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Polymannuronic acid ameliorated obesity and inflammation associated with a high-fat and high-sucrose diet by modulating the gut microbiome in a murine model

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Abstract

Polymannuronic acid (PM), one of numerous alginates isolated from brown seaweeds, is known to possess antioxidant activities. In this study, we examined its potential role in reducing body weight gain and attenuating inflammation induced by a high-fat and high-sucrose diet (HFD) as well as its effect on modulating the gut microbiome in mice. A 30-d PM treatment significantly reduced the diet-induced body weight gain and blood TAG levels ($P<0·05$) and improved glucose tolerance in male C57BL/6J mice. PM decreased lipopolysaccharides in blood and ameliorated local inflammation in the colon and the epididymal adipose tissue. Compared with low-fat and low-sucrose diet (LFD), HFD significantly reduced the mean number of species-level operational taxonomic units (OTU) per sample as well as species richness ($P<0·05$) but did not appear to affect other microbial diversity indices. Moreover, compared with LFD, HFD altered the abundance of approximately 23 % of the OTU detected ($\log_{10}$ linear discriminant analysis (LDA) score >2·0). PM also had a profound impact on the microbial composition in the gut microbiome and resulted in a distinct microbiome structure. For example, PM significantly increased the abundance of a probiotic bacterium, *Lactobacillus reuteri* ($\log_{10}$ LDA score >2·0). Together, our results suggest that PM may exert its immunoregulatory effects by enhancing proliferation of several species with probiotic activities while repressing the abundance of the microbial taxa that harbor potential pathogens. Our findings should facilitate mechanistic studies on PM as a potential bioactive compound to alleviate obesity and the metabolic syndrome.

Key words: 16S rRNA gene: Gut microbiome: Obesity: Inflammation: Polymannuronic acid: SCFA

Obesity, as an important risk factor for inflammation and clinical diseases, is becoming prevalent in various age groups of human populations, independent of their races and social status[1]. Accumulating evidence suggests that the gut microbiome plays a key role in the development of obesity and the prevention of the metabolic syndrome[2–4]. The development of obesity and local and systemic inflammation is consistently associated with overall structural changes in the gut microbiome. For example, the ratio of Firmicutes:Bacteroidetes significantly increases in obese individuals[5]. A common constituent of the gut microbiome, *Akkermansia muciniphila*, has been shown to reverse high-fat-diet-induced fat mass gain, metabolic endotoxaemia, adipose tissue inflammation and insulin resistance[6]. *Lactobacillus* and *Bifidobacterium* species in the gut microbiome are associated with inflammatory development[7,8]. Moreover, the metabolites of gut bacteria affect host physiology and pathology[9]. Lipopolysaccharides (LPS), the major component of the outer membrane of Gram-negative bacteria, such as those from the phylum Proteobacteria, activate numerous transcription factors and result in inflammation, which contributes to tissue injury[10]. Recent findings suggest that LPS can induce obesity in a germ-free murine model[11]. On the other hand, SCFA, such as acetate, propionate and butyrate, are major fermentation products of the gut microbiome[12]. In addition to contributing to host energy metabolism, butyrate is a potent signaling molecule and a strong inhibitor of inflammation[13,14].

A prebiotic is an important selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gut microbiome, and confers benefits upon host well-being and health[15]. In recent years, polysaccharides as prebiotics have been widely used to restore the disordered gut microbiome because of their non-digestible properties in the small intestine[16]. Natural polysaccharides possess a variety of biological activities. For example, mannans, which contain mannose residues and are widespread in nature, possess prebiotic properties[17]. Mannose-containing carbohydrates have been reported to exhibit activities including constipation alleviation[18] and weight loss[19]. Recently, polysaccharides of marine origin have received scientific attention because of their availability, non-toxicity and safety and easy biodegradability[20]. Polymannuronic acid (PM), also called

Abbreviations: HFD, high-fat and high-sucrose diet; LDA, linear discriminant analysis; LFD, low-fat and low-sucrose diet; LPS, lipopolysaccharides; OTU, operational taxonomic unit; PM, polymannuronic acid.

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1 → 4-linked β-D-mannuronic acid, is structurally similar to mannans[21]. PM has been shown to possess antioxidant activities and has been exploited for its medicinal potential[22]. However, little is known about its possible impact on the gut microbiome as well as its abilities to attenuate obesity and metabolic diseases. In this study, we examined the potential roles of PM as a prebiotic in ameliorating obesity and inflammation induced by a high-fat and high-sucrose diet (HFD) using a murine model.

Methods

Animal experiment

A total of fifteen C57BL/6J male mice were purchased from Vital River Laboratory and kept under controlled light conditions (12 h light–12 h dark cycle). The animals had free access to water and were fed *ad libitum* throughout the experiment. After acclimation, 4-week-old male mice were randomly assigned to one of the three experimental groups (n 5/group) for 90-d trials: the control group, fed with a standard low-fat and low-sucrose diet (LFD) for 90 d; the high-fat diet group, fed with a HFD for 90 d; and the treatment group (PM), fed with the same HFD for 90 d. During the final 30 d of the 90-d experimental period, the mice in both LFD and HFD groups were provided with PBS by daily oral administration, whereas the mice in the PM group were given a single daily dose by oral administration of 150 mg/kg body weight of PM (molecular weight, 6–8 kDa). PM with ≥97 % purity was purchased from Huahai Pharmaceutical. The standard LFD contained 7 % sucrose with 10 % of energy (kJ (kcal)) from fat, whereas the HFD contains 45 % energy (kJ (kcal)) from fat, predominantly lard and 17 % sucrose. Both diets used in the experiment were purchased from Research Diets. The detailed ingredients of the diets can be found in the online Supplementary File S1.

The body weight data of the mice were recorded daily during the experimental duration. However, the experiment proceeded to 60 d (just before the initiation of the PM treatment), blood samples and faecal samples were collected. At the end of the 90-d experiment (i.e. after the 30-d PM trial), the mice underwent overnight fasting and were anaesthetised and euthanised by cervical dislocation the following morning. Samples from the small intestine, the epicardial adipose tissue (eAT), the subcutaneous fat and the colon tissue were collected, snap-frozen in liquid N_2 and then stored in –80°C freezers until total RNA was extracted. At necropsy, the faecal samples were also collected from all three groups for SCFA measurement as well as subsequent 16S rRNA gene analysis. Faecal samples were stored in a –80°C freezer until further analysis. The blood samples and faecal samples collected at 60 d were used to generate the baseline data for TAG and SCFA, respectively. The experiment was conducted strictly according to the guidelines approved by the Ethical Committee of Experimental Animal Care at the Ocean University of China (Animal Protocol no. SCXK (Jing) 2007-0001).

Detection of serum parameters

For the glucose tolerance test, the mice were fasted overnight and then injected with 2 g/kg of glucose solution. Thereafter, the mice were anaesthetised and blood was collected by Retro-orbital Bleeding into a heparinised tube. Serum blood glucose levels were measured at 0, 30, 60 and 120 min after glucose injection. Plasma was obtained by centrifugation and stored at –80°C for further analysis. Serum TAG and glucose were measured using specific reagent kits (Beyotime Biotechnology). Serum LPS (endotoxin) levels were measured by ELISA (R&D Systems).

