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Synbiotic supplementation with prebiotic *Schizophyllum commune* derived β -(1,3/1,6)-glucan and probiotic concoction benefits gut microbiota and its associated metabolic activities

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Abstract

Synbiotics synergistically favors beneficial effects of prebiotics and probiotics towards host metabolic health by modulating gut ecosystem. In this study, we sought to examine the effects of prebiotics (*Schizophyllum commune* derived β -(1,3/1,6)-glucan), probiotics (concoction made of eight different bacterial strains) and synbiotics (prebiotics + probiotics) on gut microbiota and its associated metabolic functions through 16S rRNA gene sequences analysis. Results showed that probiotics strains used in this study were detected more in the synbiotic and probiotic treatments, while prebiotic dietary intervention increased the total bacterial abundance and metabolisms related to host immune strengthening. Probiotics and synbiotics dietary interventions enhanced similar metabolisms relating to butanediol and *s*-adenosyl-L-methionine biosynthesis. Probiotics treatment also showed depleted metabolic activities related to SCFA productions, that were not depleted in prebiotics treatment. With varying differential abundance patterns and metabolic activities across the treatments, our results suggest that synbiotic treatment provide more beneficial effects over probiotics and prebiotics.

Keywords: Synbiotics, β -(1,3/1,6)-Glucan, Gut microbiota, Butanediol biosynthesis, Adenosyl-L-methionine biosynthesis

Introduction

Gut microbiota, the virtual metabolic ‘organ’ consisting of intricate microbial ecosystem inhabiting gastrointestinal tract (GIT), has predominant and diverse effects on the traits of human health relating to physiology,

immunology, neurology and metabolomics [1, 2]. Any disruption/imbalance in the gut microbial complex has been pathologically linked with non-infectious chronic disorders such as obesity, metabolic syndrome, neurodegenerative conditions, hypertension, hypercholesteremia, cancer, diabetes, chronic inflammatory diseases such as inflammatory bowel syndrome (IBD), Crohn’s disease etc. [3, 4]. Besides host genetics and heritability, several external factors such as food, environment, medications, age, stress etc., is said to influence the host gut microbial composition [5–7]. Among these factors, the contribution of diet towards gut and systemic health is highly

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considered for a therapeutic strategy in manipulating the host gut microbiome [8–10].

Dietary prebiotics, the non-digestible food ingredients confer beneficiary effects on host health by supporting growth of probiotics, the live microorganisms that could restore and/or improve gut flora [7]. As the digestive/absorptive resistance prebiotics enters from the small intestine into the colon, gut microbes start fermenting them thereby producing nutrients and other necessary substances such as antibiotics, anti-carcinogens, inhibitors of bacterial toxin production etc. [11]. In order for the probiotics to exhibit its utmost potential, they should be accompanied with appropriate prebiotics—collectively termed as ‘synbiotics’ [12].

Synbiotics with multi-strain bioactive probiotics (*L. plantarum*, *L. fermentum* and *B. infantis*) and Triphala, a polyphenol-rich prebiotics enhanced the motility and fitness of *Drosophila melanogaster* in-addition to imposing beneficial effect on gut microflora in a simulated model of the human GIT [13]. Synbiotic administration of *L. plantarum* and inulin improved cognitive response associated gut microbial composition in Type 2 diabetes mellitus metabolic condition compared to their individual counterpart [14]. Long-term dietary intervention using the synbiotic formulation consisting of cauliflower mushroom derived β -glucan and *L. fermentum* alleviated estrogen-deficiency associated metabolic disturbances in ovariectomized rat models. It also reverses the imbalance created in the Firmicutes:Bacteroidetes ratio [15]. In another study, dietary intervention using synbiotic formulation consisting of probiotics such as *B. animalis* and *L. paracasei* together with β -glucan, the oats-derived dietary fiber in male C57BL/6J mice models attenuated high fat diet (HFD) induced obesity and other metabolic complications [16]. Further, this intervention significantly restored the gut homeostasis at the phylogenetic and functional level in terms of richness and abundance of the gut microflora, microbial metabolites such as short chain fatty acids (SCFA) and bile acids (BA), metaproteomic pathways etc. Thus, with deliberate selection of pertinent prebiotics, the functionality of probiotics can be enhanced aiding synergistic effect on desirable health conditions and re-establishment of gut homeostasis.

Herein this study, we investigated the synbiotic impact of *S. commune* derived dietary prebiotic β -(1,3/1,6)-glucan and probiotic concoction consisting eight bacterial strains towards host gut microbiota and its associated metabolic pathways.

