Role of Modified Diet and Gut Microbiota in Metabolic Endotoxemia in Mice

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Abstract

This study was aimed to investigate the effect of cultured gut microbiota (GM) from obese humans coupled HFD in inducing metabolic endotoxemia in humanized mice. In total, 30 strains were isolated from 10 stool samples of obese patients. Following morphological and biochemical *characterization, 16S rRNA gene sequencing of six abundant isolates* identified these as *Klebsiella aerogenes, Levilactobacillus brevis, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* (MZ052089- MZ052094). *In vivo* trial using above six isolates, known as human gut microbiota (HGM), was performed for six months. Sixteen mice were distributed into four groups *i.e.,* G1 (control) mice fed with chow diet, group 2 (G2) mice with HFD, group 3 (G3) mice with HFD + HGM and group 4 (G4) mice with chow diet + HGM. Body mass index (BMI) and plasma endotoxins were measured pre and post experiment. *In vivo* study revealed that HFD + HGM caused significant increase (3.9 g/cm at 20 weeks) in the body weight and BMI (0.4g/cm post experiment) of G3 mice compared to the other groups. One way ANOVA showed significantly higher level of endotoxins (2.41, 4.08 and 3.7 mmol/l) in mice groups G2, G3 and G4, respectively, indicating onset of metabolic endotoxemia. Cecal contents of experimental mice groups showed more diversified microbiota in mice groups G1 and G4 compared to G2 and G3 where high fat feeding alone and combined with obese gut microbiota caused a shift in microbial diversity as observed by all five strains belonging to either *Firmicutes* or *Bacteriodetes* phyla, respectively. In conclusion, current study reported that minor alteration in GM composition through HFD feeding and cultured GM transfer has significant impact in development of metabolic endotoxemia, possibly via modified intestinal permeability.

Introduction

Metabolic endotoxemia is a state of increased level of lipopolysaccharides (LPS) or endotoxins in the blood (Wang et al. 2010). During this condition, proinflammatory molecules such as interleukin-1, interleukin-6, tumor necrosis factors (TNF-α) are increasingly expressed. LPS are large, heat stable endotoxins, present in outer membrane of Gram -ive bacterial cell wall. It creates a permeability barrier at the bacterial surface and is a mainly responsible to confer innate resistance that Gram-negative bacteria display against various antimicrobials (Boutagy et al. 2016; Dalby et al. 2018).

Human intestine is colonized by copious amount of different bacterial phyla, and these bacteria are associated with food metabolism, energy harvesting and development of innate immunity. Among bacterial phyla as inhabitant of human gut, abundant ones include *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%), *Fusobacteria*, *Verrucomicrobia* and *Actinobacteria* (3%). *Firmicutes* is the most common with 200 genera including *Mycoplasma*, *Bacillus* and *Clostridium* (Arumugam et al. 2014). Gram- ive bacteria makes approximately 70% of total microbes and any imbalance is central to development of metabolic diseases *e.g.*, obesity and type 2 diabetes mellitus (DM) (Bailey and Holscher, 2018), due to continuous low grade inflammation also known as metabolic endotoxemia.

Regular chow diet is the normally used high fiber diet, composed of agricultural byproducts (wheat, corn, alfalfa, soybeans and supplemented with minerals, vitamins) and is palatable to mice. On the other hand, HFD contains cornstarch, amino acid supplemented casein, fiber from cellulose, sucrose or lord. Two ingredients differ between two diets. One is phytoestrogen content from soy, that is high in chow diet only and other is sucrose that is present only in HFD. Phytoestrogen content of chow diet controls anxiety, locomotion, memory, insulin-thyroid levels, lipogenesis and lipolysis *etc.* (Hooper et al. 2018). High fiber content of chow diet make it equivalent to the prebiotic, thus suppresses adiposity and other metabolic syndromes. Previous human intervention studies have performed using prebiotics such as oligofructose, inulin, galacto-oligosaccharides, resistant dextrin, insoluble dietary fiber, and whole grains. Oligofructose is found in many fruits and vegetables and is an oligosaccharide having one glucose molecule combined with several molecules of fructose. Inulin is a fructose-polymerized polysaccharide, abundantly present in vegetables (burdock and onion) and. In these studies, subjects with obesity, overweight subjects, and subjects with type 2 diabetes showed significant decrease in LPS levels in blood (Dehghan et al. 2014; Parnell et al. 2017). Likewise, other studies showed

decreased plasminogen activator inhibitor-1 (PAI-1), and improved glucose metabolism. One study concluded that galacto-oligosaccharides reduced LPS levels, and improved obesity by suppressing appetite. In mice, chronic administration of LPS resulted in hyperphagia by decreasing leptin sensitivity of afferent vagal nerves. Following galacto-oligosaccharide administration, reduced LPS levels and appetite suppression supports association between LPS and appetite (de La Serre et al. 2010).

HFD acts as strong trigger for the systemic inflammation and increased BMI. Previously, it demonstrated that increase in LPS is related to HFD consumption (Li et al. 2011). Unaware of true mechanism of increased plasma LPS linkage with HFD, causative factor was thought to be changes in intestinal microbiota, possibly a switch from Gram + ive ones to Gram – ive bacteria. Though still there is no understanding of direct effect of GM on metabolic endotoxemia, but evidently it was thought that microbial dysbiosis make the gut leaky, induce inflammation and led to endotoxemia (Li et al. 2011; Bailey and Holscher, 2018).

Increased HFD consumption modifies GM, results in increased amount of systemic level of bacterial products and increases the gut permeability for these bacterial products. A meal intake of excess HFD provokes the excess formation of LPS from the Gram -ive bacteria's cell wall, increased the excess formation of chylomicron resulting in LPS infiltration into the blood circulation (Ghoshal et al. 2009; Caesar et al. 2012). Bacterial endotoxins translocation and increased intestinal permeability are the two most important contributing factors that play an important role in the progress of metabolic endotoxemia (Fei and Zhao, 2013).