Analysis of faecal SCFA

Faecal samples were removed from the –80°C freezer, and 1200 µl of distilled and deionised water was added to each thawed sample. The samples were then mixed well by vortexing for 1 min until the materials were homogenised. The pH of the suspension was adjusted to 2–3 by adding 50 % sulfuric acid. The acidified samples were kept at room temperature for 5 min and mixed briefly every 60 s. The samples were then centrifuged at 5000 ***g*** for 10 min. The clear supernatant was transferred into two new tubes for further processing. A quantity of 50 µl of the internal standard (1 % 2-ethyl butyrate acid solution) and 500 µl of diethyl ether anhydrous were added. The tubes were mixed for 30 s and then centrifuged at 5000 ***g*** for an additional 10 min. A volume of 1 µl of the upper diethyl ether layer was injected into a GC instrument (Agilent 7820A; Agilent Technologies) for the determination of SCFA, using a published method[23].

Tissue total RNA and faecal DNA extraction

Total RNA samples were extracted from the small intestine tissue using the Trizol procedure, as previously reported[24]. Total DNA was extracted from the faecal samples collected at 90 d using a QIAamp DNA Stool Kit (Qiagen) based on the manufacturer's instructions. The concentrations of total RNA and total DNA samples were quantified using NanoDrop 2000 (Thermo Fisher). The integrity of total RNA and faecal DNA was checked using agarose gel electrophoresis.

Real-time RT-PCR

Total RNA (2 µg per sample) was converted to complementary DNA (cDNA) using M-MLV RT and random primers (Sangon Biotech). The complementary DNA (cDNA) samples were stored at –80°C until subsequent PCR for analysis.

Real-time RT-PCR was performed in an iCycler iQ5 system (Bio-Rad Laboratories). The 25·0 µl RT-PCR reaction consisted of 12·5 µl Maxima SYBR Green qPCR Master mix (Roche), 0·3 µl each of 10 µM forward and reverse primers, 5·9 µl nuclease-free water and 6·0 µl template (100 ng of cDNA template used per reaction). The thermal profile included an initial denaturation at 95°C for 3 min followed by forty-five cycles of denaturation at 95°C for 15 s, annealing at 60°C for 20 s and extension at 72°C for 30 s. *Glyceraldehyde 3-phosphate dehydrogenase* gene was used as the endogenous reference for normalisation. The expression levels of integrin subunit alpha X (*ITGAX*, or CD11 antigen-like family member C, *CD11c*), *TNFα* and *IL-10* were analysed by relative quantification using the standard curve method. The sequences of the primers used in this study can be found in the online Supplementary File S1.

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Sequencing and bioinformatics analysis

The V4 hypervariable region of the 16S rRNA gene was amplified using the primer pair 515F–806R with sample-specific barcodes. The amplification reactions were carried out using a Phusion High-Fidelity PCR Master Mix (New England Biolabs). PCR products were purified using Agencourt XP beads (Beckman Coulter, Inc.) and mixed at equal molar ratios according to their respective sample-specific barcodes. Sequencing libraries were generated using a TruSeq DNA PCR-free Sample Preparation Kit (Illumina) following the manufacturer's recommendation. The library pools were quantified and verified using a Qubit fluorometer (Thermo Scientific) and Agilent BioAnalyzer 2100 (Agilent Technologies). The library pool was then sequenced using an Illumina sequencer (Illumina) to generate 2×250 bp paired-end reads.

Quality filtering and quality control procedures for raw sequence reads were performed using the QIIME pipeline under specific filtering conditions to obtain high-quality clean sequences. Paired-end reads were first merged using FLASH (version 1.2.7) with default parameters[25]. Chimeric sequences were then identified and removed. In brief, the processed sequences were compared with the reference Gold database – a FASTA file containing the ChimeraSlayer reference database in the Broad Microbiome Utilities, version microbiomeutil-r20110519, using UCHIME Algorithm[26]. Operational taxonomic unit (OTU) calling was performed using Uparse software (version 7.0.1001)[27]. Sequences with ≥97 % similarity were assigned to the same OTU. A representative sequence from each OTU was screened for further annotation using the Greengene database[28]. α Diversity analysis was conducted using the QIIME pipeline (version 1.7.0)[29]. β Diversity analysis was performed based on weighted and unweighted UniFrac matrix calculated in QIIME. Principal coordinates analysis (PCoA) was performed using the WGCNA stats package and ggplot2 package in R (version 2.15.3). Hierarchical clustering was performed according to the unweighted pair group method with arithmetic mean algorithm using average linkage in the QIIME pipeline.

Statistical analysis

All values in the tables and figures are expressed as mean values with their standard errors. Statistical comparisons of the results were performed by Tukey's *post hoc* test following ANOVA using SPSS version 18.0 (IBM). $P < 0{\cdot}05$ was considered statistically significant. In addition, the linear discriminant analysis (LDA) effect size (LEFSe) method[30] was used to test significant differences of microbiome features between two dietary treatments; and the cutoff value is the absolute $\log_{10}$ LDA score $> 2{\cdot}0$.

Results

Polymannuronic acid attenuated the symptoms associated with the metabolic syndrome in high-fat and high-sucrose diet -fed mice

HFD-feeding led to a significant increase in body weight (Fig. 1(a)) and induced obesity in mice. At the baseline level (60 d, just before the PM administration), the mean body weight for the LFD group was 27·30 g, whereas the mean body weight for the HFD group reached 33·46 g ($P = 0{\cdot}0029$). The mean body weight for the third group to be used for the PM trial at this point was also significantly higher than that of the LFD group. However, the two HFD groups did not differ in mean body weight ($P > 0{\cdot}05$). HFD also elevated blood TAG levels and impaired glucose tolerance at 60 d (Fig. 1(c)–(e)). At the conclusion of the 30-d PM trial (i.e. at 90 d of the experiment), HFD-induced body weight gain was significantly reduced by PM ($P = 0{\cdot}0054$). The mice in the PM group had significantly reduced food intake (online Supplementary File S1). The contents of the subcutaneous fat as well as the epididymal fat were also significantly lower in the PM group compared with the HFD group without PM administration (Fig. 1(b)). Moreover, a 30-d PM treatment resulted in an improved glucose tolerance as evidenced by reduced blood glucose levels (Fig. 1(d)) and blood TAG levels ($P = 0{\cdot}0290$; Fig. 1(e)). Together, these findings suggest that PM was able to reduce body weight gain and blood TAG levels, and to improve glucose tolerance in the HFD-induced obese mice.

