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Original Research

Bacillus coagulans TCI711 Supplementation Improved Nonalcoholic Fatty Liver by Modulating Gut Microbiota: A Randomized, Placebo-Controlled, Clinical Trial

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A B S T R A C T

Background: Nonalcoholic fatty liver disease (NAFLD) has become one of the major problems of chronic liver disease worldwide. It not only causes damage to the liver but also engenders chronic hepatitis and cirrhosis. Recent studies have shown that regulating *Bacillus coagulans* can improve NAFLD.
Objectives: This trial explores whether *B. coagulans* TCI711 (BCT) could ameliorate NAFLD.
Methods: A total of 57 patients with NAFLD were recruited through FibroScan liver fibrosis scanner and divided into placebo ($n = 28$) and BCT-supplemented groups ($n = 29$). Specifically, 1 BCT probiotic capsule was supplemented daily for 8 wk. Furthermore, the blood, stool, and fatty liver content were then examined.
Results: Parameters evaluated for liver and kidney indicators showed no side effects after supplementing BCT. A significant reduction of 8.7% in the fatty liver was achieved by effectively suppressing the grade of fatty liver as revealed by controlled attenuation parameter. BCT also regulated gut microbiota profiles, with significant increases observed in *Bifidobacterium*, *Eubacterium*, *Ruminococcaceae*, and *Sellimonas* compared with the baseline.
Conclusions: BCT may improve NAFLD by regulating gut microbiota, and parameters evaluated for liver and kidney indicate no side effects.

Keywords: *Bacillus coagulans*, controlled attenuation parameter, fatty liver, gut microbiota, nonalcoholic fatty liver disease.

Introduction

Nonalcoholic fatty liver disease (NAFLD), commonly referred to as fatty liver, is currently the most prevalent chronic liver condition worldwide [1]. Its incidence ranges between 15% and 30% in Western countries, whereas in regions such as Taiwan, Southeast Asia, Korea, Japan, and China, it is steadily increasing and estimated to reach between 15% and 45% [1]. A normal liver contains ~3%–5% of its weight in fat. When the fat content exceeds 5%, the histology shows >30% of hepatocytes exhibiting fat deposition, which is termed as "fatty liver" [2]. Various causes contributes to the development of NAFLD, including viral hepatitis, obesity, hyperlipidemia, diabetes, hypertension, medication usage, and endocrine disorders [3]. To date, there are no specific medications to address NAFLD. The primary approach involves identifying the underlying etiology of NAFLD and administering targeted treatments, such as specific lipid-lowering agents, antidiabetic medications, or vitamin E, all

Abbreviations: BCT, *B. coagulans* TCI711; CAP, controlled attenuation parameter; CRE, creatinine; GOT, Glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HbA1c, glycated hemoglobin; HFD, high-fat diet; HS, hepatic steatosis; NAFLD, nonalcoholic fatty liver disease; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B; PCR, polymerase chain reaction; TC, total cholesterol; TE, transient elastography; TG, triglycerides.

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of which have shown efficacy in improving NAFLD and liver function [4]. Drug therapy aims mainly to prevent further damage to hepatocytes and inhibit factors contributing to NAFLD. Some studies suggest that dietary adjustments are crucial in managing NAFLD [5,6]. Limiting the total energy (calorie) intake to 30% is recommended for the dietary strategy concerning NAFLD. This implies a daily reduction of ~500–1000 calories [7]. Some studies suggest that the Mediterranean diet, which is plant-based and rich in olive oil, nuts, fruits, vegetables, and fish containing monounsaturated fatty acids, can reduce liver fat, inflammation, diabetes, and even cardiovascular diseases [8]. The study indicates that certain daily habits such as snacks, sugary beverages, processed meats, saturated fats, and foods with added sugars (especially fructose) can worsen NAFLD [9]. Therefore, their regular consumption should be minimized.

The gut microbiota plays a vital role in the occurrence and progression of NAFLD [10]. The intestinal tract is the largest digestive organ of the human body and is called the second brain of the human body. As the intestine contains a variety of digestive juices, it is the major site for the absorption of various dietary nutrients. Besides, gut microbiota is important in maintaining healthy homeostasis in the intestine [11]. In recent years, mounting research has focused on the gut-liver axis and found that the intestinal mucosal barrier, gut microbiota, and its metabolites are closely related to NAFLD pathogenesis [12]. Therefore, appropriate supplementation of specific probiotics can affect the liver's metabolic function and slow down NAFLD severity [13].

