration of total SCFAs in the mediuM was in the following order: GLU > TPS > BLK (Table 1). The main ingredients of the SCFAs produced in the fermentation medium were acetic, propionic, and *n*-butyric acids. Acetic, propionic, and *n*-butyric acids in the fermentation medium of TPS group increased significantly from 0 to the 24th hour ($p < 0.05$), and *i*-butyric acid was not detected at 0 and 6th hour, but increased significantly at 12 and 24th hour ($p < 0.05$); the contents of *n*-valeric and *i*-valeric acids increased during the fermentation, and there was no discrepancy at 6 and 12th hour ($p > 0.05$), although it did increase significantly at 24th hour ($p < 0.05$).

Acetic, propionic, and *n*-butyric acids, which are the main ingredients of SCFAs, were more abundant in the medium of TPS and GLU groups than in the BLK group at the 6, 12, and 24th hour. Among medium of these groups, the content of acetic acid was in the order of GLU > TPS > BLK, the contents of propionic and butyric acids were in the order of TPS > GLU > BLK, and the differences were significant at the 6, 12, and 24th hour ($p < 0.05$). *i*-butyric acid was not detected at 0 and 6th hour, but at the 12 and 24th hour, the order of content was

Table 1. Contents of SCFAs in the medium at different fermentation time points.

SCFA	Group	Content (mM)			
		0 h	6 h	12 h	24 h
Acetic acid	BLK	8.3 ± 0.09^{AB}	8.5 ± 0.39^{aAB}	7.93 ± 0.42^{aAC}	8.84 ± 0.13^{aB}
	TPS		9.51 ± 0.03^{bC}	10.01 ± 0.20^{bD}	11.66 ± 0.38^{bE}
	GLU		11.4 ± 0.59^{bC}	11.68 ± 0.12^{cC}	12.36 ± 0.25^{cD}
Propionic acid	BLK	6.30 ± 0.14^{A}	6.05 ± 0.14^{aA}	5.60 ± 0.19^{aA}	5.85 ± 0.40^{aA}
	TPS		6.95 ± 0.05^{bB}	7.34 ± 0.17^{bC}	8.16 ± 0.19^{bD}
	GLU		7.09 ± 0.34^{bB}	6.26 ± 0.22^{cC}	6.68 ± 0.41^{cC}
n-butyric acid	BLK	3.30 ± 0.07^{A}	3.24 ± 0.04^{aA}	3.01 ± 0.11^{aA}	3.12 ± 0.09^{aA}
	TPS		3.60 ± 0.03^{bC}	3.79 ± 0.08^{bD}	4.09 ± 0.04^{bE}
	GLU		3.57 ± 0.15^{bB}	3.60 ± 0.02^{bB}	3.67 ± 0.13^{cB}
i-butyric acid	BLK	ND	ND	0.58 ± 0.01^{aA}	1.01 ± 0.02^{aB}
	TPS		ND	0.45 ± 0.02^{bA}	0.56 ± 0.15^{bB}
	GLU		ND	0.47 ± 0.10^{bA}	0.85 ± 0.05^{cB}
n-valeric acid	BLK	0.52 ± 0.13^{A}	0.78 ± 0.10^{aB}	1.14 ± 0.05^{aC}	1.20 ± 0.02^{aD}
	TPS		0.5 ± 0.04^{aAB}	0.62 ± 0.15^{bAB}	0.74 ± 0.05^{aBC}
	GLU		0.51 ± 0.23^{aA}	1.42 ± 0.09^{aB}	1.00 ± 0.02^{aC}
i-valeric acid	BLK	0.64 ± 0.01^{A}	0.74 ± 0.03^{aB}	1.20 ± 0.04^{aC}	2.05 ± 0.02^{aD}
	TPS		0.65 ± 0.02^{aA}	0.74 ± 0.09^{bAB}	0.83 ± 0.11^{aB}
	GLU		0.60 ± 0.05^{aA}	1.00 ± 0.03^{cB}	1.72 ± 0.06^{aC}

Means followed by different lowercase superscripts in a column are significantly different for each SCFA among different groups at the same time point ($p < 0.05$). Means followed by different uppercase superscripts in a row are significantly different for each SCFA among different time points ($p < 0.05$). ND indicates that SCFAs were below the detection minimum threshold. SCFAs, short-chain fatty acids; BLK, ultrapure water addition group; TPS, tea polysaccharides addition group; and GLU, glucose addition group.