Polymannuronic acid ameliorated local and systematic inflammation induced by high-fat and high-sucrose diet

We measured serum LPS as an indicator of systemic inflammation. HFD-feeding significantly increased serum LPS levels. Compared with the mice fed LFD, HFD increased serum LPS levels by approximately 75 % ($P < 0{\cdot}0005$, Fig. 2(a)). PM as a dietary supplement significantly decreased serum LPS levels down to the level observed in the mice fed LFD. Furthermore, the mRNA expression levels of several pro-inflammatory cytokines (Fig. 2(b)–(d)) such as *TNFα* and *CD11c* were significantly increased by HFD in the colon and eAT tissues, respectively. PM treatment was able to knock down their mRNA expression to the level observed in the LFD group. On the other hand, mRNA levels of the anti-inflammatory cytokine *IL-10* were significantly down-regulated by HFD, whereas PM restored its expression back to the baseline levels observed in the mice fed a standard LFD diet ($P = 0{\cdot}0001$; Fig. 2(c)). Overall, our results suggest that HFD tended to promote local and systemic inflammation, whereas PM was able to ameliorate HFD-induced inflammation.

Polymannuronic acid altered faecal SCFA profiles

HFD significantly decreased the faecal concentrations of propionate and butyrate as compared with the LFD-fed controls. The total SCFA content was also decreased by HFD. PM not only increased total SCFA levels but also had a significant impact on both propionate and butyrate levels. PM was able to restore the repressed butyrate levels by HFD to a level similar to that in the LFD group (Fig. 3).

High-fat and high-sucrose diet altered the microbial composition of the gut microbiome

To assess the effects of dietary treatments on the microbial composition of the gut microbiome, the V4 hypervariable

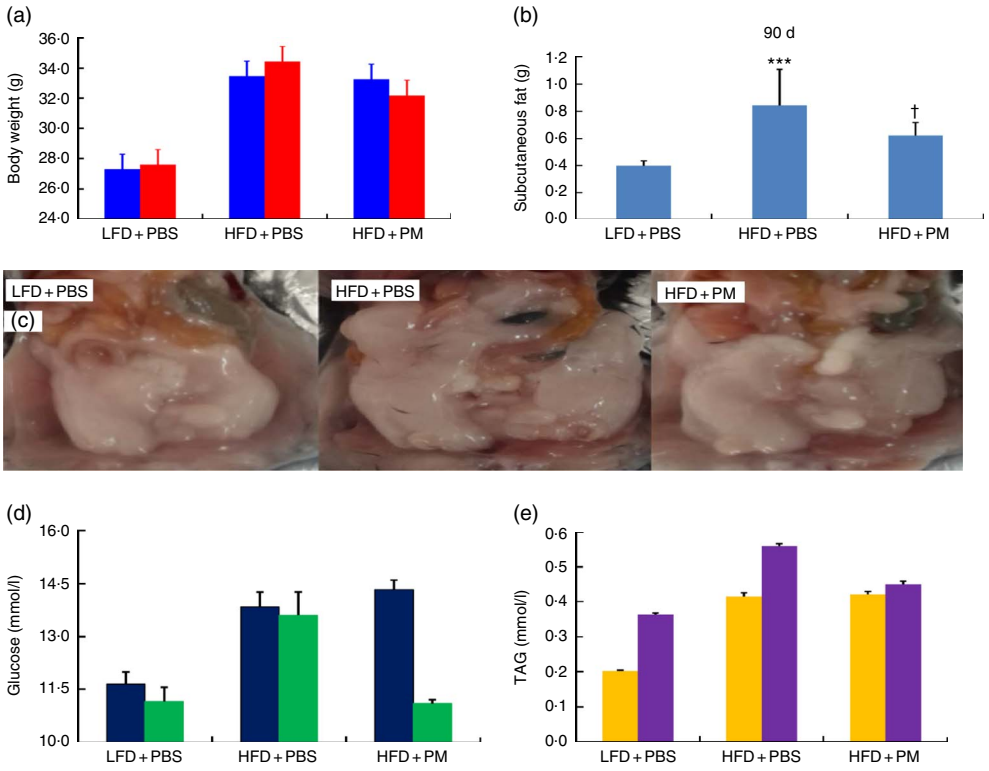

Fig. 1. Polymannuronic acid (PM) reduced a high-fat and high-sucrose diet (HFD)-induced body weight gain and subcutaneous fat accumulation. (a) The body weight of the mice fed a standard low-fat and low-sucrose diet (LFD), a HFD and a HFD supplemented with PM (HFD + PM). The baseline data at 60 d of the experiment (just before the PM administration) were collected without oral administration of PBS or PM. The 90-d data were collected from the samples at necropsy (i.e. after a 30-d PM daily oral administration (HFD + PM) or a time-matched daily oral administration of PBS (LFD + PBS and HFD + PBS). (b) Subcutaneous fat weights (g). (c) Fat morphology. (d) Blood glucose values at 30 min after glucose injection. (e) TAG levels. a: ■, 60 d; ■, 90 d; d: ■, 60 d; ■, 90 d; e: ■, 60 d; ■, 90 d. *** $P<0·001$ (LFD + PBS *v.* HFD + PBS), † $P<0·05$ (HFD + PBS *v.* HFD + PM).

region of the 16S rRNA gene was sequenced. A total of 1 061 641 raw reads were generated from faecal samples (mean 70 776 (SD 6176) per sample). After rigorous quality control procedures, including denoising and filtering, approximately 40 % quality sequences were used for subsequent analysis. Collectively, a total of 632 species-level OTU were detected in the study (online Supplementary File S2). Compared with mice fed a low-fat diet, HFD significantly reduced the number of OTU per sample from 384·80 (SD 36·85) in the control LFD to 251·40 (SD 111·34) ($P<0·05$). The number of OTU per sample remained repressed in the mice fed HFD supplemented with PM for 30 d (224·20 (SD 34·68)). Likewise, species richness was significantly reduced by HFD. Nevertheless, other microbial diversity indices, such as Shannon's, Simpson's and Pielou's evenness, remained unchanged by HFD. Of note, rarefaction curves did not appear to reach a plateau in this study. This suggests that whereas deeper sequencing may reveal rare OTU, the majority of microbial diversity has been captured in this study.

LEFSe analysis detected ninety-four taxa that were significantly impacted by HFD (absolute $\log_{10}$ LDA score >2·0). Compared with LFD, HFD significantly repressed the abundance of sixty-six taxa, whereas the abundance of twenty-eight taxa was elevated by HFD. Selected taxa significantly impacted by HFD are shown in Fig. 4(a). For example, at the phylum level, the abundance of Actinobacteria, Verrucomicrobia and TM7 was significantly decreased by HFD, compared with LFD. The abundance of several key genera, such as *Akkermansia*, *Bifidobacterium*, *Coprococcus*, *Faecalibacterium*, *Lactobacillus*, *Lactococcus* and *Ruminococcus* was significantly repressed by HFD. On the other hand, HFD increased the abundance of several genera from the families Enterococcaceae and Enterobacteriaceae (LDA score >2·0). Moreover, HFD induced a profound change in the gut microbiome at the species level. The abundance of approximately 23 % of the OTU in a given sample was significantly altered by HFD. HFD repressed the abundance of fifty OTU but elevated the abundance of sixteen OTU, compared with LFD. Among them, at least twenty-one OTU were named species. For example, the abundance of an OTU assigned to *A. muciniphila* was reduced 300-fold by HFD. Similarly, an OTU related to *Bifidobacterium pseudolongum* was decreased from 26·91 to 6·41 % by HFD. On the other hand, HFD-feeding resulted in a significant expansion of *Escherichia coli* and *Clostridium* populations. At least two OTU assigned to *E. coli* were significantly increased by HFD; the abundance of one *E. coli* OTU (OTU no. 2) increased from 0·8 % in the mice fed LFD to 22·70 % in the HFD-fed mice, whereas the abundance of another OTU related to *E. coli* was increased 9-fold by HFD. The population of a human pathogen, *Clostridium perfringens* (OTU no. 4), was also significantly expanded under the HFD feeding; its abundance increased from 0·92 % in the mice fed LFD to 11·52 % in the HFD-fed mice. Moreover, the abundance of