Materials and methods

Description of prebiotic, probiotic and synbiotic feed used

Prebiotics: 1% β -(1,3/1,6)-glucan isolated as exopolysaccharide upon fermenting *Schizophyllum commune*

(Quegen Biotech Co. Ltd. Republic of Korea) with a concentration of 3 g per kilogram of normal diet feed. Probiotics: concoction (with a concentration of 15 g per kilogram of normal diet feed) containing eight different bacterial strains. The probiotics was formulated and procured from CKD Bio, Seoul, Republic of Korea. Dietary supplement with probiotic concoction containing Bifidobacterium, Lactobacilli and Streptococcus thermophiles were chosen, as they have the ability to re-configure gut microbiota with beneficial microbes. The characteristics of prebiotic fiber and probiotic bacterial loads were described in Fig. 1. Synbiotics: blend of prebiotics and probiotics in the normal diet feed at concentrations indicated in Fig. 1.

Animal model

Five-weeks old ICR mice (a general-purpose mouse model) were used in this study. Experimentation involving animals were ethically approved and were in accordance with the guidelines framed by Animal care and Use Committee (ACUC No. 2019-0019), Jeju National University. With an adaptation period of 2-weeks to our laboratory ambience, the mice were randomly sorted into four groups with n=6 per group as follows: normal diet, prebiotic diet, probiotic diet and synbiotic diet. Ad libitum supply of food and water was provided to the mice at all time-points of the experiment. Food, water and mice bedding in the cages were changed twice a week during the entire experimental time scale of 5 weeks.

Experimental design

The experiment was carried out as depicted in Additional file 1: Figure S1. At the end of the experimental duration, mice were sacrificed through inhalation anaesthetics. A day prior to the sacrifice, fecal samples collected from individual mice were taken for the analysis of fecal microbiota.

Histology

Upon sacrificing the mice, intestinal samples were collected and fixed in formalin solution. Tissue processing was carried out using alcohol/xylene solution followed by embedding them in hot paraffin wax. Using microtome, intestinal tissue was sectioned and stained using Haematoxylin–Eosin-method. Microscopic analysis of the stained section was done using Olympus BX51 microscope at the indicated magnification scale. Thickness of mucosal and sub-mucosal layer of the intestine tissue was quantified using ImageJ software.

Microbial community analysis

Fecal DNA was obtained using PowerFecal DNA extraction kit (QIAGEN, Germany). V4 region of 16S rRNA

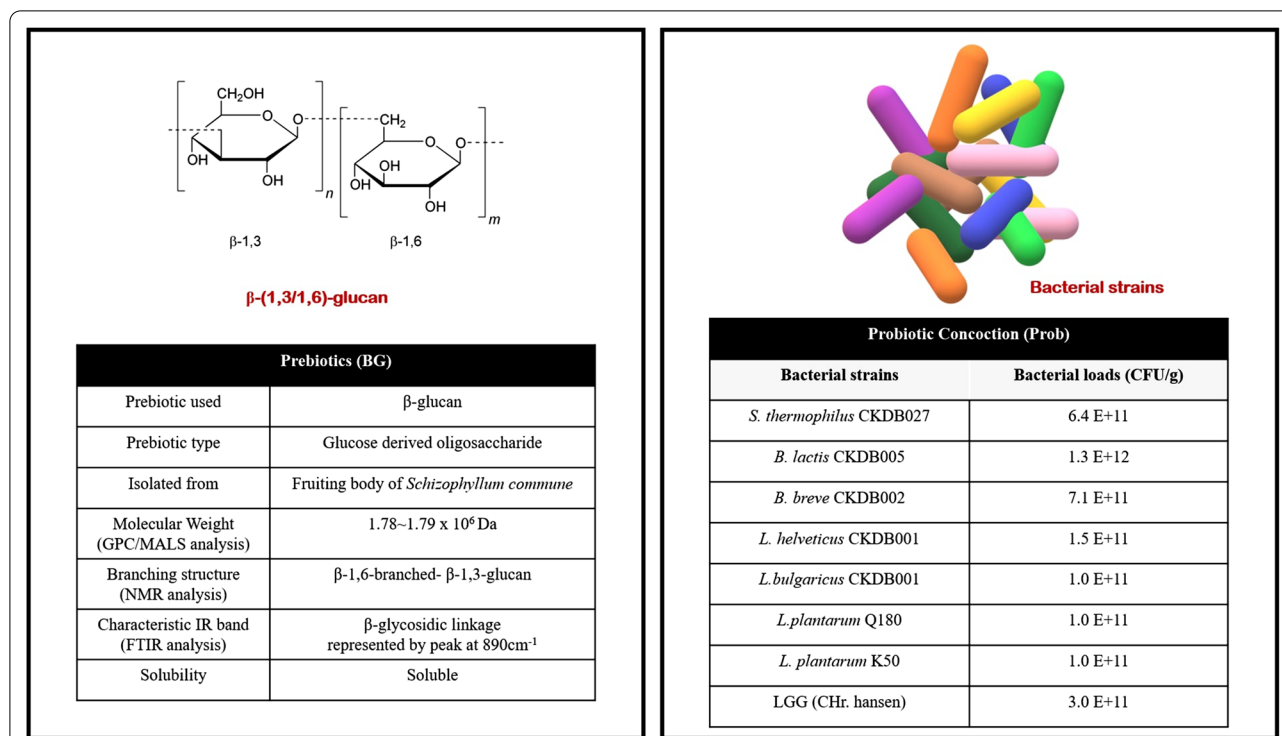


Fig. 1 Description of prebiotics and probiotics. GPC gel permeation chromatography, MALS multi-angle light scattering, NMR nuclear magnetic resonance, FTIR Fourier transform infra-red, CFU colony-forming units