Bacillus coagulans, also known as *Lactobacillus sporogenes*, are beneficial bacteria commonly called a probiotic, *B. coagulans*. It is a Gram-positive, spore-forming bacterium capable of surviving harsh conditions, such as those found in the stomach, allowing it to reach the intestines in a viable state [14]. *B. coagulans* has been known as a probiotic with various activities, such as modulating microbiota by increasing lactic acid bacteria, decreasing coliforms, and regulating the host immune system [15]. *B. coagulans* could ameliorate bile acid metabolic dysfunction and NAFLD in rats fed a cholic acid-supplemented diet [16]. Although a few clinical studies have revealed an improving effect of *B. coagulans* TCI711 (BCT) on NAFLD, it deserves further extensive research. Therefore, in this study, BCT was isolated from apple pomace to explore whether it can improve NAFLD.

Methods

Isolation and identification of BCT strain

The plate (man-rogosa-sharpe + 0.5% cystine) was directly coated with diluted fresh juice from an apple pomace, and cultured in an anaerobic environment of 37°C for 2 d. The colonies on the plate were then selected for streaking. A single colony was stored on a solid medium as a source of colony polymerase chain reaction (PCR), and lactic acid bacteria 16S and yeast internal transcribed spacer were amplified by a colony PCR. The PCR products were analyzed and compared by National Center for Biotechnology Information basic local alignment search tool, and the strain was identified and confirmed to be *Weizmannia coagulans* TCI711 (formerly *Bacillus coagulans* TCI711).

The manufacturing process of BCT strain

All test materials were provided by TCI Co., Ltd. The culture medium was prepared with specific ingredients, including yeast peptone, glucose, $MgSO_4$-$7H_2O$, NaCl, KH_2PO_4, K_2HPO_4, manganese gluconate, and water, which was sterilized at 121°C for 30 min. After sterilization, the medium was cooled to 37°C. Inoculation with TCI711 seed culture initiates fermentation, with carefully controlled pH and dissolved oxygen levels throughout. The fermentation process was scaled up successively, from a small bioreactor to a 750 L bioreactor, and the optical density was monitored until it reached the desired value. The biomass was mixed with a premixed excipient consisting of skim milk powder, lactose, and trehalose at 50°C. The mixture was cooled to 4°C before mixing with the biomass while coating/encapsulation. The coated/encapsulated biomass underwent lyophilization, freezing at −40°C, and subsequent sublimation of water. After grinding into a fine powder, the material was sieved and vacuum-sealed in polyethylene packages. The final product was stored at −18°C to maintain stability and efficacy.

Clinical trial design

The clinical trial adopted a randomized, double-blind, placebo-controlled design. The study was approved by the Taipei Medical University Institutional Review Board (N202208021), and was registered on ClinicalTrials.gov Identifier (NCT05635474). This study recruited 57 subjects with NAFLD. All subjects signed a subject consent form. The inclusion criteria were as follows: *1*) aged between 20 and 75 y old, with no history of hepatitis B, hepatitis C, cirrhosis, liver fibrosis, or weight-loss surgery; *2*) controlled attenuation parameter (CAP) for liver steatosis ≥220 dB/m; *3*) no antibiotic usage within the past 3 mo; *4*) no participation in any experiments or research projects within the past 3 mo; *5*) no consumption of any nutritional supplements during the study period; *6*) maintaining regular dietary habits and avoiding alcohol intake throughout the study. The exclusion criteria included: *1*) subjects known to be allergic to probiotics, drugs, or food, or have difficulty in digestive tract absorption or disorders; *2*) pregnant and breastfeeding. Subjects were divided into a BCT (n = 29) and placebo group (n = 28). The subjects were informed to consume 1 capsule every day for 8 wk. Blood, stool, and liver assessments were performed at weeks 0, 4, and 8, for each subject.

Preparation of test sample

The BCT capsule included *Bacillus coagulans* (100 mg, 1.66×10^9 CFU/g), maltodextrin, magnesium stearate, and silicon dioxide. The placebo capsule included maltodextrin, magnesium stearate, and silicon dioxide. The mass was the same between these groups.