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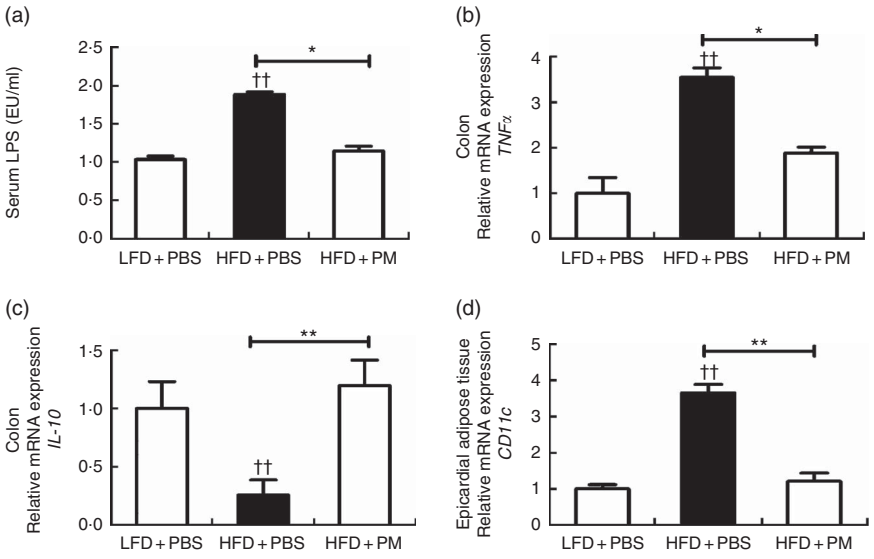

Fig. 2. Polymannuronic acid (PM) alleviated local and systematic inflammation. (a) Serum lipopolysaccharide (LPS) levels, (b) *TNFα* mRNA expression in the mouse colon, (c) *IL-10* mRNA expression in the mouse colon and (d) *CD11c* mRNA expression in the mouse epicardial adipose tissue (eAT). Values are means (n 5), with standard errors represented by vertical bars. * $P < 0{\cdot}05$ (high-fat and high-sucrose diet (HFD) + PM *v.* HFD + PBS), ** $P < 0{\cdot}01$, †† $P < 0{\cdot}01$ (low-fat and low-sucrose diet (LFD) + PBS *v.* HFD + PBS).

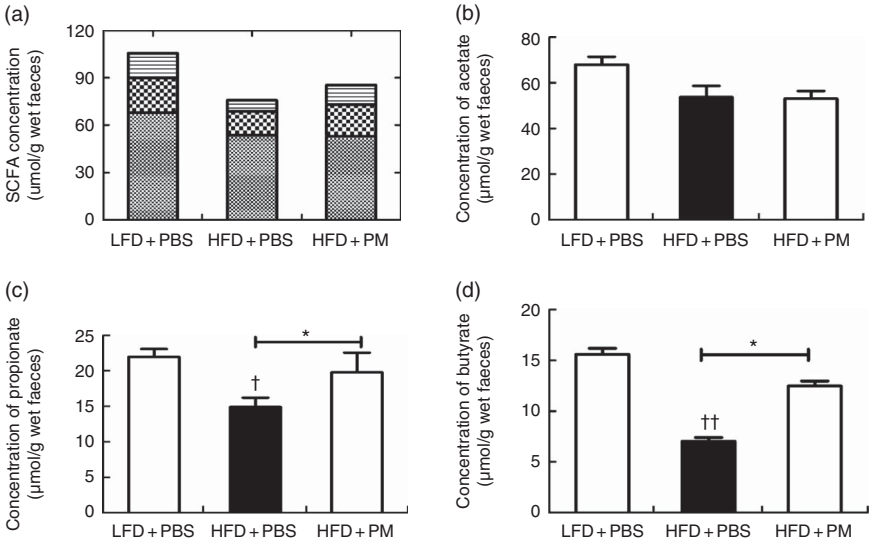

Fig. 3. Polymannuronic acid (PM) increased the faecal concentration of SCFA (y-axis: μmol/g of wet faeces). (a) Total SCFA (▩, acetate; ▨, propionate; ▤, butyrate), (b) acetate, (c) propionate and (d) butyrate. Values are means (n 5), with standard errors represented by vertical bars. † $P < 0{\cdot}05$, †† $P < 0{\cdot}01$ based on a modified t test between low-fat and low-sucrose diet (LFD) + PBS and high-fat and high-sucrose diet (HFD) + PBS groups, respectively, * $P < 0{\cdot}05$ based on a modified t test between HFD + PM and HFD + PBS groups.

an OTU related to *Enterococcus* (OTU no. 5) was significantly increased from 1·46 to 14·80 % by HFD. As a result, those OTU of potential human pathogens became the most abundant in the gut microbiome of the HFD-fed mice, accounting for approximately 49 % of all sequences.

Polymannuronic acid induced a significant change in the microbial composition

PM as a diet supplement for 30 d had a significant impact on the gut microbiome composition and led to changes in the abundance of sixty-four taxa at absolute $\log_{10}$ LDA scores > 2·0; Fig. 4(b)). Nevertheless, the dietary treatment by PM did not seemingly restore the reduced microbial species richness by HFD. At the phylum level, PM altered the abundance of three phyla: Actinobacteria, Bacteroidetes and Proteobacteria (Fig. 5(b)). The abundance of Actinobacteria in the LFD mice was 31·69 (SD 2·31) %, which was significantly repressed by HFD to 8·37 (SD 0·29) % (LDA log score 5·13). PM restored the HFD-repressed abundance of Actinobacteria to a level similar to that observed in the LFD mice. However, the abundance of Bacteroidetes and Proteobacteria (Fig. 5) was significantly