gene was amplified using 515F (5'-GTGCCAGCMGCC GCGGTAA-3') and 806R (5'-GGACTACHVGGGTWT CTAAT-3') primer set. Two-step PCR was done to prepare MiSeq library and sequenced at Macrogen (Seoul, South Korea) according to the manufacturer's instruction. Sequence data were processed using MOTHR [17] according to the standard operational protocol (https://mothur.org/wiki/miseq_sop/) with a slight modification of adding singleton removal after pre.cluster [18] sub-routine. Silva.nr_v132 [19] was used for alignment and RDP version 11.5 [20] was used for taxonomic classification. Operational taxonomic units (OTUs) were assigned using opti.clust algorithm [21] with a sequence distance 0.03.

MOTHR was used for calculating ecological indices (Chao and Shannon) and non-metric multidimensional scaling (NMDS). NMDS was plotted using vegan R package to draw ellipse with 95% confidence level. PICRUSt2 [22, 23] was used to predict metabolic activities based on 16S rRNA gene sequences.

Statistical analysis

Results of histology analysis was given as average \pm standard error and significance was observed using student t-test method with $p < 0.05$ considered to be statistically significant. MOTHR was used to conduct analysis of

molecular variance (AMOVA) to examine significant difference between groups on NMDS plots. Differential abundance analysis was performed using LEfSe [24] and ALDEx2 [25] for OTUs and predicted metabolic activities, respectively. Spearman correlation analysis was performed to identify associations between differentially abundant OTUs and predicted metabolic activities. ANOVA was applied to compare ecological indices.