Blood examination

The collected blood was centrifuged in a refrigerated centrifuge (Hettich Zentrifugen, Universal 320R) at 1300 g and 4°C for 20 min to separate the upper plasma from the lower blood cells. The plasma was then divided into microcentrifuge tubes and stored for glycated hemoglobin (HbAlc), creatinine (CRE), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), triglyceride (TG), total cholesterol (TC), HDL cholesterol concentrations, and LDL cholesterol.

FibroScan examination

The CAP score was used to measure and quantify hepatic steatosis (HS). The CAP (dB/m) determines the degree of ultrasound attenuation because of hepatic fat at the standardized frequency of 3.5 MHz through a vibration-controlled elastography [17]. This procedure was implemented on FibroScan® (Echosens) and was recorded for each subject. We used conventional probe M, and subjects were fasted for 2 h before testing. Following the recommended cut-off values, NAFLD was classified as absent (<248), mild (248–267), moderate (268–279), and severe (≥280) [18]. The F score categorizes the degree of liver fibrosis in patients with NAFLD into 4 levels, that is, F0–F1 = 0–6.9: nonadvanced fibrosis, F2 = 7–8.5: between nonadvanced and advanced fibrosis, F3–F4 = 8.51–13.99: advanced fibrosis, and F4 > 14: cirrhosis [19]. The CAP system classifies HS into 4 grades: S0 (no HS:liver fat content <10%, CAP < 220 dB/m), S1 (mild HS: liver fat content = 10%–33%, CAP = 220–230 dB/m), S2 (moderate HS: liver fat content = 33%–66%, CAP = 230–290 dB/m), and S3 (severe HS: liver fat content >66%, CAP = 290–400 dB/m) [20].

Fecal sample collection and gut microbiome analysis

The subjects collected fecal samples at home using a validated protocol by BIOTOOLS Co., LTD [21]. The preservation solution in the collection tubes can ensure that the stool specimens are stored at room temperature within 1 mo after collection without affecting the quality of the specimens. Subjects returned the stool samples within 1 wk after the test which was stored at −20°C until sent to BIOTOOLS Co., Ltd for inspection. Total genomic deoxyribonucleic acid (DNA) from samples was extracted using the column-based method (for example QIAamp PowerFecal DNA Kit, Qiagen). DNA concentration was determined and adjusted to 5 ng/uL for the following process. For the 16S rRNA gene sequencing, the V3–V4 region was amplified by a specific primer set (F: 5′-CCTACGGGNGGCWGCAG-3′, R: 5′-GACTACHVGGGTAT CTAATCC-3′) according to the 16S Metagenomic Sequencing Library Preparation procedure (Illumina). In brief, 12.5 ng of gDNA was used for the PCR reaction carried out with KAPA HiFi HotStart ReadyMix (Roche) under the PCR condition: 95°C for 3 min; 25 cycles of: 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; 72°C for 5 min, and hold at 4°C. The PCR products were monitored on 1.5% agarose gel. Samples with bright main strip around 500 bp were chosen and purified using the AMPure XP beads for the following library preparation. The Sequencing library was prepared according to the 16S Metagenomic Sequencing Library Preparation procedure (Illumina). In brief, a secondary PCR was performed using the 16S rRNA V3-V4 region PCR amplicon and Nextera XT Index Kit with dual indices and Illumina sequencing adapters (Illumina). The indexed PCR product quality was assessed on the Qubit 4.0 Fluorometer (Thermo Scientific) and Qsep100TM system. An equal amount of the indexed PCR product was mixed to generate the sequencing library. Finally, the library was sequenced on an Illumina MiSeq platform, and paired 300-bp reads were generated.

Statistical analysis

The data were assessed for non-normal distribution using the Kolmogorov–Smirnov test. Therefore, nonparametric statistical analysis methods were employed. The comparison of measurement results for parameters among groups and between groups was analyzed by Kruskal–Wallis H test, Mann–Whitney U test, Wilcoxon rank-sum test, and chi-square test by IBM SPSS 26.0, as $P < 0.05$ was considered statistical significance.