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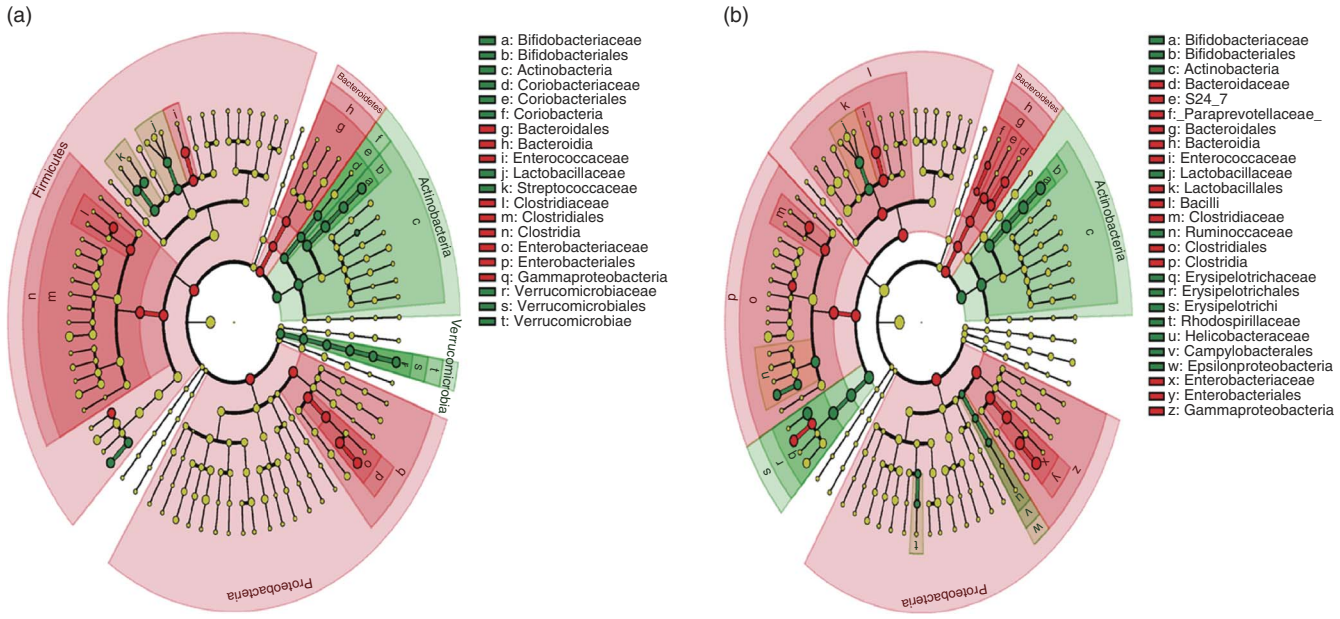

Fig. 4. Cladograms displaying the taxa with significantly different abundance. (a) Low-fat and low-sucrose diet (LFD + PBS) *v.* high-fat and high-sucrose diet (HFD) + PBS (■). (b) HFD + PBS *v.* HFD supplemented with polymannuronic acid (PM) for 30 d (HFD + PM, ■). Only the taxa with absolute $\log_{10}$ LDA scores ≥4·0 are displayed.

decreased by PM treatment. PCoA of the unweighted UniFrac matrix showed distinct clustering of the samples within each group; the separation of the treatment groups was clearly evident (Fig. 6(a)). PCoA analysis was also able to show a clear separation among the three groups (Fig. 6(b)). Together, our data suggest that PM was able to induce a distinguishable microbiome composition.

At the genus level, PM resulted in a significant reduction in the abundance of several genera, such as *Bacteroides*, *Clostridium* (Fig. 7(a)), *Coprococcus*, *Dorea*, *Enterococcus* (Fig. 7(b)), *Oscillospira* and *Sutterella*. At the species level, at least thirty-four OTU were significantly impacted by PM, including ten OTU with their abundance significantly increased by PM, compared with the mice fed a HFD diet (Table 1). For example, three named species, *B. pseudolongum* (Fig. 7(c)), *L. reuteri* and *Rominococcus bromii* were significantly more abundant in the PM group compared with the HFD group. On the other hand, the *E. coli* population was significantly decreased by PM from the elevated level in the HFD group (Fig. 7(d)).

In this study, we identified a total of thirty-three OTU that belong to nine genera, such as *Anaerostipes* and *Butyricicoccus*, which harbor butyrate-producing bacteria. Among them, the two OTU assigned to *Roseburia* and *Anaerofustis*, OTU no. 60 and OTU no. 286, respectively, were significantly increased by PM compared with HFD. The abundance of OTU no. 60 was at least 60 % higher in the PM group than in the HFD as well as in the LFD groups. In our data set, the OTU sequences sharing >97 % sequence homology to *Faecalibacterium prausnitzii* were readily detectable in the mouse faeces; and its abundance was significantly reduced by HFD, compared with the mice fed a LFD diet. However, PM as a dietary supplement had no apparent impact on the abundance of this potentially novel *Faecalibacterium* isolate.

Discussion

The gut microbiome plays a key role in the development of obesity and intestinal inflammation[31,32]. It is well documented that HFD can have a profound impact on the structure and function of the gut microbiome[4,33,34]. In this study, HFD significantly reduced the mean number of species-level OTU and species richness but had little effect on other microbial diversity indices, such as Shannon's and Simpson's. HFD significantly impacted the abundance of six phyla (LDA $\log_{10}$ score >2·0), including the five most abundant phyla, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria and Verrucomicrobia. HFD significantly increased the abundance of both Firmicutes and Bacteroidetes, resulting in a reduced ratio of Firmicutes: Bacteroidetes, from a ratio of approximately 80 in the mice fed a low-fat diet to a ratio of approximately 20 in the HFD-fed mice. The observation of a lowered Firmicutes:Bacteroidetes ratio by HFD was in agreement with a previous report[35]. Moreover, HFD significantly altered the abundance of approximately 23 % of OTU present in a given sample, resulting in a distinct change in the microbiome structure. For example, HFD significantly reduced the abundance of an OTU assigned to *A. muciniphila* (OTU no. 9), from 6·06 % in the mice fed LFD to 0·02 % in the HFD-fed mice (LDA $\log_{10}$ score >4·0). The species *A. muciniphila*, a mucin-degrading bacterium, can reverse high-fat diet-induced metabolic disorders, including fat-mass gain, metabolic endotoxaemia, adipose tissue inflammation and insulin resistance[6]. Detrimental effects of HFD on overall health also become evident by a marked increase in the abundance of several genera that harbor human pathogens, such as *Clostridium*, *Enterococcus* and *Escherichia*. Intriguingly, HFD led a 4-fold reduction in the abundance of an OTU that shares >97 % sequence homology with a known butyrate-producing bacterium, *F. prausnitzii*[36]. This observation may