Both dietary modulations and gut microbiota are the most significant contributions to human health. Turnbaugh et al. (2006) transferred the human fecal microbiota into germ free mice to create humanized mice, which were successfully colonized with donor's microbiota. Switching this humanized mice to HFD for a single day resulted in changed metabolic pathways and microbiome. The authors observed that though colonization establishes the initial microbial community, however diet can significantly alter this primary community. Further, humanized mice showed high adiposity index following feeding with HFD. Their study established an animal model to study the effect of genetic and environmental factors on the GM and host metabolism. However, Turnbaugh and colleagues (2006) used fecal microbiota from normal healthy individuals and there is difference in the gut microbiota and metabotypes of lean *vs* obese individuals (Waldram et al. 2009). Additionally, variation in gut microbiota has been reported across populations of different ethnicity. For example, Jain et al. (2018) revealed microbial community with dominant *Firmicutes*, *Actinobacteria* and underrepresented *Bacteroides* in Indian and Chinese population. While, Brooks et al. (2018) documented 12 microbial genera and families from two US-based 1,673 individuals. Majority of these include the family *Christensenellaceae*, overlap with closely related taxa and form clusters showing similar metabolic processes.

Hence, with all this background the current study seeks to establish whether a chow diet administered therapeutically to mice inoculated with fecal microbiota from obese individuals, is able to change the gut microbiota to a composition associated with healthy individuals using an *in vivo* approach (mice). The study also aims to clarify whether a chow diet reduces adiposity and metabolic endotoxemia in such mice possibly by acting as prebiotic.

Materials And Methods

In total, clinically diagnosed obese (n = 10) volunteer subjects were selected from the Mayo Hospital Lahore and general population. All subjects gave fully written informed consent about participation in study which was approved by Institutional Bioethics Committee (IBC) vide letter no. IBC 2431, GC University, Lahore.

Sample collection and inoculum preparation

Fresh stool sample in sterile stool collection vials were collected from obese subjects maintaining a temperature of 5 ± 1°C during transportation. Fecal inoculum was prepared by mixing sample in saline solution (Possemiers et al. 2004) and streaking on various media including MacConkey agar, nutrient agar, xylose lysine deoxycholate agar (XLD), tryptone soya agar (TSA) and brain heart infusion agar (BHI) was performed at 37°C for 3 days following Lau et al. (2016).

Morphological, biochemical and genetical characterization of human gut microbiota (HGM)

Using a traditional culture based method, isolated strains were morphologically characterized on brain heart infusion agar and different biochemical tests such as catalase, citrate utilization, urease, H_2S production, methyl red test, indole, voges proskauer and denitrification were also performed (Gerhardt et al. 2006). This approach general emphasizes on easy to culture microbes of gut, and offer advantage of growth so fastidious microbes due to use of specific media. There is the limitation that it offers only 10–50% of gut microbes to be cultured (Eckburg et al. 2014). Using universal primers 16S-27-F (5' AGAGTTTGATCCTGGCTCAG-3') and 16S-1522-R (5'- AAGGAGGTGATCCAGCCGCA-3'), PCR was used to amplify 16S rRNA gene in a thermal cycler under standard conditions. The amplified product sequenced by Axil scientific, Singapore. Sequenced data was examined using BLAST software and a phylogenetic tree was constructed in neighbor joining method using MEGA X 10.2.5.

Animal housing conditions (*in vivo* study)

All animals were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Department of Zoology, GC University, Lahore, Pakistan. The study was approved by the Institutional Bioethics Committee via approval number IBC-180920 following guidelines designed by Committee for the Purpose of control and supervision of experiments on animals, Pakistan. Twenty albino mice were taken from the animal house of department of Zoology GC University Lahore and were mated. Ampicillin (1g/L) was mixed in drinking water of mother one week before birth. 16 pups were selected and treated with ampicillin (1g/L) in water till three weeks of age (Ellekilde et al. 2014). Following slight modification of protocol by Ellekilde et al. (2014), mice were kept on same diet in temperature controlled conditions (22°C) for another three weeks. After that, six weeks old mice were distributed into four groups, each group comprising of 2:2, male-females ratio. Mice were fed with chow diet (G1), HFD (G 2), HFD + HGM (G3) and chow diet + HGM (G4). Body weight was measured weekly. Adiposity in terms of body mass index (BMI) and endotoxins were measured at the start and end of experiment (6 and 24 weeks respectively) (Wang and Liao, 2014).

Preparation and transfer of HGM inoculum

The bacterial inoculum was prepared by taking isolated HGM and making dilution of 1: 10 with 50% glycerol. The inoculum was stored at -80°C, making small aliquots. At the day of experiment, it was further diluted in ratio of 1:5 and 0.15 ml was given 3 times to each mouse (Ellekilde et al. 2014). The mice from both G3 and G4 were colonized with the HGM via oral route using force feeding method.

Chow diet and high fat diet (HFD) feeding

Mice from groups (G1; control) mice and G4) were fed with chow diet (energy contents: 20% protein, 70% carbohydrate and 10% fat. Mice from both groups (G2 and G3) were fed with HFD (energy contents: 13% protein, 6% carbohydrate and 81% fats) (Lamont et al. 2016).

Measurement of plasma endotoxins

Plasma endotoxins were measured pre (week 6) and post experiment (after 24 weeks). Briefly, blood was collected from the tail of mice at week 6, transferred to EDTA tube to prevent clotting and plasma endotoxin concentration was

measured. While, post experiment (after 24 weeks), mice were sacrificed and blood was collected from the left ventricle of heart via cardiac puncture. Plasma endotoxin concentration was measured following method by Wang and Liao (2012).