Results

Safety assessment of BCT supplementation

Table 1 shows the basic information of the subjects. Table 2 shows the basic information of the subjects at weeks 4 and 8 of the trial. The number of females was greater than that of males in the placebo group; the body weight and BMI were slightly higher than those of the TCI711 group. Table 3 reveals no significant differences in the functional indicators of liver (GOT and GPT), renal (CRE), and lipid profiles (TG, TC, and LDL-C), as well as HbA1c after the TCI711 administration. This suggested that parameters evaluated for liver and kidney indicators show no side effects of TCI711.

BCT can effectively improve NAFLD

The FibroScan liver fibrosis scanneroffers a rapid and noninvasive means to detect the presence of fatty liver and liver fibrosis. Among these, the CAP for liver steatosis is a crucial quantitative indicator for assessing fatty liver content [22]. The results revealed that after 8 wk of administering the TCI711, there was a significant reduction of 5.2% in the fatty liver content (Figure 1A). Transient elastography (TE) studies using the standard M probe encountered a high rate of TE failure between 5% and 22% in obese patients with high BMI (>30 kg/m^2) [23]. After excluding severely obese subjects, it was observed that subjects with a BMI <30 had a significant reduction of 8.7% in fatty liver in TCI711 group when compared with placebo (Figure 1B). Fatty liver content can be graded according to the CAP indicating fatty liver degeneration. The higher the grade,

TABLE 1
Basic information of subjects

Variable	BCT group (n =29)	Placebo group (n =28)
Sex (male)	15	8
Sex (female)	14	20
Age (y)	47.4 ± 12.5	51.2 ± 11.8
Height (cm)	166.1 ± 9.4	162.9 ± 9.1
Weight (kg)	73.0 ± 14.7	76.5 ± 16.9
BMI (kg/m^2)	26.2 ± 3.7	29.2 ± 6.1
Systolic pressure (mmHg)	132.9 ± 14.4	134.0 ± 19.1
Diastolic pressure (mmHg)	78.5 ± 10.4	79.8 ± 11.3
Pulse (bpm)	76.7 ± 11.8	78.6 ± 10.2
PAC-SYM (score)	6.2 ± 8.2	5.6 ± 3.8
Energy (kcal)	1814.5 ± 456.7	1779.3 ± 431.0
Carbohydrate (g)	199.6 ± 48.8	200.9 ± 59.9
Fat (g)	76.4 ± 24.8	75.8 ± 18.0
Protein (g)	68.5 ± 17.6	69.9 ± 12.9
Dietary fiber (g)	5.2 ± 4.8	4.4 ± 2.6
Exercise		
None	19	18
<150 min/wk	5	6
≥150 min/wk	5	4

The data are presented as the mean ± SD.
Abbreviations: BCT, *Bacillus coagulans* TCI711; PAC-SYM, patient assessment of constipation-symptom.

TABLE 2
Basic information of subjects during the trial at 4 and 8 wk

Variable	BCT group		Placebo group	
	Week 4	Week 8	Week 4	Week 8
Body weight (kg)	72.8 ± 14.4	72.7 ± 14.4	76.3 ± 17.1	76.3 ± 17.2
BMI (kg/m^2)	26.2 ± 3.5	26.2 ± 3.6	29.0 ± 6.0	28.9 ± 6.0
Systolic pressure (mmHg)	130.3 ± 11.2	132.8 ± 11.7	132.7 ± 20.7	130.8 ± 20.2
Diastolic pressure (mmHg)	79.2 ± 9.9	80.7 ± 9.7	79.1 ± 13.9	79.5 ± 15.1
Pulse (bpm)	80.1 ± 10.4	78.1 ± 12.8	80.0 ± 10.5	78.6 ± 8.8
PAC-SYM (score)	4.3 ± 6.6	5.5 ± 7.0	5.0 ± 5.3	5.1 ± 5.3
Energy (kcal)	1800 ± 398.4	1775.5 ± 418.8	1767.0 ± 354.2	1744.6 ± 331.0
Carbohydrate (g)	199.8 ± 43.1	194.4 ± 50.6	198.7 ± 51.3	197.4 ± 45.1
Fat (g)	76.0 ± 24.0	71.6 ± 22.1	74.4 ± 15.8	74.0 ± 20.4
Protein (g)	69.6 ± 14.4	70.3 ± 17.5	71.8 ± 12.7	70.8 ± 18.6
Dietary fiber (g)	5.4 ± 4.4	5.1 ± 4.6	4.7 ± 2.3	4.7 ± 2.7
Exercise				
None	19	19	18	18
<150 min/wk	5	6	6	7
>150 min/wk	5	4	4	3
MET levels				
Category 0	18	18	17	20
Category 1	7	8	4	5
Category 2	4	2	6	2
Category 3	0	1	1	1

The data are presented as the mean ± SD.
Abbreviations: BCT, *Bacillus coagulans* TCI711; MET, metabolic equivalent task; PAC-SYM, patient assessment of constipation-symptom.