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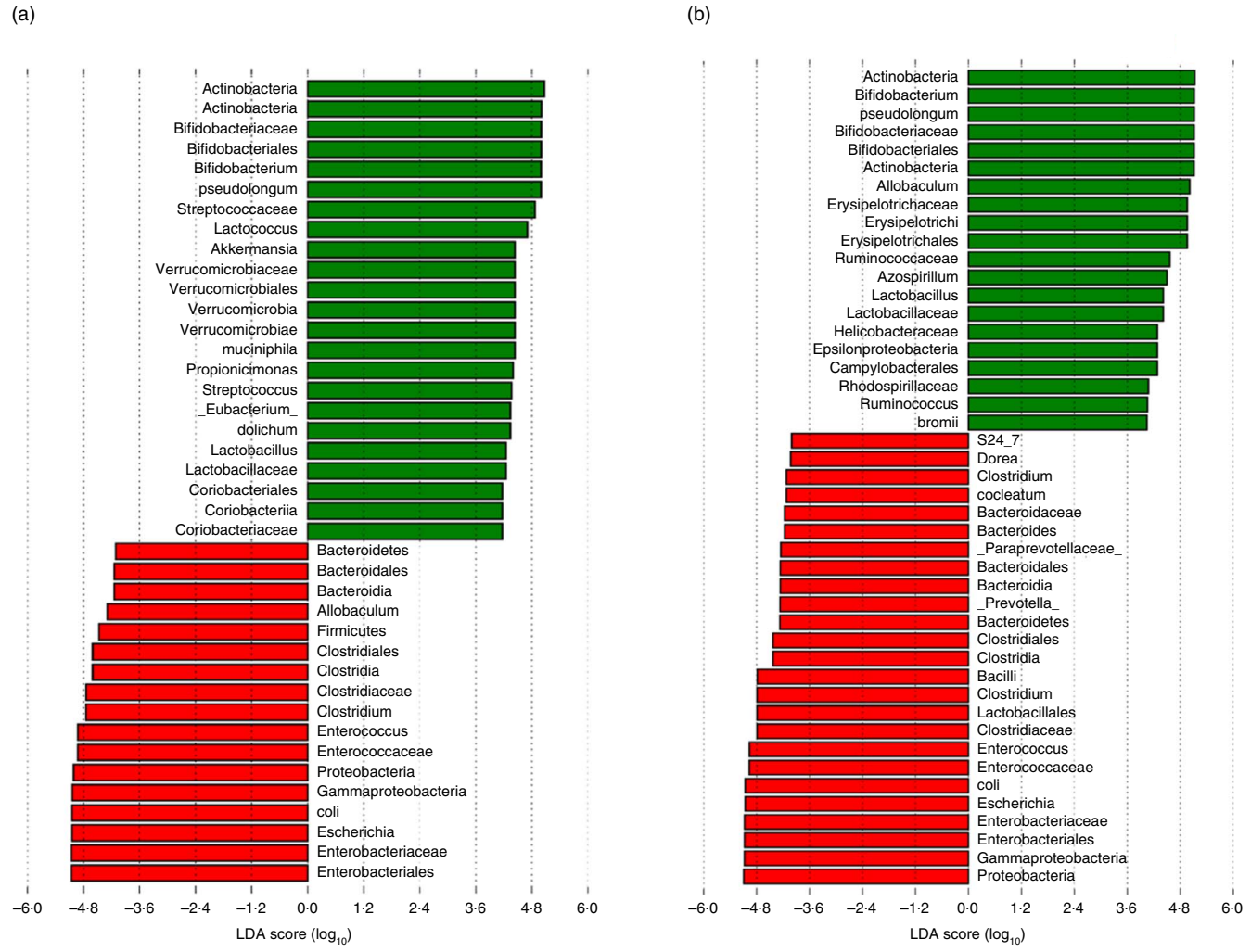

Fig. 5. Select microbial taxa significantly impacted by a high-fat and high-sucrose diet (HFD) and polymannuronic acid supplementation (PM). The statistical test was performed using the linear discriminant analysis (LDA) effect size method. However, the absolute $\log_{10}$ LDA scores >2·0 were considered significant, only those taxa with the absolute $\log_{10}$ LDA scores ≥4·0 are listed in the charts. (a) Low-fat and low-sucrose diet (LFD + PBS, ■) *v.* HFD + PBS. (b) HFD + PBS (■) *v.* HFD + PM (■).

help explain the significantly lower butyrate biosynthesis in the faeces of the HFD-fed mice.

Chronic inflammation is one of the primary mechanisms underlying adverse health effects of obesity[37]. Previous studies suggest that inflammation and obesity are associated with the alteration of gut permeability[38]. LPS is one of the key contributing factors affecting gut permeability and gut inflammation[39]. In this study, serum LPS as well as mRNA expression levels of several pro-inflammatory cytokines such as *TNFα* and *CD11c* were significantly increased in the adipose tissue of the HFD-induced obese mice, compared with the mice fed LFD. Moreover, our results show that PM was able to lower serum LPS levels. A 30-d PM dietary intervention significantly decreased the gene expression of several pro-inflammatory cytokines at the mRNA level. Moreover, PM was able to increase the gene expression level of an anti-inflammatory cytokine, *IL-10*. We hypothesise that PM might have the potential to ameliorate the metabolic syndrome by attenuating chronic inflammation induced by HFD.

In this study, we measured faecal concentrations of SCFA. Our results demonstrated that HFD significantly decreased two of the three major SCFA, propionate and butyrate ($P < 0{\cdot}05$), while slightly reducing acetate concentration as well as total SCFA (Fig. 3). Furthermore, PM was able to partially restore the levels of total SCFA repressed by HFD. Previous reports suggest that SCFA protect against HFD-induced obesity and insulin resistance[40] by decreasing PPARγ expression. Propionate and butyrate are known to increase intestinal gluconeogenesis and lead to beneficial effects on glucose and energy homoeostasis[41]. A common receptor-independent mechanism, such as PPARγ-dependent switch from lipid synthesis to utilisation, may explain the beneficial effects of SCFA. SCFA are important to gut health. SCFA also modulate the function of the adipose tissue, skeletal muscle and liver tissue[12]. Furthermore, butyrate possesses potent anti-inflammatory properties and regulates numerous biological processes because of its abilities to serve as a signaling molecule[42]. Acetate plays an important role in controlling inflammation and in combating