BLK > GLU > TPS, and the differences between the BLK and TPS groups, and between the BLK and GLU groups, were significant ($p < 0.05$). The order of *n*-valeric and *i*-valeric acid contents in the groups of medium was BLK > GLU > TPS, which was not significant at the 6th hour ($p < 0.05$), but increased significantly at the 12 and 24th hour ($p > 0.05$), respectively.

In summary, TPS group could be metabolised into SCFAs by microbiota in the fermentation medium. The contents of acetic, propionic, and *n*-butyric acids were higher than those in the medium of BLK group ($p < 0.05$). Propionic and *n*-butyric acids were significantly higher than the medium of GLU group ($p < 0.05$), but *i*-butyric, *n*-valeric, and *i*-valeric acids were at lower levels than in the medium of BLK group.

Effects of tea polysaccharide on faecal microbiota
Diversity index analysis

The rarefaction curve reflects the depth of sample sequencing, and the Shannon-Wiener curve reflects the microbial diversity of each sample at different sequencing numbers. Figure 1 shows that the curves became smoother as the depth of sample sequencing increased, thus indicating that no new OTUs would be found with more sequencing, and the sequencing depth was sufficient to reflect the information of most microbiota in the samples.

Effects of tea polysaccharides on OTU quantity and microbiota diversity

The OTU number and microbiota diversity in each group are shown in Table 2. The OTU number represents the relative abundance of species (Zhang *et al*., 2010). The alpha diversity index can estimate the diversity of species. The Chao 1 estimator and observed species index can estimate the community richness in samples. The Shannon index and PD whole tree index can estimate the community diversity in samples. The OTU number of the medium of TPS group was the highest ($p < 0.05$) among all groups, thus suggesting that TPC contributed to the improvement of microbiota diversity. This was

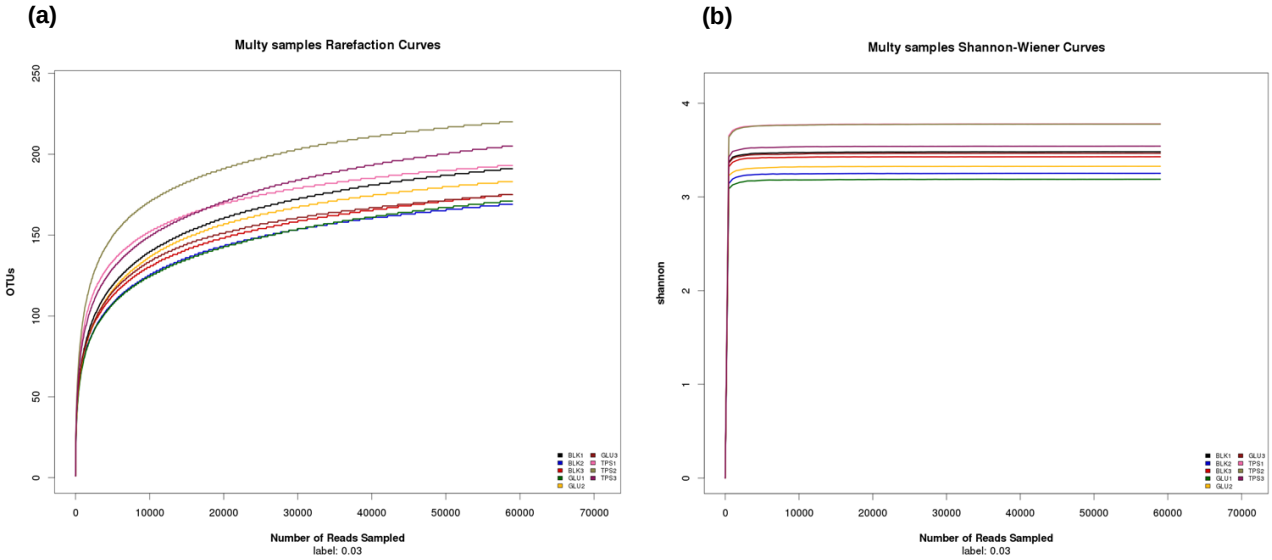

Figure 1. Index of sample diversity. **(a)** Rarefaction curve; **(b)** Shannon-Wiener curve.