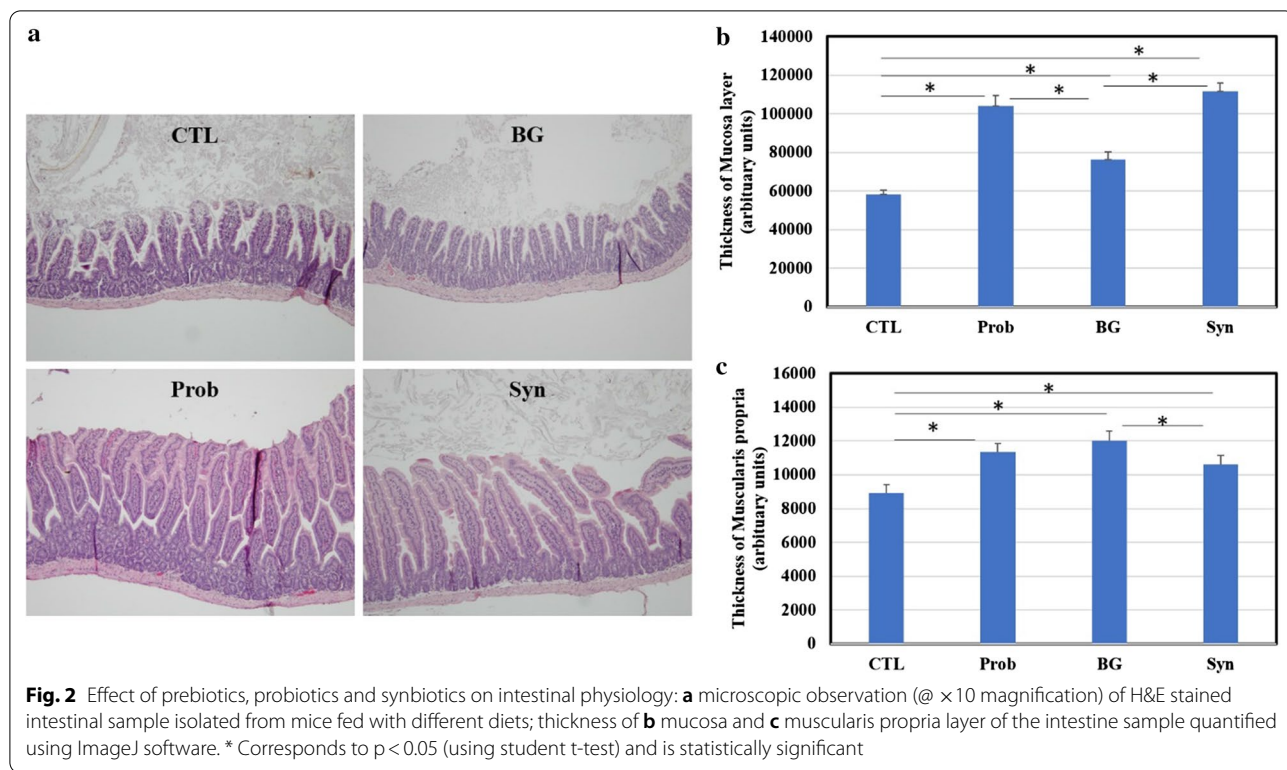
Results

Effects of treatments on gut architecture

Mice groups fed with prebiotics, probiotics and synbiotics does not have any dysbiosis in the mucosal as well as muscularis propria layer (Fig. 2). A statistically significant increase in the thickness of mucosal and muscularis propria was observed in the mice group fed with prebiotics, probiotics and synbiotics when compared to that of the normal diet group.

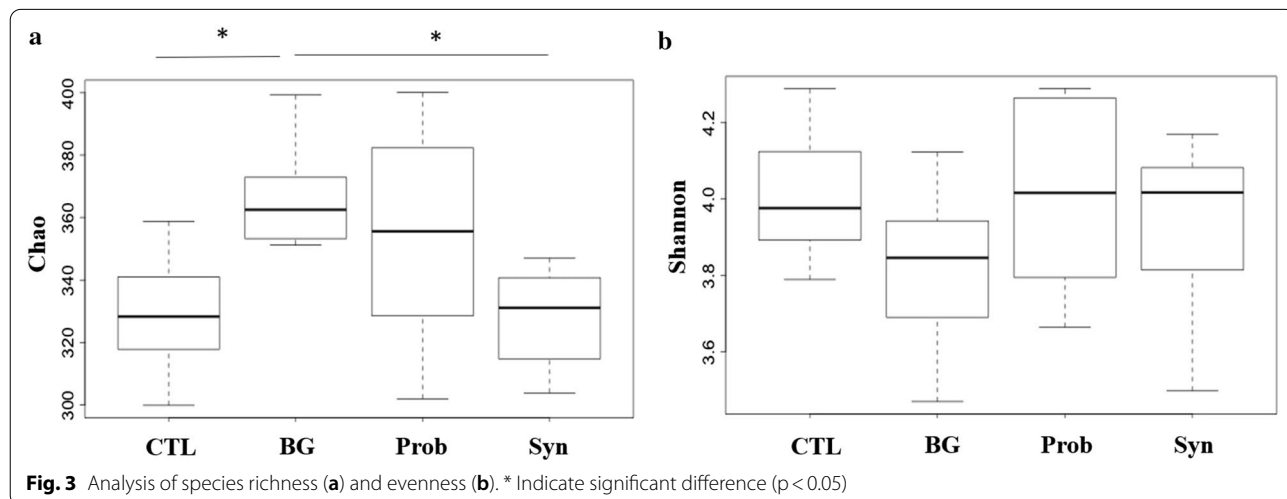
Effects of treatments on microbial communities

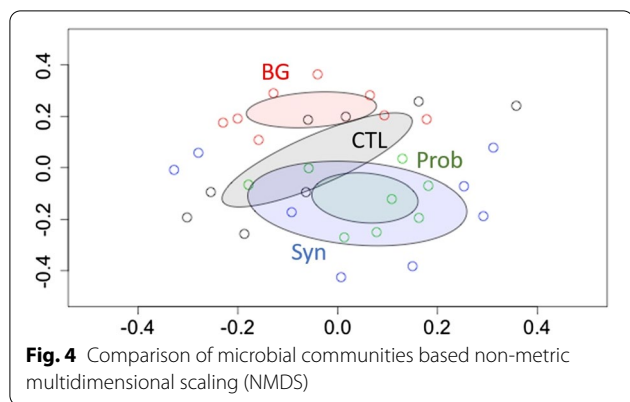
Good's coverage obtained for sequenced data showed greater than 99% (Additional file 2: Figure S2), indicating enough sequencing depth was achieved in this study. Our results from Additional file 3: Figure S3 showed that there was not significant difference among the treatments in the microbial composition at the phylum (Additional



file 3: Figure S3A), family (Additional file 3: Figure S3B), and genus level (Additional file 3: Figure S3C). All the samples showed higher occurrence of Firmicutes, Bacteroidetes and Actinobacteria at the phylum level, and members of Porphyromonadaceae and Lachnospiraceae, Lactobacillaceae, and Bacteroidaceae were prominent at the family level. Accordingly, *Lactobacillus* and *Bacteroides* and unclassified genera of those families showed higher abundance compared to the other genera.

Ecological indices have shown a significant rise in microbial richness (chao) after beta glucan (BG) treatment against control ($p < 0.05$) and synbiotics ($p < 0.05$), although microbial diversity (Shannon) remained unaffected in all the administered treatments (Fig. 3). β -Diversity analysis has shown distinct distribution for different groups, though probiotic and synbiotic sample groups were quite similar as shown by non-metric multidisciplinary scaling (NMDS) (Fig. 4). Analysis of





against probiotic ($p < 0.001$) and synbiotic ($p < 0.001$) (Table 1).