TABLE 3
Blood biochemical indices after supplementation of BCT

Variable	BCT group		Placebo group	
	Week 0	Week 8	Week 0	Week 8
GOT (U/l)	20.3 ± 7.0	20.0 ± 6.9	22.3 ± 8.6	21.4 ± 8.3
GPT (U/l)	26.4 ± 15.9	25.5 ± 15.5	30.3 ± 18.4	28.6 ± 17.5
CRE (mg/dL)	0.76 ± 0.17	0.75 ± 0.18	0.72 ± 0.18	0.72 ± 0.16
TG (mg/dL)	151.8 ± 107.3	138.0 ± 97.6	137.1 ± 66.6	139.0 ± 66.7
TC (mg/dL)	179.9 ± 35.7	183.8 ± 37.5	193.6 ± 35.1	196.6 ± 36.6
HDL-C (mg/dL)	49.1 ± 12.6	52.7 ± 15.0*	48.7 ± 8.6	51.5 ± 10.8*
LDL-C (mg/dL)	100.4 ± 34.6	103.4 ± 34.1	117.4 ± 34.1	117.4 ± 34.9
HbA1c (%)	5.8 ± 0.7	5.9 ± 0.8	5.8 ± 0.5	5.9 ± 0.5

The data are presented as the mean ± SD.
Abbreviations: BCT, *Bacillus coagulans* TCI711; CRE, creatinine; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triglyceride.
* Significant ($P < 0.05$) differences from week 0.

the greater the fat content in the liver. Table 4 shows that after 8 wk of TCI711 supplementation, a significant inhibition in fatty liver was observed in the terms of reduced F score and CAP. Table 5 shows that 6 subjects transitioned from severe to moderate grade, and 1 subject shifted from mild to absent in the TCI711 group, implying its fatty liver reduction potential.

BCT-regulated gut microbiota

Next, we explored whether BCT can improve fatty liver by regulating gut microbiota profiles. Specifically, after 8 wk of BCT supplementation, subjects' feces were collected and analyzed by next-generation sequencing for intestinal microbial distribution. The *Bifidobacterium* (Figure 2A) and *Eubacterium* (Figure 2B) were significantly increased when compared with baseline (week 0). The abundance of *Ruminococcaceae* is negatively correlated with the levels of liver transaminases (GLT and GPT) and the extent of HS and inflammation. *Sellimonas* is a potential biomarker of intestinal homeostasis [24]. The *Ruminococcaceae* (Figure 2C) and *Sellimonas* (Figure 2D) were significantly increased in comparison to baseline (week 0). These results indicate that BCT supplementation may modulate intestinal microbial distribution.

Discussion

This study showed that BCT may improve NAFLD by regulating intestinal flora, and the parameters evaluated showed no adverse effects. To the best of our knowledge, this is also the first trial to simultaneously use the FibroScan liver fibrosis scanner to distinguish the degree of fatty liver and analyze gut microbiota through fecal collection. The current NAFLD treatment strategies such as supplements or drugs focused on reducing metabolic risk factors, with lifestyle modification techniques [25]. The gut microbiota is an endogenous factor contributing to the development of NAFLD [26]. The NAFLD may be associated with small intestinal bacterial overgrowth, which induces liver damage by producing gut-derived