Table 2. OTU number and α-diversity of microbiota in the fermentation medium.

Group	OTU number	Observed species	Chao1	Shannon	PD whole tree
BLK	181.67 ± 10.02^{b}	178.20 ± 9.75^{b}	208.06 ± 13.04^{b}	4.88 ± 0.18^{b}	13.76 ± 0.77^{ab}
TPS	206.33 ± 15.52^{a}	203.90 ± 15.26^{a}	228.84 ± 7.47^{a}	5.33 ± 0.20^{a}	15.04 ± 0.85^{a}
GLU	175.67 ± 6.43^{b}	173.03 ± 6.19^{b}	197.50 ± 2.98^{b}	4.80 ± 0.20^{b}	13.18 ± 0.62^{b}

Means followed by different lowercase superscripts in a column are significantly different by Tukey's Test ($p < 0.05$). BLK, ultrapure water addition group; TPS, tea polysaccharides addition group; and GLU, glucose addition group.

consistent with the maximum number of OTUs in the medium of TPS group with the same sequencing volume, as shown in Figure 1. The observed species index and Chao1 estimator also showed that the species richness of the medium of TPS group was the highest of all the groups ($p < 0.05$). Not only the relative abundance of microbiota, but also the species evenness in the community influenced the Shannon index and PD whole tree index. The Shannon index and PD whole tree index of the medium of TPS group were greater than those of the BLK and GLU groups ($p < 0.05$), thus showing that the relative abundance and diversity of microbiota was the highest in all groups. In conclusion, TPS could increase microbiota diversity in the fermentation medium.

Effects of tea polysaccharides on microbial composition in fermentation medium

Effects of different treatments on microbial composition in fermentation medium

Principal component analysis (PCA) was performed based on the OTU information to analyse the microbial composition of the fermentation medium with different treatments. The difference in the microbiota of each group with different treatments was selected based on the Euclidean distance.

PCA of microbiota in fermentation medium is shown in Figure 2.

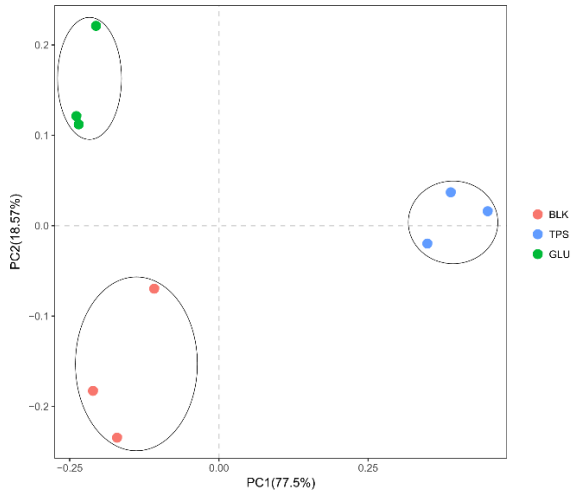

Figure 2. PCA of microbiota in the fermentation medium.

The diversity of microbiota among the different groups was significant. This indicated that different treatments had meaningful impacts on the microbiota. The distances between the fermentation medium of TPS and BLK groups, and between the fermentation medium of TPS and GLU groups, were greater than those between the BLK and GLU groups. The BLK and GLU groups were adjacent in PC1, which explained 77.5% of the total variance, thus indicating that the microbiota in fermentation medium of the TPS group was different from that of the BLK and GLU groups, and that the fermentation medium of GLU and BLK groups had similar microbiota.