Differentially abundant OTUs were identified using LEfSe and summarized in Fig. 5. Results in Fig. 5 showed that probiotic and synbiotic treatment significantly increased *Bifidobacterium*, *Streptococcus*, *Lactobacillus* and unclassified species of the family Porphyromonadaceae. BG treatment increased unclassified member of the family Lachnospiraceae, while probiotic treatment increased the genera *Atopostipes*, *Corynebacterium*, *Jeotgalicoccus* and *Aerococcus*. Distinctly, synbiotic treatment increased *Parabacteroides*. On the other hand, all the treatments showed a decrease of diverse unclassified members of Lachnospiraceae and Porphyromonadaceae.

Table 1 Analysis of molecular variance (AMOVA)

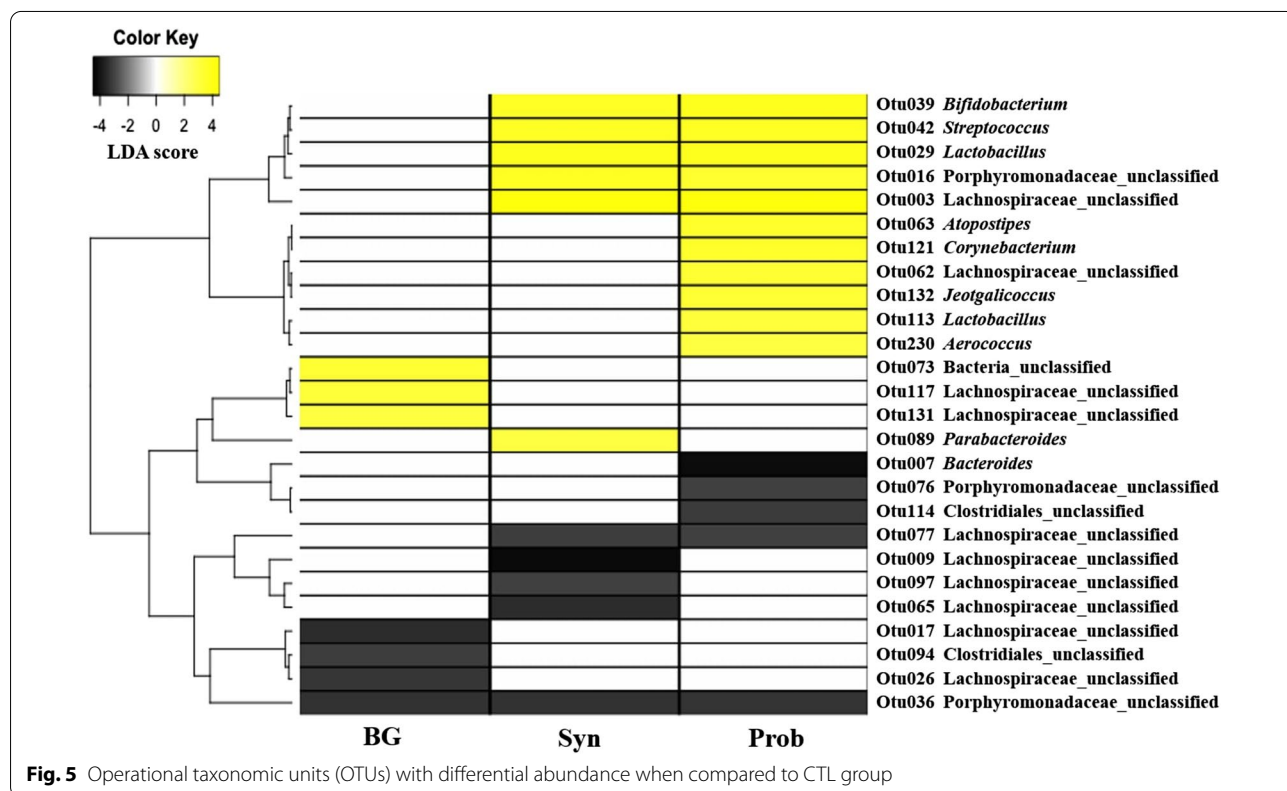
Fs	CTL	BG	Prob
CTL			
BG	1.45		
Prob	2.38**	4.42***	
Syn	1.79	3.65***	1.50