Determination of bacterial diversity in cecal content of Diet + HGM treated mice

Cecal contents were collected from postmortem mice of all four groups separately and slurries were made by homogenizing the cecal content with 0.1 M phosphate-buffered saline (pH 7). Using Qiagen fecal Mini Kit (Germany), genomic DNA was isolated separately from intestinal contents, quantified, normalized to 1ng/µl and used as template. Following optimization, 16S rRNA gene fragments were amplified using primers 341F: 5'-CCTACGGGNGGCWGCAG-3' and 805R: 5'-GACTACHVGGGTATCTAATCC-3'. Obtained fragments were purified using Gen elute Kit and sequenced. Using NCBI BLAST website, sequences were blast and strains were identified upto taxonomically on the basis of E-value. Phylogenetic tree was constructed and the genetic distance of each strain was determined.

Statistical Analysis

Statistical analysis was performed using the statistical software SPSS Version 15.0 (Windows Evaluation Version). T-test (Paired samples t-test) and one way ANOVA followed by post hoc Turkey test was used to analyze all data at P ≤ 0.05.

Results

Morphological, biochemical and genetic characteristics of fecal bacteria

Total 30 bacteria were isolated from human stool samples. Out of these, 17 morphologically different strains were observed (Table S1). Gram staining revealed that 80% of strains were Gram negative. Biochemical characterization revealed that most of these bacteria belong to six genera *Bacillus* sp. (1 strain), *Clostridia* (3 strains), *Enterobacter* (1 strain), *Escherichia* (3 strains), *Lactobacillus* (5 strains) and *Staphylococcus* (4 strains) (Table 1). Out of these genera, four belong to phylum Firmicutes (*Bacillus*, *Clostridia, Lactobacillus* and *Staphylococcus*) and two were from Proteobacteria (*Enterobacter* and *Escherichia*). 16S rRNA gene sequencing identified six abundant isolates belonging to species as *Klebsiella aerogenes, Levilactobacillus brevis, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* (Accession numbers: MZ052089- MZ052094). Phylogenetic tree of isolates is shown in Fig. 1.

Table 1

Biochemical characterization fecal bacteria isolated from obese patients

Bacterial strains	Citrate	H_2S Pro.	Urease	Cat.	Nit. Red	VP	Starch	MR	Indole	Carbohydrate Fermentation		
										Glucose	Sucrose	Lactose
OB-1	+	-	-	+	+	+	-	-	-	+	+	-
OB-2	-	-	-	-	+	-	+	+	-	+++	+	+++
OB-3	-	-	-	+	-	-		+	+	+	+	+
OB-4	+	-	+	+	+	+	+	+	-	+	+	+
OB-5	-	-	-	-	+	-	+	+	-	+++	+	+++
OB-6	-	-	-	+	-	+		+	+	-	+	+
OB-7	-	-	-	+	-	+		+	+	-	+	+
OB-8	+	-	+	+	+	+	+	+	-	+	+	+
OB-9	-	-	+	-	+	-		+	-	+	+	+
OB-10	-	-		-	+	-	+	+	-	+++	+	+++
OB-11	-	-	-	-	+	-	+	+	-	+++	+	+++
OB-12	-	-	-	+	-	+		+	-	+	+	-
OB-13	+	-	+	+	+	+	+	+	-	+	+	+
OB-14	-	-	+	-	+	-		+	-	+	+	+
OB-15	-	-	-	-	+	-	+	+	-	+++	+	+++
OB-16	-	-	+	-	+	-		+	-	+	+	+
OB-17	+	-	+	+	+	+	+	+	-	+	+	+

OB, Obese patients; H_2S Pro., H_2S Productio; Cat., Catalase; Nit. Red., Nitarate reduction; VP., Voges proskauer; MR, Methyl Red

Body weight and BMI

Body weight of experimental animals measured over a period of 24 weeks revealed that there was non significant increase (3 g/cm^2) in body weight of G3 mice (HFD + HGM) and G4 mice (Chow diet + HGM) compared to control group (G1; Chow diet) after 16 weeks irrespective to gender. This increase was continuous and non significant during other weeks. No difference was observed in mice treated with chow diet over the whole experimental period (Fig. 2). However, significant difference in BMI (4 g/cm^2) (P 0.05) of experimental mice of G3 (HFD + HGM) was observed compared to control group (G1; Chow diet) at the end of experiment. G4 mice (Chow diet + HGM) also showed significant increase in BMI compared to G1 (Chow diet) (Fig. 3).

Endotoxins levels

Levels of endotoxins in treated versus control mice shown in Fig. 4. It was observed that mice in groups (G3 and G4) exhibited significantly elevated concentration of plasma endotoxins compared to control group (G1). Highly significant difference (P ≤ 0.001) was observed in G3 (HFD + HGM) as well as G4 mice (Chow diet + HGM), when compared with control group (G1) and G2 (HFD). Overall, levels of endotoxins were observed to follow the trend: G3 (HFD + HGM) > G4 (Chow diet + HGM) > G2 (HFD) > G 1 (Chow diet) (Fig. 4)

Diversity of cecal bacteria from diet + HGM treated mice

To determine the effect on gut microbiota, 20 isolates (five isolates per group), belonging to five phyla *viz*., *Fimicutes, Proteobacteria, Actinobacteria, Verrucomicobia* and *Bacteriodetes* were identified via 16S rRNA gene sequencing. It was observed that mice of groups G1 (Chow diet) showed a more diversified microbiota (*Bifidobacterium bifidum, B. longum, Akkermansia muciniphila, Flavobacterium* sp., *Prevotella copri*) belonging to phyla *Actinobacteria*, *Verrucomicobia* and *Bacteriodetes* compared to G2 (HFD) where all five isolates (*Lactobacillus acidophilus, L. gasseri, L. plantarum, Enterococcus* sp., *Bacillus* sp.) belonged to phylum Firmicutes. Gut microbiota of G3 (HFD + HGM), showed five isolates (*Parabcetriodes gordonii, Bacteriodes vulgatus, B. gallinarum*, *Bacteriodes* sp., *B. faecis*) belonging to phylum *Bacteriodetes*, whereas in mouse group G4 (Chow diet + HGM), among five isolates, two strains (*Ruminococcus bromii, Clostridium* sp.) belonged to *Firmicutes* and three strains (*Enterobacter aerogenes, Escherichia coli, Shigella* sp.) belonged to *Proteobacteria* (Fig. 5).