Effect of tea polysaccharides on microbiota in fermentation medium

The faecal microbiota consisted of primarily four phyla: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria (Figure 3). Firmicutes and Bacteroidetes accounted for more than 82.38%, thus suggesting that Firmicutes and Bacteroidetes could reflect the changes in microbiota at the phylum level. When compared with the fermentation medium of BLK and GLU groups, the relative abundance of Firmicutes in the fermentation medium of TPS group increased, and the relative abundance of Bacteroidetes decreased, thus indicating that TPS altered the microbiota in the fermentation medium at the phylum level.

A heat map of the top 20 microbiota at the genus level was generated (Figure 4) to assess the microbiota distribution in different groups. *Bacteroides* and *Faecalibacterium* were the two main microbiota at the genus level in all groups. In addition, the dominant microbiota in the fermentation medium of TPS group was *Ruminococcus* 1, *Eubacterium rectale* group, and *Alistipes*. The microbiota relative abundance of the fermentation medium of TPS group, which was higher than that of the BLK group, included *Ruminococcus* 1, *Eubacterium rectale* group, *Ruminococcus* 2, *Butyricimonas*, *Fusicatenibacter*, *Phascolarctobacterium*, *Megamonas*, *Lachnoclostridium*, *Roseburia*, *Parasbutterfly*, and *Tyzzerella* 3. Except for *Megamonas* and *Phascolarctobacterium*, the other microbiota showed significant differences between the fermentation medium of TPS and BLK groups ($p < 0.05$). This result suggested that TPS altered the composition of microbiota at the genus level.

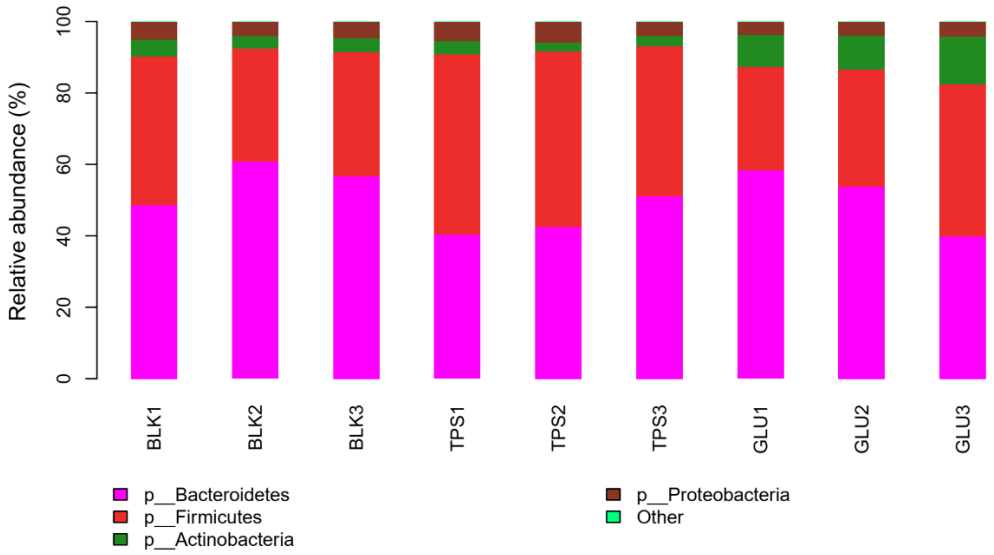

Figure 3. Microbiota in the fermentation medium at the phylum level.