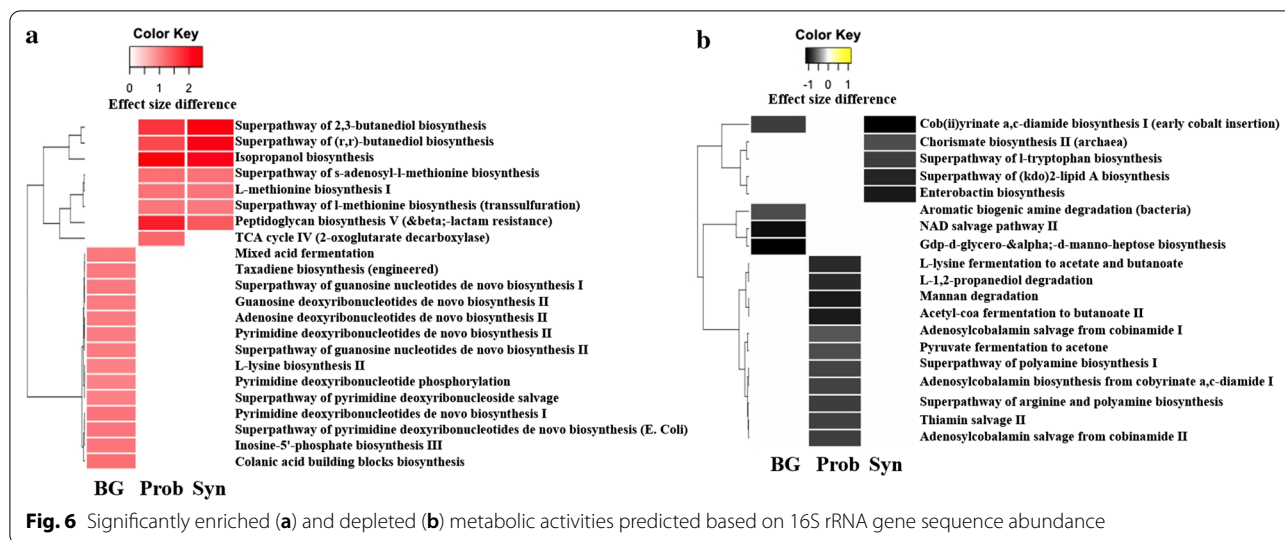
* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$

molecular variance (AMOVA) also had shown a significant difference in diversity induced by BG treatment

Effects of treatments on predicted intestinal metabolic functions

PICRUSt 2 was used to predict metabolic activities based on microbial community. We have applied differential abundance comparison using ALDEx2 to identify significantly enriched or depleted metabolic activities. Results in Fig. 6 show that BG have shown enriched metabolic activities related with biosynthesis of nucleobases, mixed acid fermentation and colanic acid, whereas synbiotic and probiotic treatments enriched metabolic activities related to butane diol and methionine biosynthesis





(Fig. 6a). In addition, probiotic treatment also increased the 2-oxoglutarate decarboxylase biosynthesis (Fig. 6a).

PICRUSt analysis also showed that probiotics treatment significantly decreased various metabolic activities where each treatment showed unique patterns (Fig. 6b). Our results suggest that synbiotic treatment decreased metabolic activities of biosynthesis of chorismite, tryptophan and enterobactin, BG decreased aromatic biogenic amine degradation, NAD salvage pathway II and mannoheptose biosynthesis and a number of metabolisms was depleted by probiotics treatment, including fermentation for SCFA production, mannan degradation, biosynthesis of polyamine and adenosylcobalamin (vitamin B12) production.

Associated bacteria with the significantly altered pathways

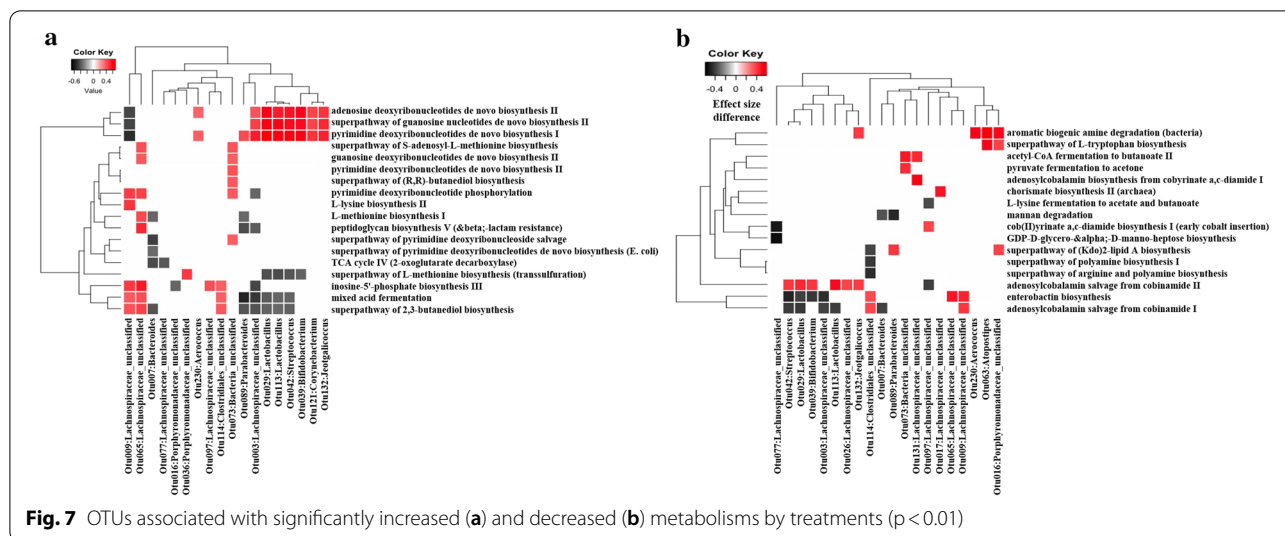
Spearman correlation analysis have calculated positive correlations between significantly ($p < 0.05$) enriched de novo biosynthesis pathways of nucleobases (i.e. adenosine, guanosine and pyrimidine deoxynucleotide) with *Lachnospiraceae_unclassified* (OTU003), *Lactobacillus* (OTU029, 113), *Streptococcus* (OTU042), *Bifidobacterium* (OTU039), and *Cornebacterium* (OTU121). In addition, metabolisms related with 2–3 butanediol synthesis and mixed acid fermentation were also found positively correlated with *Clostridiales_unclassified* (OTU114) and *Lachnospiraceae_unclassified* (OTU009, 065). Negative correlations were also observed between *Lachnospiraceae_unclassified* (OTU009) and biosynthesis of nucleobases. Likewise, the abundance of *Parabacteroides* (OTU089), *Lachnospiraceae_unclassified* (OTU003), *Lactobacillus* (OTU029, 113) and *Streptococcus* (OTU042) were negatively correlated with the