Discussion

In the present study, 11 strains with purified on the basis of variations in colour, shape and internal characteristics from obese human fecal samples. On the basis of biochemical characterization, great diversity of bacterial strains belonging to different genera *Bacteriodes, Escherichia*, *Enterobacter, Lactobacillus* and *Staphylococcus etc.* were observed. 16S rRNA gene sequencing identified further six isolates upto species level such as *K. aerogenes, L. brevis, E. coli, S. aureus, B. cereus* and *B. subtilis*. Gut microbiota plays important role in metabolic functioning including synthesis of macronutrients, catabolism of dietary toxins and fermentation of indigestible food substance. Our observation of compositional changes in gut microbiota with increased *Firmicutes* level in the obese patients is consistent with the findings of Cani et al. (2012) and Jakobsson et al. (2014), who observed that decreased Bacteriodetes and increased Firmicutes led to metabolic disorders in humans. Likewise, Caricilli and Saad (2013) conducted a study on obese and control individual and showed increase in phyla *Firmicutes* and *Actinobacteria* and decrease in *Bacteriodetes*. However, this is in contrast to findings by Li et al. (2018), who reported the positive role of *Firmicutes/Bacteriodetes* role in improving gut dysbiosis and controlling metabolic endotoxemia. There are controversial findings on the observed *Firmicutes/Bacteriodetes* ratio in obese *vs* lean individuals. This might be due to the fact that experimental approach to identify gut microbial community was different in various studies depending upon culture dependent or culture independent sequencing methods. This study used cultured dependent approach and its importance can be judged by the fact that properties of uncultured organisms present in gut can only be inferred from their cultured relatives. Yousi et al. (2019) used culture dependent method to isolate human gut bacteria from fecal sample. They found that there is a major proportion of Bacteroides and Firmicutes. Lau et al. (2016) used both culture dependent and culture independent methods and revealed that most of the bacteria detected by culture independent method can detected by using culture dependent method as well. Johnson et al. (2017) also supported the similar notion and emphasized the fact that great extent of uncultured biology remains to be better characterized using culture dependent approach. Previously, Leser et al. (2002) isolated gut microbiota using culture independent method and found Bacteroides and Firmicutes as major phyla but more diversity. This suggests that both culture dependent and culture independent method can be adopted for the gut bacteria isolation and culture dependent is more useful for observing bacteria physiology and other related features.

Our study identified a significant difference of weight gain of mice treated with HFD + HGM compared to control after 16 weeks over experimental period of 24 weeks. Mean energy intake was comparatively higher in mice treated with HFD alone and combined with HGM. It might lead to increased adiposity thus increased weight gain compared to those mice which received low energy intake from chow diet. Chow diet didn't show any effect in restoring the microbial composition comparable to healthy humans, making it not an effective prebiotic (assuming chow diet as a prebiotic), as reported by Bäckhed et al. (2004) who used germ-free and colonized normal and knockout mice fed with a standard, polysaccharide-rich rodent-chow diet indicated that this host-microbe mutualistic association allows the extracted energy to be stored in adipocytes via pathway that involves microbial regulation of the intestinal epithelial expression of fasting-induced adipocyte protein (Fiaf), a circulating inhibitor of lipoprotein lipase (LPL). Microbial fermentation of dietary polysaccharides to monosaccharides and short-chain fatty acids in the distal gut and their subsequent absorption stimulate *de novo* synthesis of triglycerides in the liver. Microbial suppression of Fiaf in the gut epithelium results in reduced levels of this circulating LPL inhibitor, increased LPL activity in adipocytes, and enhanced storage of liver-derived triacyglycerols in fat cells. Diet changes can modify measurably the composition of the human gut microbiota within days. David et al. (2012) reported that the gut microbiotas of humans were significantly altered only 2 days after the subjects switched to an animal-derived diet. When the subjects returned to their regular diets after 4 days of the animal-derived diet, their gut microbiota returned to a composition similar to the baseline composition. Dalby et al. (2018), who reported that mice fed with HFD for 8 weeks showed increase adipose tissue index and significant weight gain compared to those who had low calorie diet. In another study, Turnbaugh et al. (2006) transferred gut microbiota from obese mice to leaner mice and observed weight gain in the recipient mice. Likewise, significant increase in BMI index of HFD + HGM treated consistently support the fact that HFD and GM are related factors with strong effect on BMI (Cani and Delzenne, 2011).

Continuous use of high fat meal causes an elevation level of plasma endotoxin in individuals, hence inducing various metabolic disorders (Cani et al. 2008). Endotoxins (LPS) are continuously produced in the body in the normal range from gut microbiota but feeding on high fat diet for a long period increased their concentration in the body. In line with previous study, we observed a significant elevation in endotoxin levels of all our experimental mice groups treated with high energy diet and obese gut microbes (Boutagy et al. 2016; Bailey et al. 2018). This elevation was robust in mice treated with combination of HFD and HGM. Potential explanation for the observed findings could be the fact that increased HFD intake and obese HGM are both triggering factors, responsible for increased intestinal permeability as well as increased release on inflammatory factors leading to metabolic endotoxemia (Yousi et al. 2019). Similarly, Manco et al. (2010) established that modified gut microbiota alters LPS which mainly regulates inflammation and other metabolic disorders.