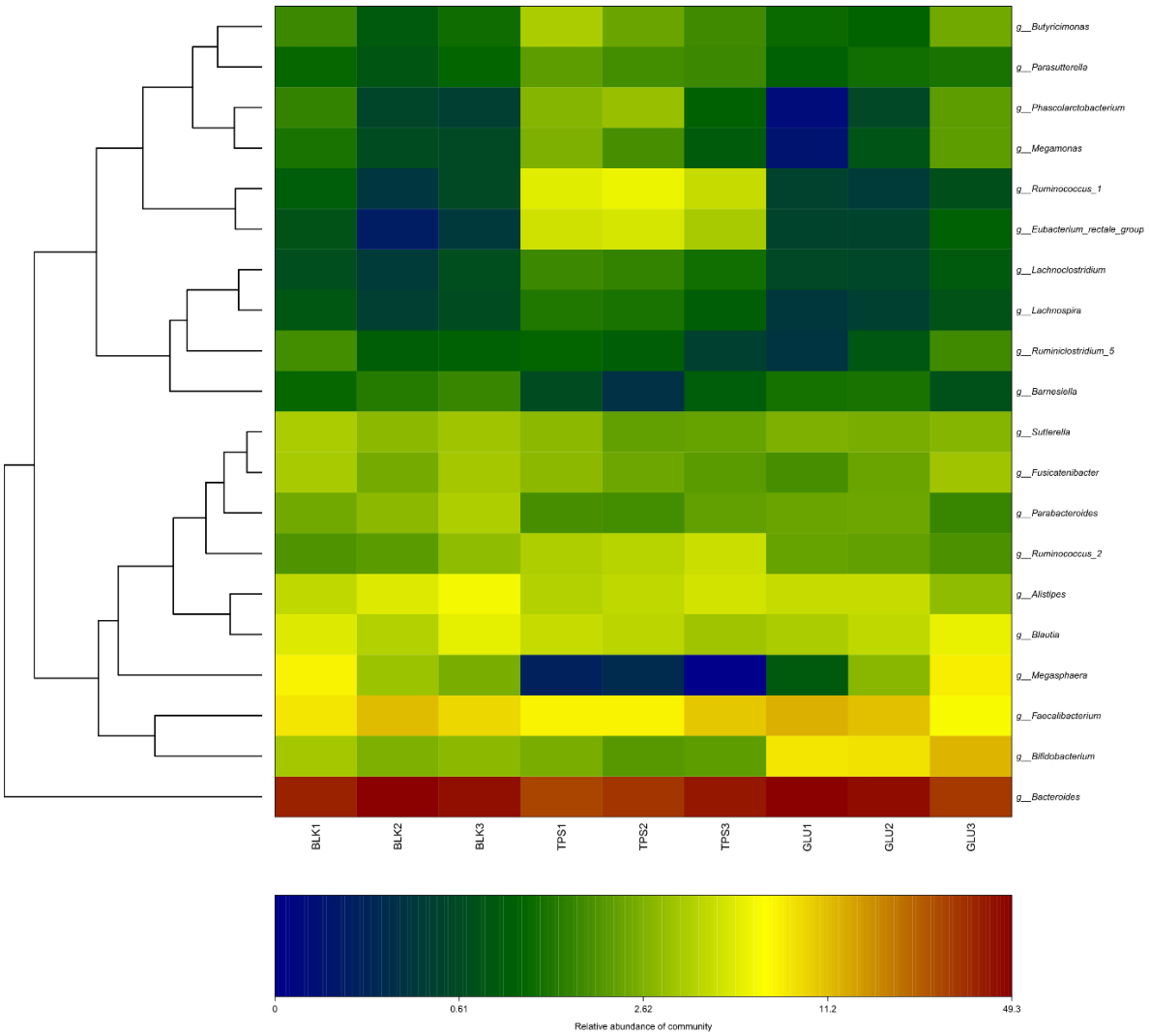

Figure 4. Heat map of the top 20 microbiota in the fermentation medium at the genus level.

Biomarkers of fermentation medium

LDA Effect Size (Segata *et al*., 2011) (LEfSe) analysis can demonstrate comparisons between groups to find species with significant differences in abundance.

There were 15 biomarkers in the two groups, 11 in the TPS group and four in the GLU group (Figure 5). As classified by species, the biomarkers of the fermentation medium of TPS group were mainly distributed in *o*_Clostridiales and *o*_Lachnospiraceae. Biomarkers of fermentation medium of GLU group were allocated to *c_Actinobacteria*, *o_Bifidobacteriales*, *f_Bifidobacteriaceae*, and *g_Bifidobacterium*.