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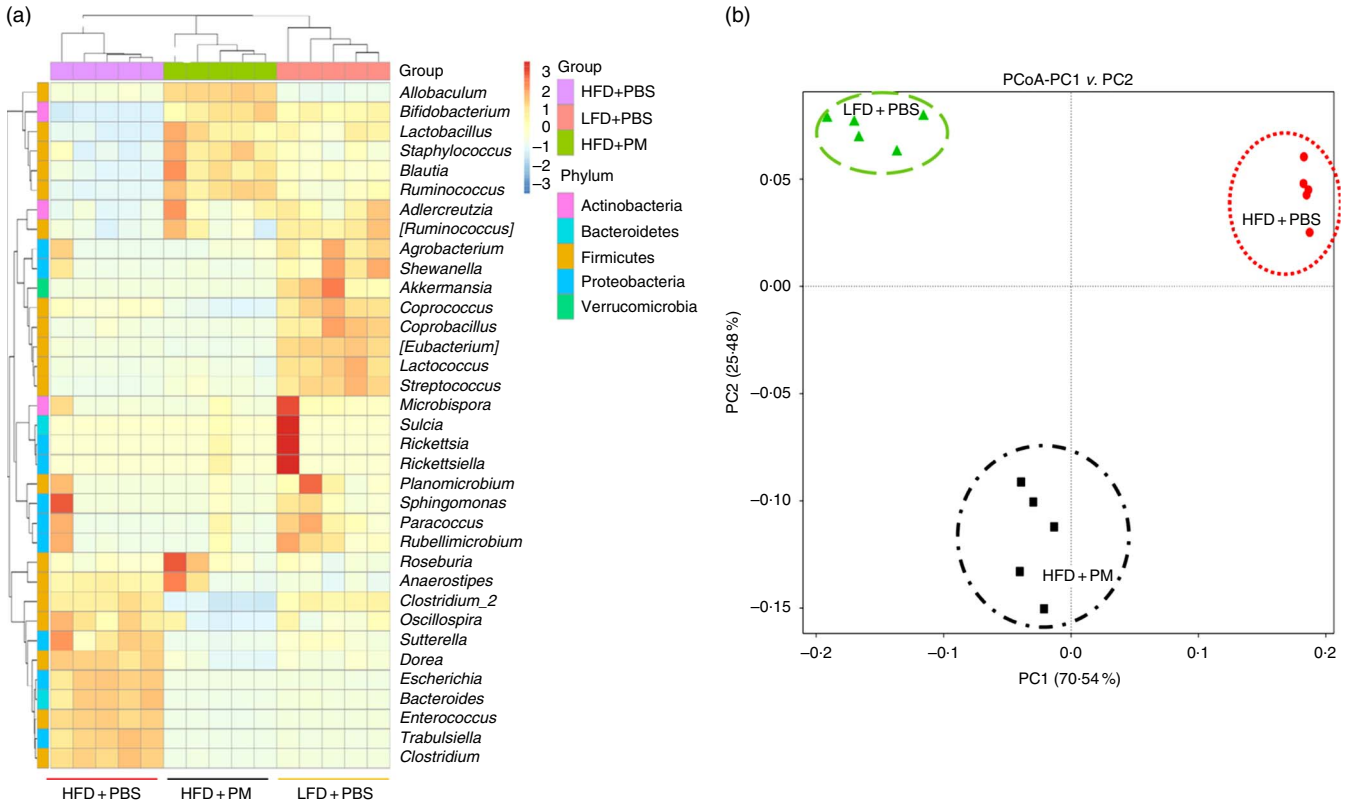

Fig. 6. Overall structural changes of the gut microbiome in response to a high-fat and high-sucrose diet (HFD + PBS) and HFD supplemented with polymannuronic acid (PM) for 30 d (HFD + PM). (a) Clustering analysis. (b) Principal coordinates analysis (PCoA) shows a distinct separation of the microbiome structure of the animals in each treatment group. LFD + PBS, low-fat and low-sucrose diet.

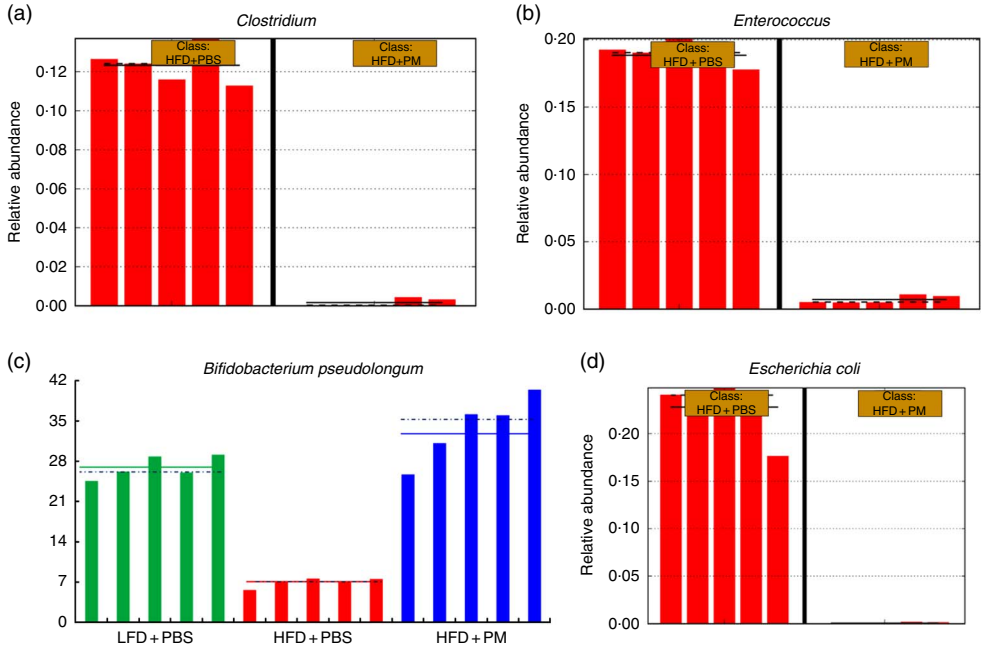

Fig. 7. Select microbial genera and species displaying significant differences in their relative abundance in the gut microbiome: (a) *Clostridium*, (b) *Enterococcus*, (c) *Bifidobacterium pseudolongum* and (d) *Escherichia coli*. x-axis: Individual samples; y-axis: relative abundance percentage (c) or fraction ((a), (b) and (d)); straight line, Mean abundance value of the group; dotted line, median of the group. LFD + PBS, low-fat and low-sucrose diet; HFD + PBS, high-fat and high-sucrose diet; HFD + PM, HFD supplemented with polymannuronic acid (PM) for 30 d.

Table 1. Operational taxonomic units (OTU) significantly impacted by polymannuronic acid (PM) in the gut microbiome in mice*
(Mean values with their standard errors)