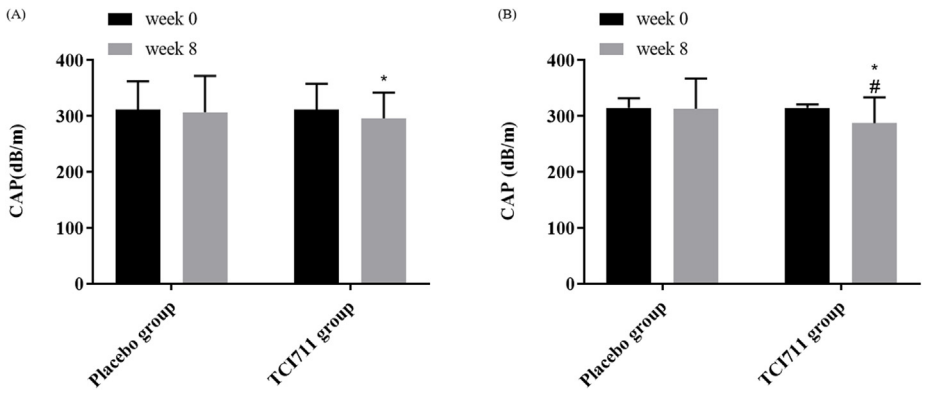

FIGURE 1. *B. coagulans* TCI711 decreased the fatty liver content. (A) The subjects ($n = 57$) were supplemented with 1 *B. coagulans* TCI711 capsule or placebo daily for 8 wk, thereafter a FibroScan liver fibrosis scanner was used to analyze the fatty liver. (B) Further excluding 14 subjects with a BMI ≥30 kg/m^2, the remaining subjects ($n = 43$) received 1 B. coagulans TCI711 capsule or placebo daily for 8 wk. *, compared with baseline (week 0) ($P < 0.05$). #, compared with the placebo group ($P < 0.05$).

TABLE 4
Effect of BCT on liver fibrosis

Variable	BCT group		Placebo group	
	Week 0	Week 8	Week 0	Week 8
F score (Kpa)	4.6 ± 1.0	4.5 ± 0.9	5.0 ± 2.0	5.4 ± 2.2
CAP (dB/m)	312.2 ± 46.3	296.1 ± 46.5*	311.6 ± 51.6	307.0 ± 65.3

The data are presented as the mean ± SD.
Abbreviations: BCT: *Bacillus coagulans* TCI711; CAP, controlled attenuation parameter; F score, Fibrosis score.
* Significant ($P < 0.05$) differences from week 0.

TABLE 5
Effect of BCT supplementation on the fatty liver grade

Steatosis grade	BCT group		Placebo group	
	Week 0	Week 8	Week 0	Week 8
S0	0	1	0	0
S1	2	1	0	1
S2	2	8	8	7
S3	17	11	14	14

Abbreviations: BCT: *Bacillus coagulans* TCI711; S0, no steatosis (<10% fat); S1, mild steatosis (10%–33% fat); S2, moderate steatosis (34%–66% fat); S3, severe steatosis (>67% fat).

lipopolysaccharides and tumor necrosis factor-alpha (TNF-α) [27,28], leading to increased endotoxin absorption and thereby compromising the intestinal barrier integrity [29]. Therefore, the regulation of gut microbiota appears to be a novel therapeutic approach for treating NAFLD [30].

Probiotics can regulate the gut microbiota and promote intestinal mucosal barrier function and mucosal recovery [31]. The gut and the liver are closely associated, forming the gut-liver axis [32]. The interplay in this axis depends on 2 factors, that is, an intact intestine and a liver that can handle immunologic responses and the metabolism of endogenous and exogenous compounds [33]. *B. coagulans* is via natural food sources. It is present in fermented foods such as sauerkraut, kimchi, and yogurt [14]. At present, many kinds of *B. coagulans* containing functional foods such as pasta, chocolate, and ice cream are available in the market. This is attributed to the spore-forming nature of *B. coagulans* guarantees in offering stability and vitality in functional food, when compared with other probiotic bacterial strains [34]. A 4-wk study using a mixture of 3 probiotics (*Streptococcus, Bifidobacterium, and Lactobacillus*) in ob/ob mice showed reductions in fatty liver content and inflammation via downregulating nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) activity [33,35]. Oral supplementation of *bifidobacteria* in high-fat diet (HFD)-induced obese mice can also prevent NAFLD and obesity, thereby improving metabolic health [36,37]. In a clinical trial, oral synbiotic (combination of pro/prebiotics) administered to patients with NAFLD for 2–3 mo, improved liver enzyme levels, TNF-α, and oxidative stress markers [38]. The oral administration of *B. longum* with fructo-oligosaccharides, combined with lifestyle modification, ameliorated the serum profile of aspartate aminotransferase, LDL cholesterol, TNF-α, and endotoxins while improving insulin resistance, steatosis, and NASH activity index [39]. In NAFLD rats, supplementation of *B. coagulans* for 8 wk reduced liver and blood glucose and TG levels [40].