To assess the effect of HFD and HGM on experimental mice microbiota, we sacrificed mice and used cecal contents to determine the microbial diversity via 16S rRNA gene sequencing. It was observed that high fat feeding alone and combined with obese gut microbiota caused a shift in microbial diversity as observed by isolation of all five strains belonging to *Firmicutes* phylum in HFD fed group and *Bacteriodetes* phylum in HFD plus HGM fed group. Comparatively, mice of chow fed groups (alone and combined with HGM) showed a more diversified microbiota, where isolates belonging to phyla *Firmicutes, Proteobacteria, Actinobacteria, Verrucomicrobia* and *Bacteriodetes* were screened. Previous studies have shown that microbial flora changes in response to modified diet (chow diet/HFD) and may cause metabolic diseases by mediating metabolic endotoxemia (Jakobsson et al. 2014). Li et al. (2018) also demonstrated that improved gut microbiota by increasing *Firmicutes:Bacteriodetes* ratio had beneficial impact on HFD induced metabolic endotoxemia.

Conclusion And Future Perspective

Together, this study concluded that high fat feeding and obese gut microbiota triggered are involved in the development of metabolic endotoxemia, thus providing direct evidence about the role of both factors in development of various

metabolic diseases triggered by mediating endotoxemia induced inflammation. It also showed that though chow diet maintains microbial diversity but is unable to restore the obese gut health by changing the gut microbiota to a composition associated with healthy individuals. Additionally, it also didn't reduce adiposity and metabolic endotoxemia, making it not an effective prebiotic. It is further evident from our finding that consumption of small number of obese gut microbiota and high fat feeding can result in significant changes in gut dysbiosis and metabolic changes in mice. This is similar to observations made by Messer et al. (2018), who reported that microbes of gut community have close functional relations and even small number of microbes and/or their functions could have serious impacts on total community. That'In contrast, future studies using probiotics combined with chow diet might suggest whether diet changes are able to restore gut microbiota induced adiposity and metabolic endotoxemia.

Declarations

Conflict of interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authors contributions

IL conceived the idea, designed experiments and wrote the manuscript. HS performed experiments and collected data. SR, MM, MF, SA helped in analyzing data and revised the manuscript. CR and NA helped in experimentation. All authors approved the final version of manuscript.

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Figures

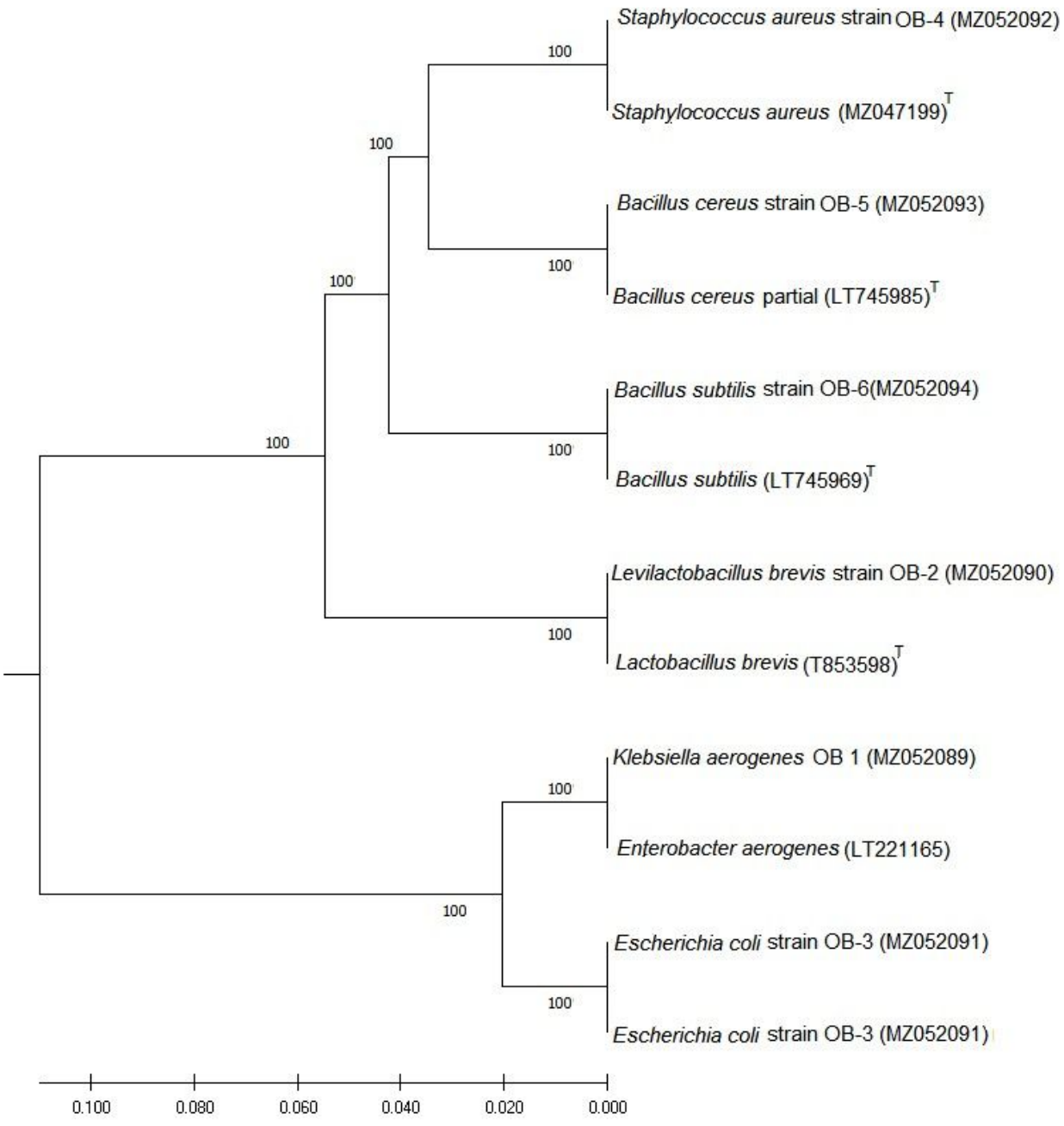

Figure 1

Phylogenetic tree based on 16S rRNA gene sequence showed genetic variability among the 06 human fecal bacteria and type strains. The tree was constructed using MEGA X software by UPGMA method. Briefly, human stool samples were obtained from 10 obese (OB) patients and purified on different media under aerobic and anaerobic conditions. On the basis of visual examination, six morphologically different and abundant isolates were subjected to 16S rRNA gene sequencing.