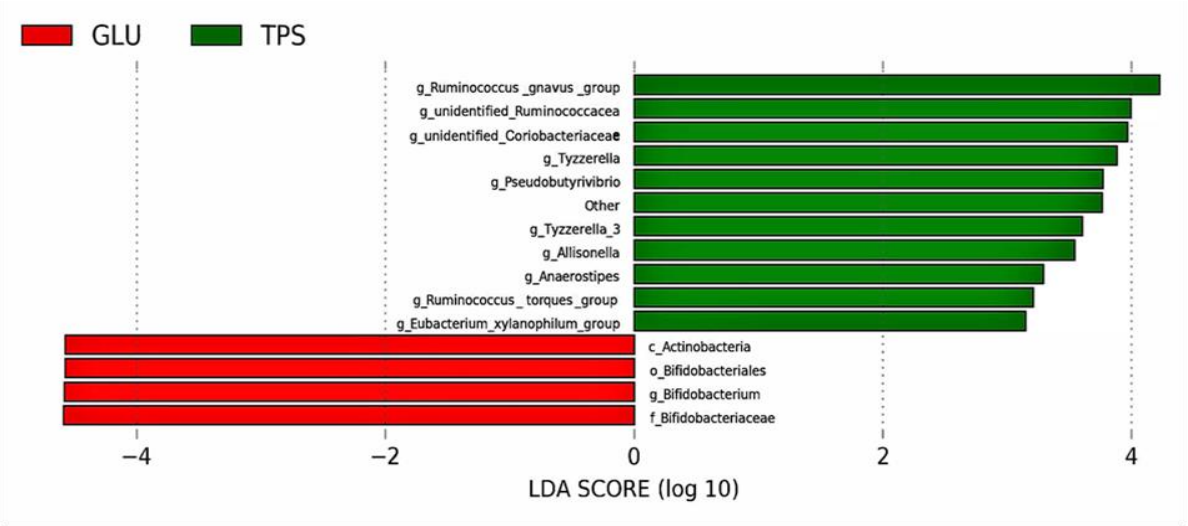

Figure 5. LEfSe analysis of the LDA distribution histogram based on the classified information.

Discussion

The mode of connection of adjacent glycosides on the polysaccharide backbone (*i.e.*, the glycosidic bonds) is important, and determines the physiological activity of polysaccharides (Xie and Nie, 2010). Enzymes encoded by human genes cannot degrade TPS, while CAZy, which is encoded by the genes of intestinal microbiota, can degrade TPS into monosaccharides, and metabolise them into SCFAs. Therefore, SCFAs may be the bridge for the physiological activity of TPS.

In the present work, TPS were metabolised into SCFAs, which was consistent with the results of Chen *et al.* (2018). The contents of acetic, propionic, and *n*-butyric acids in the fermentation medium of TPS group were higher than those in the BLK group during fermentation. However, the contents of *i*-butyric, *n*-valeric, and *i*-valeric acids in the fermentation medium of TPS and GLU groups were lower than those in the BLK group. The reason might be that intestinal microbiota turn to fermentable organics such as amino acids from dietary or endogenous proteins and dietary fats to provide energy when the supply of carbon source in the intestine is insufficient (Wall *et al*., 2009). The endogenous mucoprotein was metabolised to branched fatty acids by microbiota, such as *i*-butyric, 2-methyl butyric, and *i*-valeric acids. The medium of the BLK group was carbon-free, but the microbiota could produce SCFAs such as *i*-butyric and *i*-valeric acids with a small amount of endogenous proteins present in the faecal samples. Therefore, the contents of *i*-butyric and *i*-valeric acids in the medium of BLK group were higher than those in the TPS and GLU groups.

The results of this experiment showed that TPS could increase the diversity of microbiota in fermentation medium, which was consistent with Kong *et al.* (2009) and Cheng *et al.* (2017). The results of PCA showed that the microbiota structure in the fermentation medium of TPS group was significantly diverse from those in the BLK and GLU groups. As the only carbon source in the fermentation medium, TPS promoted the growth of microbiota that secrete CAZy, and increased the diversity of microbiota in the medium, which resulted in a different microbiota from the BLK and GLU groups.