	LFD + PBS		HFD + PBS		HFD + PM			
OTU_ID	Mean	SEM	Mean	SEM	Mean	SEM	log_{10} LDA score	Annotation
OTU_1	26·91	1·96	6·41	0·74	33·83	5·63	5·01	Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|*Bifidobacterium pseudolongum*
OTU_18	0·83	0·28	1·35	0·08	2·38	0·64	3·64	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_6	6·07	0·67	1·69	0·09	2·05	0·26	3·35	Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|*Streptococcus*
OTU_25	0·11	0·05	0·29	0·05	0·72	0·22	3·33	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|[*Ruminococcus*] *gnavus*
OTU_40	0·50	0·08	0·33	0·02	0·57	0·16	3·00	Actinobacteria|Coriobacteriia|Coriobacteriales|Coriobacteriaceae|*Adlercreutzia*
OTU_67	0·09	0·05	0·02	0·01	0·22	0·08	2·96	Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|*Lactobacillus reuteri*
OTU_14	4·97	0·27	0·65	0·12	0·18	0·04	3·30	Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|[*Eubacterium*] *dolichum*
OTU_21	1·90	0·22	0·29	0·03	0·13	0·03	2·91	Firmicutes|Clostridia|Clostridiales
OTU_61	0·06	0·02	0·03	0·01	0·10	0·03	2·57	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_58	0·09	0·03	0·22	0·05	0·04	0·02	2·96	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|*Oscillospira*
OTU_105	0·01	0·01	0·00	0·00	0·04	0·02	2·35	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_33	0·22	0·02	0·41	0·04	0·03	0·02	3·20	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_46	0·20	0·02	0·11	0·02	0·02	0·01	2·71	Firmicutes|Clostridia|Clostridiales
OTU_87	0·05	0·01	0·08	0·02	0·02	0·01	2·51	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|*Oscillospira*
OTU_52	0·09	0·03	0·18	0·04	0·01	0·00	2·87	Firmicutes|Clostridia|Clostridiales
OTU_248	0·00	0·00	0·00	0·00	0·01	0·01	2·54	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_249	0·01	0·01	0·00	0·00	0·00	0·01	2·42	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Ruminococcus
OTU_78	0·08	0·01	0·03	0·02	0·00	0·00	2·19	Bacteroidetes|Bacteroidia|Bacteroidales|[Paraprevotellaceae]|[*Prevotella*]
OTU_464	0·00	0·00	0·00	0·00	0·00	0·00	2·86	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_29	0·05	0·02	1·26	0·24	0·00	0·01	3·70	Bacteroidetes|Bacteroidia|Bacteroidales|S24-7
OTU_578	0·01	0·01	0·09	0·03	0·00	0·00	2·64	Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|*Escherichia coli*
OTU_167	0·00	0·00	0·01	0·00	0·00	0·00	2·49	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae
OTU_63	0·02	0·01	0·16	0·03	0·00	0·00	2·80	Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|Bacteroides
OTU_489	0·02	0·01	0·06	0·03	0·00	0·00	2·36	Firmicutes|Bacilli|Lactobacillales|Enterococcaceae
OTU_193	0·00	0·00	0·01	0·00	0·00	0·00	2·37	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|*Ruminococcus*
OTU_225	0·00	0·00	0·01	0·00	0·00	0·00	2·33	Firmicutes|Clostridia|Clostridiales
OTU_143	0·00	0·00	0·02	0·01	0·00	0·00	2·22	Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|*Proteus*
OTU_119	0·02	0·01	0·01	0·01	0·00	0·00	2·36	Firmicutes|Clostridia|Clostridiales|Dehalobacteriaceae|*Dehalobacterium*
OTU_184	0·00	0·00	0·01	0·00	0·00	0·00	2·19	Firmicutes|Clostridia|Clostridiales
OTU_252	0·00	0·00	0·01	0·00	0·00	0·00	2·42	Firmicutes|Clostridia|Clostridiales|Lachnospiraceae
OTU_191	0·01	0·00	0·00	0·00	0·00	0·00	2·68	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae
OTU_242	0·00	0·00	0·01	0·00	0·00	0·00	2·58	Bacteroidetes|Bacteroidia|Bacteroidales|S24-7
OTU_210	0·00	0·00	0·01	0·00	0·00	0·00	2·53	Firmicutes|Clostridia|Clostridiales
OTU_636	0·00	0·00	0·01	0·00	0·00	0·00	2·70	Firmicutes|Clostridia|Clostridiales|Ruminococcaceae

LFD, low-fat and low-sucrose diet; HFD, high-fat and high-sucrose diet; LDA, linear discriminant analysis.
* The statistic test was performed using the LDA effect size algorithm.

pathogen invasion[43]. Likewise, propionate was considered to have a potentially important role in protecting against diet-induced obesity[44]. SCFA are major microbial fermentation products of dietary fibre. Significant changes in SCFA profiles observed in the PM group in this study suggest that PM may have potential to modulate the microbial composition of the gut microbiome.

Indeed, we observed a significant impact of PM on the microbial composition and structure of the gut microbiome. At the phylum level, PM was able to restore the HFD-repressed abundance of Actinobacteria to its pre-stressed level as observed in the mice fed a low-fat diet (Fig. 4). In addition, PM treatment resulted in a significant reduction in the abundance of both Bacteroidetes and Proteobacteria. At a species level, PM led to a marked alteration of the abundance of thirty-four OTU (Table 1). Among thirty-three OTU from the genera known to harbor butyrate-producing bacteria[45], two OTU, OTU no. 60 from the genus *Roseburia* and OTU no. 286 from *Anaerofustis*, were significantly increased by PM. Nevertheless, overall populations of the butyrate-producing bacteria as a functional group impacted by PM were small, accounting for only 0·12 % of all sequences. This suggests that PM may regulate gut butyrate production via metabolic cross-feeding rather than a direct expansion of butyrate-producing bacteria. For example, PM significantly increased the abundance of an OTU assigned to *R. bromii* (OTU no. 20), from HFD-repressed 0·04 to 1·94 %, a level much higher than that observed in the mice fed a low-fat diet (0·24 %). *R. bromii* itself is not a butyrate-producing bacterium. However, in coculture with other dominant amylolytic species, *R. bromii* strongly stimulates utilisation of resistant starch[46], suggesting that it may play a pivotal role in the degradation of resistant starch in the colon and may indirectly affect biosynthesis and microbial ecosystem output of SCFA in the gut.

PM treatment resulted in a 5-fold expansion of an OTU assigned to *B. pseudolongum* (LDA log_{10} score = 5·01), from

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6·41 % in the HFD group to 33·83 % in the PM group (Fig. 7(c)). After the expansion, *B. pseudolongum* became the most abundant species in the HFD mice supplemented with PM. Interestingly, this species is also the most predominant *Bifidobacteridium* in the intestine of FOS-supplemented mice[47]. Furthermore, oral administration of *B. pseudolongum* to BALB/c mice results in a higher excretion of viable bifidobacteria and a positive impact on antigen-induced cytokine production. In addition, PM significantly increased the abundance of a probiotic bacterium, *L. reuteri*. Together, our findings suggest that PM may exert its immunoregulatory effects via enhancing proliferation of several bacterial species with probiotic activities.

Of note, PM had a marked impact on the *E. coli* population in the gut. As Fig. 7(d) shows, compared with the mice fed LFD, HFD led to a 28-fold increase in the abundance of the two OTU assigned to *E. coli*. As a result, *E. coli* became one of the most abundant species in the gut of the HFD-fed mice, accounting for approximately 22·80 % of all 16S sequences. PM as a dietary supplement resulted in a 290-fold reduction in *E. coli* abundance, to a level ten times lower than that in the mice fed LFD. Similarly, PM significantly decreased the abundance of several key genera that harbor human pathogens, such as *Clostridium* and *Enterococcus* (Fig. 7(a) and (b)).

In conclusion, our findings suggest that PM was able to reduce HFD-induced body weight gain, adiposity index, fat mass and serum TAG levels in mice. Moreover, PM significantly decreased blood LPS levels and increased the expression of several anti-inflammatory cytokines at the mRNA level. PM had a profound impact on the gut microbiome, significantly altering the abundance of approximately 14 % species-level OTU. Furthermore, PM as a dietary supplement resulted in a significant expansion of several probiotic species while repressing the abundance of the genera that harbor pathogens, suggesting that PM may exert its immunoregulatory effects by restoring the structure and function of the gut microbiome altered by HFD. Our initial findings should facilitate subsequent mechanistic studies on how PM may alleviate obesity and inflammation. Our results suggest that seaweed-derived PM has the potential to be used as a bioactive compound with desirable health benefits.

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Conceived and designed the experiment: F. L. and Q.-J. T. Performed the experiment: F. L., X. W., H. S., Y. W. and C. X. Analysed the data and wrote the manuscript: F. L. All authors reviewed and approved the manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517000964

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