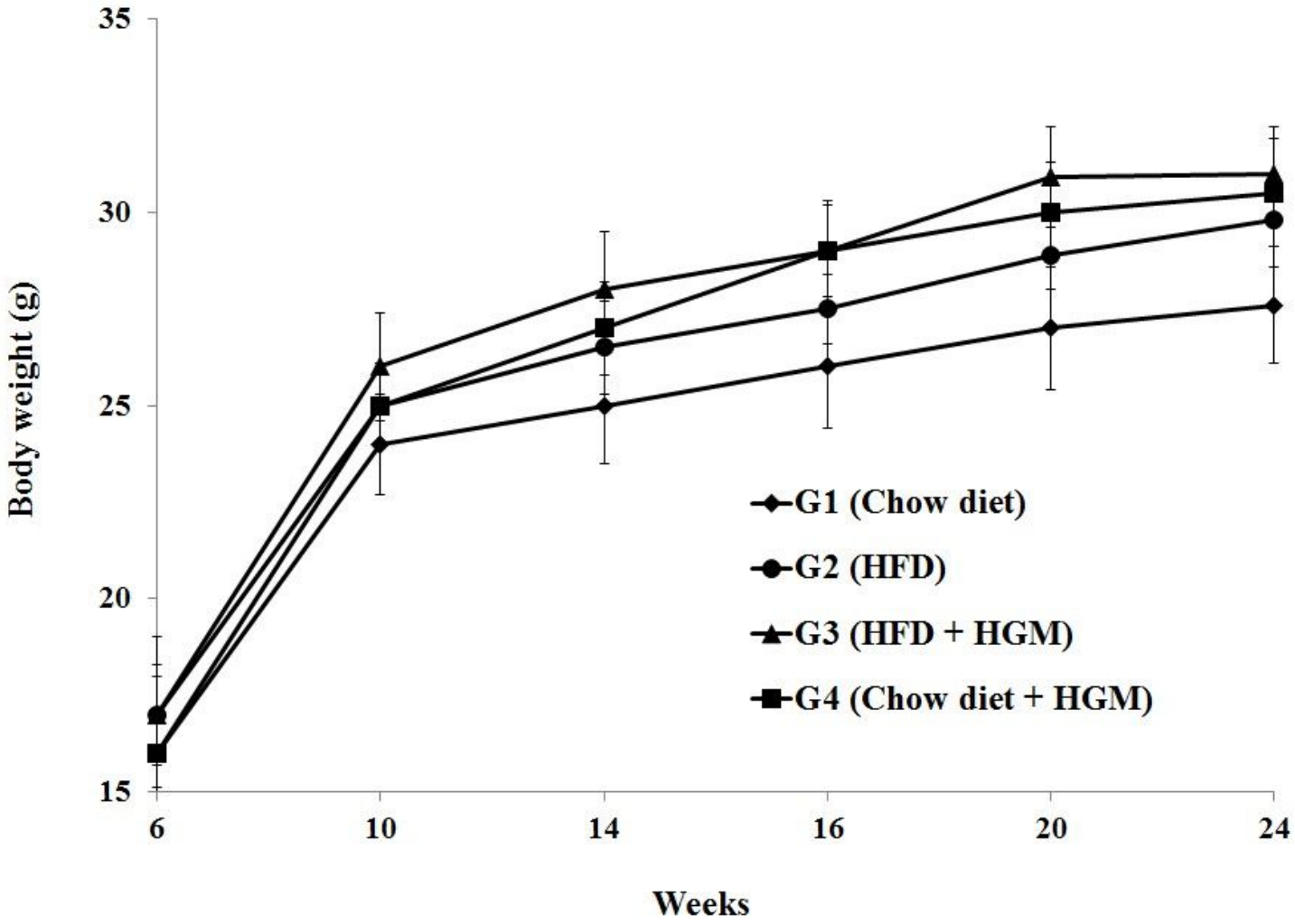

Figure 2

Effect of diet (chow/high fat diet; HFD) alone and combined with human gut microbiota (HGM) on body weight of mice (n=4/group). Values are represented as mean ± standard error of mean (sem), mice. *$p < 0.05$