The transformation of TPS to SCFAs was linked to the existence of microbiota, which in turn stimulated the selective enrichment of some SCFA-producing microbiota. At the phylum level, the

relative abundance of Firmicutes in the fermentation medium of TPS group was higher than that in the BLK and GLU groups, and the relative abundance of Bacteroides was reduced. Bacteroides is Gram-negative bacteria, and the main component of the cell wall is lipopolysaccharide (LPS). After the death of Gram-negative bacteria in the intestinal tract, the cell wall degrades and releases LPS, which induces inflammation and the cascade reaction of immune stimulation. It has been reported that immune regulation (Monobe *et al*., 2010; Yuan *et al*., 2015) is one of the physiological activities of TPS, which might be associated with Gram-negative bacterial decline in the intestine. At the genus level of microbiota with the top 20 relative abundances, *Ruminococcus* 1 (Cheng *et al*., 2018), *Euconflictum rectale* group (Duncan *et al*., 2004), *Butyricimonas* (Yao *et al*., 2015), *Roseburia* (Sakamoto *et al*., 2009), *Lachnospira* (Vanegas *et al*., 2017), and *Parasbutterflyella* (Du *et al*., 2018) were associated with producing SCFAs, of which the abundance in the fermentation medium of TPS group was higher than that in the BLK group ($p < 0.05$). In addition, *Megamonas* (Scupham *et al*., 2008) and *Phascolarctobacterium* (Watanabe *et al*., 2012), associated with SCFA production in the fermentation medium of TPS group, were also higher than those in the BLK group, but the difference was not significant ($p > 0.05$).

In addition, 11 biomarkers in the fermentation medium of TPS group were identified by LEfSe analysis. Classification by species revealed that the microbiota with noteworthy differences in the fermentation medium of TPS group was mainly distributed in Clostridiales and Lachnospiraceae. Recent studies have shown that Lachnospiraceae may be associated with the degradation of intestinal carbohydrates into SCFAs and gas (CO_2, CH_4, *etc.*) (Duncan *et al*., 2010). *Eubacterium xylanophilum* group belongs to Lachnospiraceae, and can ferment xylan to form formic, acetic, and *n*-butyric acids (Van Gylswyk and Van der Toorn, 1985). Xylan is polypentapentate, and connected by β-D-(1,4) xylosidic bonds. Fermentation of xylan by the *E. xylanophilum* group suggested that the bacterium may secrete CAZy, which is a prerequisite for intestinal microbiota to use TPS as energy. *Anaerostipes* are Gram-positive anaerobic bacteria, and also belong to Lachnospiraceae, which metabolise lactic into butyric acid (Muñoz-Tamayo *et al*., 2011). In summary, when compared with the medium of BLK group, the medium of TPS group selectively enriched the microbiota producing SCFAs, and the result of the change in microbiota increased the content of SCFAs, which was consistent with the result of SCFA analysis.

The hypoglycaemic effect (Wang *et al*., 2010) was thought to be an important physiological activity of TPS, and the mechanism may be linked to SCFAs. Tea polysaccharides are one of the main components of coarse tea. It is a tradition for the Chinese and Japanese to use coarse tea to combat diabetes. Tea polysaccharides is metabolised and converted to SCFAs by gut microbiota in the intestines. Some beneficial metabolism is mediated by propionic and butyric acids in the intestinal epithelium. These metabolic pathways are sensed in the portal vein, and signalled through the intestinal-brain nerve circuit to improve insulin sensitivity and glucose tolerance (Vadder *et al*., 2014). Recent studies have also shown that SCFAs affect the proliferation or production of T_{reg} cells by inhibiting GPCRs or histone deacetylase (HDAC), thereby affecting immune regulation and exerting antitumor effects (Arpaia *et al*., 2013; Smith *et al*., 2013; Singh *et al*., 2014).

Conclusion

In the present work, anaerobic culture experiments *in vitro* showed that the faecal microbiota could ferment tea polysaccharides to produce SCFAs, while the structure of the faecal microbiota was modified. The SCFA-producing microbiota enriched in the fermentation medium of TPS group confirmed the correlation between the faecal microbiota and SCFA production.

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