mixed acid fermentation and 2-3 butanediol biosynthesis (Fig. 7a).

On the other hand, association with differentially abundant bacteria and significantly ($p < 0.05$) depleted metabolisms were investigated (Fig. 7b). Decrease in aromatic biogenic amine degradation and tryptophan synthesis was positively correlated with the *Porphyromonadaceae_unclassified* (OTU016) and *Atopostipes* (OTU063). Similarly, adenosylcobalamine salvage from cobinamide-II was associated with the *Lactobacillus* (OTU029, 113), *Streptococcus* (OTU042), *Bifidobacterium* (OTU039), while adenosylcobalamine salvage from cobinamide-I was found to be positively correlated with the *Clostridiales_unclassified* (OTU114) and *Lachnospiraceae_unclassified* (OTU009). Additionally, decrease in enterobactin and adenosylcobalamine salvage from cobinamide-I were negatively correlated with the *Lactobacillus* (OTU029, 113), *Streptococcus* (OTU042) and *Lachnospiraceae_unclassified* (OTU003) (Fig. 7b).

Discussion

Gastrointestinal (GI) tract organs such as stomach, small intestine and colon along with other accessory organs such as liver, pancreas etc., are involved in the functions of digestive system such as breakdown of foods, nutrient absorption and excretion of solid waste apart from housing trillions of microorganisms with proper barrier levels [26]. Cross-section of GI tract in the intestinal organ consists of mucosa, submucosa, muscularis propria, serosa, mesentery etc. from the lumen to the outer wall. Mucosa, the innermost layer, consists of epithelial layer, lamina propria and muscularis mucosa. Organised into crypts and villi, the single, continuous epithelium layer contains diverse cells such as enterocytes, Paneth cells, goblet cells



etc. Among these cell types, goblet cells secrete mucin, the heavily glycosylated proteins involved in the first line of defence against gut microbial encroachment into the intestinal wall. Muscularis propria is in charge of the peristaltic propulsion of food through the intestine, the defects of which leads to intestinal motility disorders and obstruction disorders [27, 28]. Thus, the architecture conservation of the GI tract compartments is of prime importance. From Fig. 2, it was observed that prebiotics, probiotics and synbiotics used in this study conserved the intestinal architecture and even resulted with increased thickness of mucosal and muscularis propria layer thickness, thereby attenuating gut motility disorders, and defending microbial encroachment into intestinal walls.

Microbial ecology among different treatment have shown that effects of probiotics and synbiotics were not of much difference (Fig. 3a, b), but microbial richness was significantly increased after BG treatment, as BG is a fine prebiotic and was expected to be utilized as carbon source by diverse microbes [29]. According to the differential abundance tests, our results showed only synbiotics and probiotics groups increased the abundance of numerous beneficial microbes i.e. *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, and *Lachnospiraceae* [30–32]. In addition, synbiotic treatment also increased the abundance of *Parabacteroides* (OTU089) and had higher count of unclassified species of the Clostridiales (OTU114), and *Bacteroides* (OTU007), compared to probiotic treatment. Some of the Clostridiales have been reported to be butyrate producers [33]. Some species of *Parabacteroides* and *Bacteroides* have been recently reported to be protective against inflammation in the gut such as IBS [34]. *Bacteroides* has been also reported to benefit their host by excluding

pathogens to colonize in the gut [35]. These beneficial gut microbes were not increased by BG treatment.