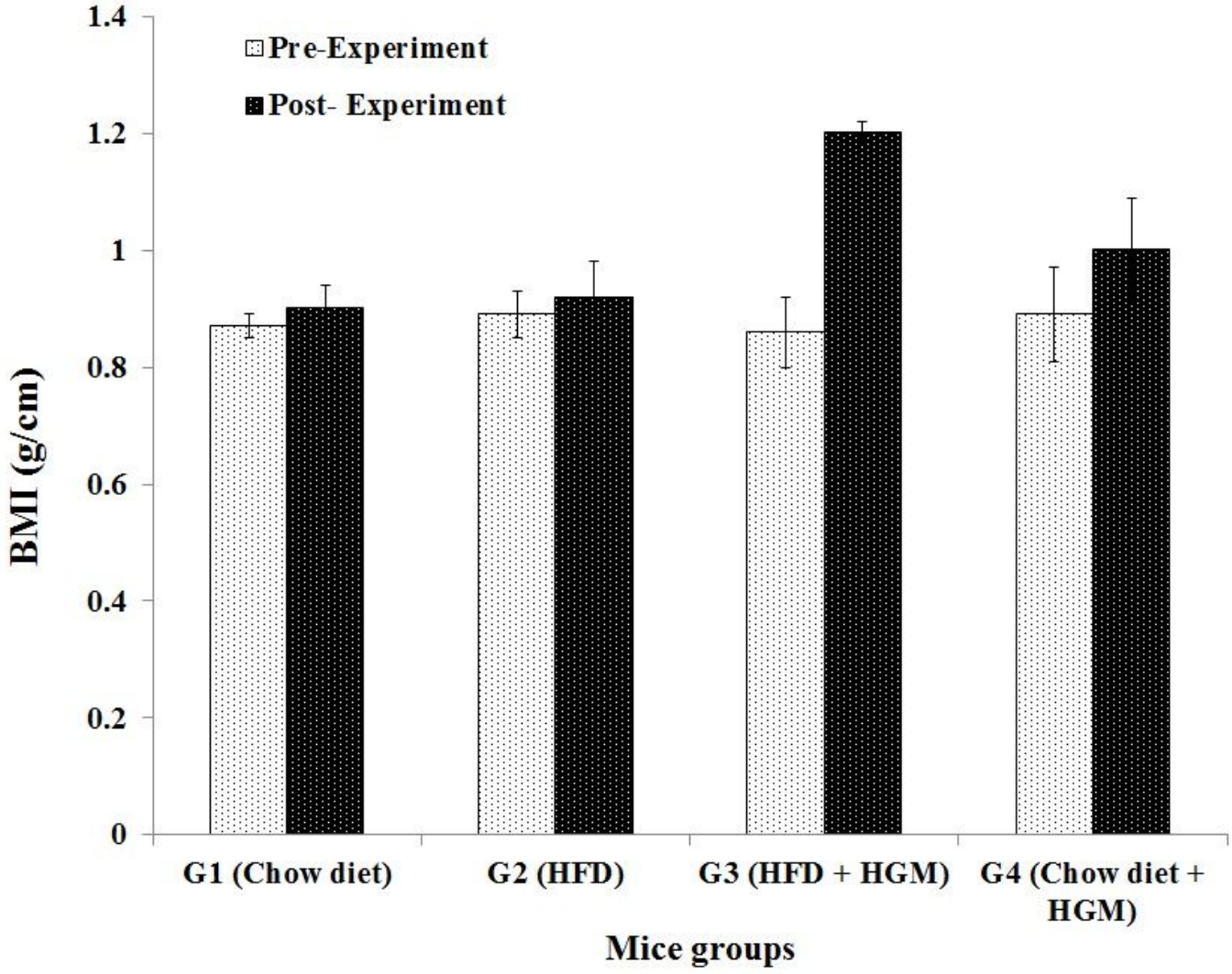

Figure 3

Effect of diet (chow/high fat diet; HFD) alone and combined with human gut microbiota (HGM) on body mass index (BMI) of mice (n=4/group) pre (6 weeks) and post experiment (24 weeks). Values are represented as mean ± SEM, One-way analysis of variance (ANOVA) with post hoc Tukey was used to analyze data in SPSS (Version 13.0). Bars with no common superscript are significantly different ($p<0.05$).

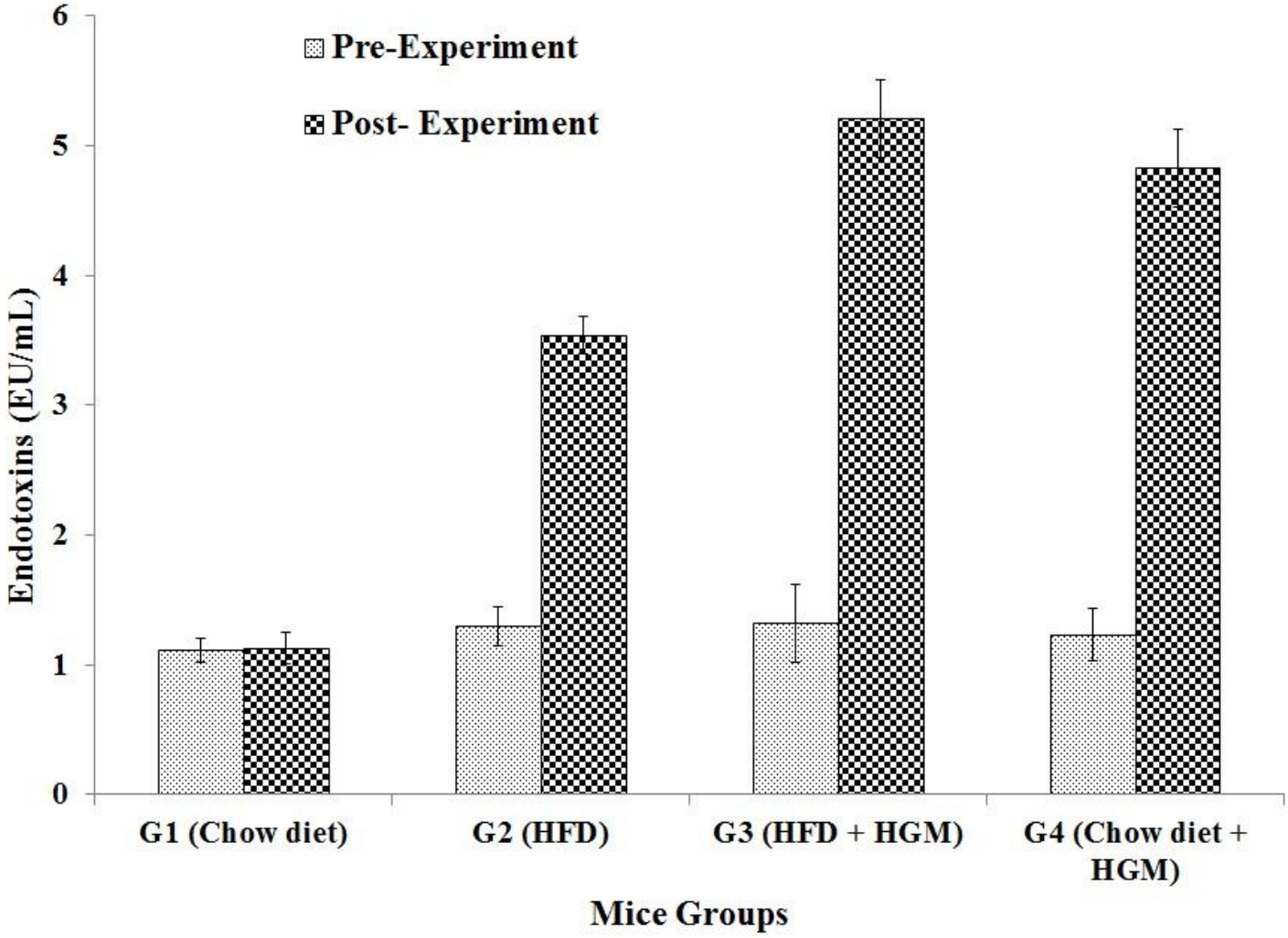

Figure 4

Effect of diet (chow/high fat diet; HFD) alone and combined with human gut microbiota (HGM) on endotoxins level mice (n= 4mice/group). Values are represented as mean ± SEM. One-way analysis of variance (ANOVA) with post hoc Tukey was used to analyze data in SPSS (Version 13.0). Bars with no common superscript are significantly different ($p<0.05$).

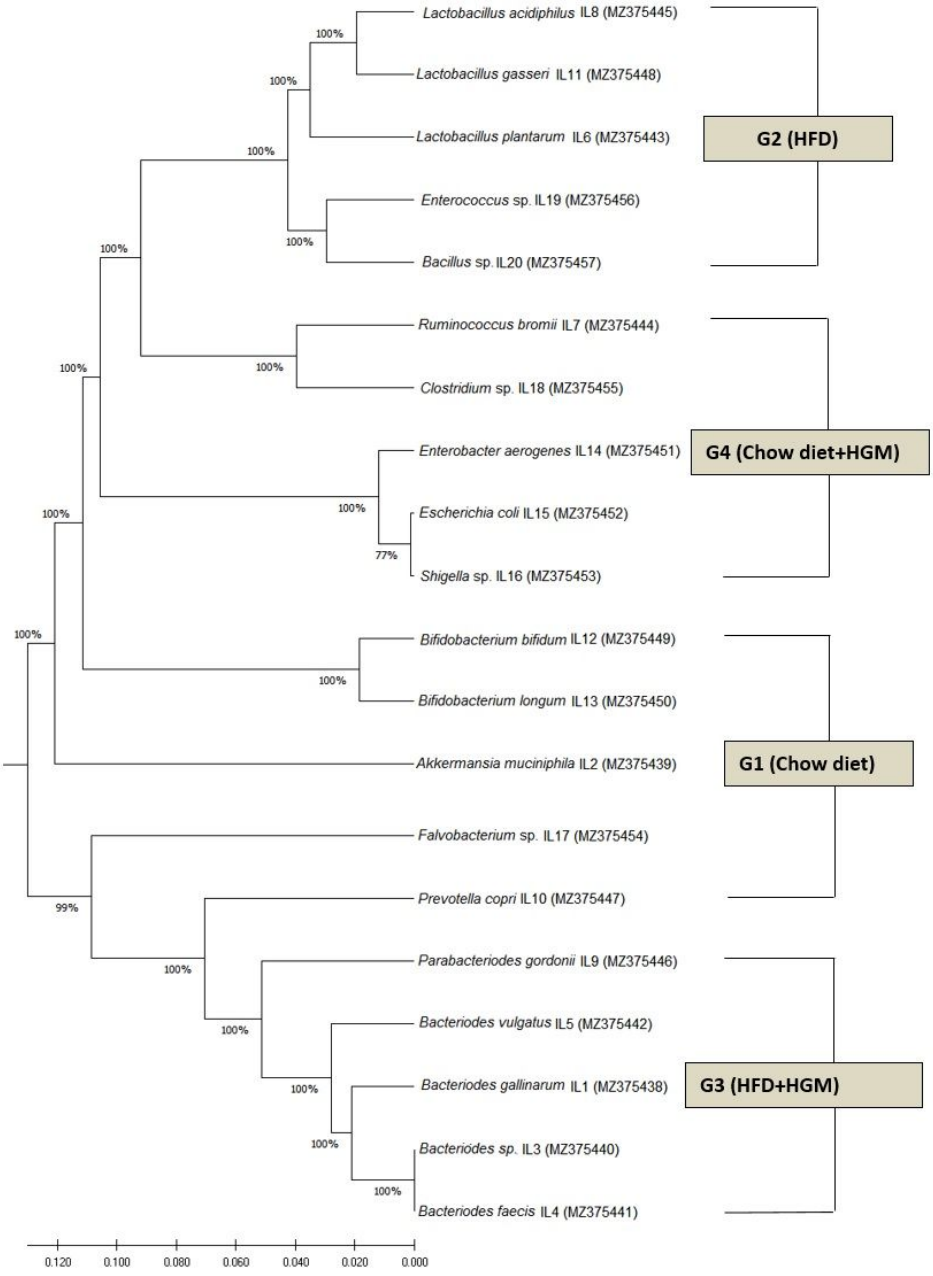

Figure 5

Phylogenetic tree based on 16S rRNA gene sequence showed genetic variability among 20 mice cecal strains. Mice of groups G1 (Chow diet) showed a more diversified microbiota belonging to phyla Actinobacteria, Verrucomicobia and Bacteriodetes compared to G2 (HFD) where all five isolates belonged to phylum Firmicutes. Gut microbiota of G3 (HFD + HGM), showed five isolates belonging to phylum Bacteriodetes, whereas in mouse group G4 (Chow diet + HGM), among five isolates, two strains belonged to Firmicutes and three strains belonged to Proteobacteria.

Supplementary Files

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

- TableS1.docx