We found that BG treatment mostly enhanced the metabolisms related to biosynthesis of nucleotides (Fig. 6a). Perhaps, there may have been an increase in absolute abundance of intestinal bacteria. It has been reported that the use of beta-glucan supplement increased number of probiotics strains, which leads to improvement of intestinal flora [36]. However, in this study, beta-glucan was treated to mice where gut dysbiosis was not induced. Therefore, our results could be expected. Colanic acid was also increased by BG. It has been reported that colanic acid promotes mitochondrial fission and enhances the mitochondrial unfolded protein response under stress conditions, suggesting that anti-aging potentials in the BG treatment [37]. On the other hand, metabolic prediction analysis showed quite a similar response for probiotic and synbiotic treatment, which includes biosynthesis of methionine and peptidoglycan. It has been reported that 2-oxoglutarate decarboxylase provides succinyl-CoA for methionine biosynthesis, which leads to peptidoglycan biosynthesis [38], therefore, these enriched metabolic activities are related to each other. Methionine is an important sulfur containing amino acid which have nutritional and structural importance and simultaneously it is also the precursor of the enzyme *s*-adenosyl-L-methionine [39]. Enzyme *s*-adenosyl-L-methionine have numerous role including liver protection against the reactive oxygen species generated from the pathogens and alcohol consumption [39] and catalyzes *trans*-sulfuration reaction which is important for membranous structure and functions [39]. Butanediol biosynthesis was also enriched by probiotics and

synbiotics, suggesting that extended fermentation of pyruvate in gut and possible anti-obesity effects [40] (Fig. 6a).

PICRUSt 2 analysis also revealed depletion in large number of beneficial metabolisms after probiotic treatments which includes adenosylcobalamin biosynthesis, polyamines and butyric acid (Fig. 6b). Butyric acid is perhaps most well-studied beneficial products in gut, while adenosylcobalamin, also known as vitamin B12, and polyamines have been reported as key modulators contributing in the structure and function of human gut microbial communities [41, 42]. Therefore, the use of probiotics may have unbeneficial side effects. In contrast, synbiotic treatment did not show decrease in these metabolic activities but showed decrease in enterobactin and tryptophan synthesis. Tryptophan biosynthesis is a feedback inhibition process and it has been reported increased superpathway of L-tryptophan biosynthesis did not increase the amount of serum tryptophan [43]. Enterobactin is required by microbes for iron acquisition [44] and Cob(II) yrininate a,c-diamide biosynthesis I is involved in vitamin B12 synthesis using cobalt. Depletion of these could be caused by beta-glucan as they are known to bind to metal ions [45]. BG treatment also depleted aromatic biogenic amine degradation, NAD salvage pathway II and manno-heptose biosynthesis. Biogenic amines in the gut are important metabolites that play a crucial role in the regulation of intestinal functions such as digestion, absorption and immunity [46]. Depletion of the biogenic degradation may increase the availability of biogenic amines. Moreover, blocking NAD metabolisms has been reported to limit intestinal inflammation [47]. To support this, manno-heptose has been reported to increase under intestinal inflammation [48]. Therefore, BG treatment alone may increase host immunity. Together, the differential abundance of the predicted metabolic activities indicated unique metabolic consequences among the treatments: BG showing increased bacterial abundance and immunity; probiotics and synbiotics showing increased butanediol and *s*-adenosyl-L-methionine contributing to beneficial effects such as anti-obesity and liver protection. Probiotics, however, seems to deplete some of the metabolic activities related to vitamin B12 biosynthesis, which was not the case for synbiotics.

Correlation analysis between abundance of bacteria and metabolic predictions indicate association of taxa with metabolic activities. Our results showed enriched nucleobase biosynthesis were mostly associated with probiotic strains such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, which were negatively associated with methionine biosynthesis, acid fermentation and butanediol biosynthesis (Fig. 7a). On the other hand, depleted metabolic activities are mostly associated with diverse

unclassified species of *Lachnospiraceae*. Probiotic strains were also negatively associated with enterobactin biosynthesis and vitamin B12 biosynthesis (Fig. 7b). As it has been intensively discussed, there are some possibilities that multi-strain of probiotics may less benefit in comparison to the single-strain probiotics [49]. However, it should be noted that this disadvantage was not observed in synbiotic treatment while maintaining the beneficial effects of probiotics treatment.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-020-00572-4>.

Additional file 1: Figure S1. Schematic representation of the experimental design.

Additional file 2: Figure S2. Ranges of Good's coverage obtained in this study.

Additional file 3: Figure S3. Microbial composition at the phylum (A), family (B) and genus (C).

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Authors' contributions

Conceptualization: TU and MC; formal analysis: VS, KM and CH; funding acquisition: TU and MC; project administration: YMK; resources: SHK and JL; supervision: SP, TU and MC; writing—original draft: VS, KM, TU and MC. All authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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