Furthermore, *B. coagulans* significantly inhibited oxidative stress markers in liver tissue [41]. Bacillus-treated HFD-fed mice also exhibited significantly suppressed chronic inflammation in the liver and skeletal muscle, which was observed to be associated with reduced HFD-induced intestinal permeability and enhanced adiponectin production [42]. In addition, *Bacillus* treatment significantly reversed HFD-induced HS [42]. A study showed that the *B. coagulans* supplementation in patients with NAFLD for 12 wk significantly reduced TNF-α and NF-κB activity [16]. Consistent

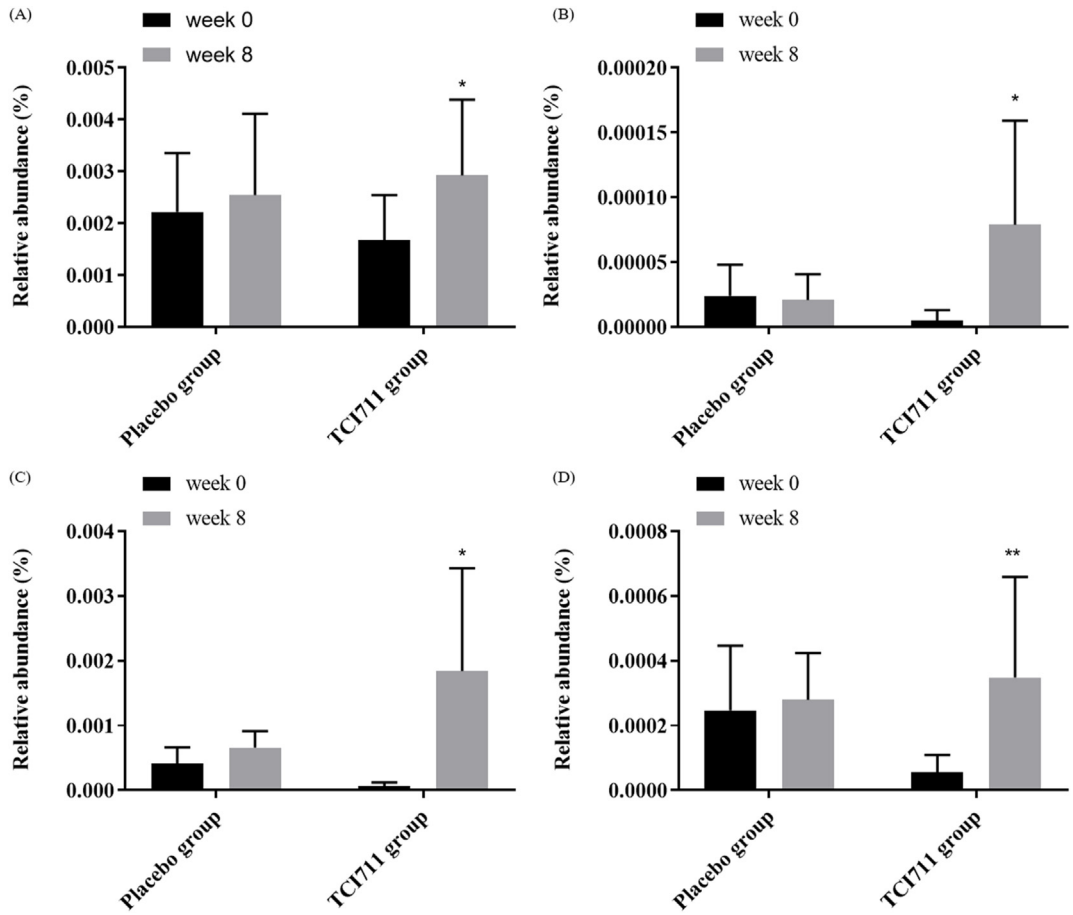

FIGURE 2. *B. coagulans* TCI711 regulated gut microbiota. The subjects ($n = 43$) received 1 *B. coagulans* TCI711 capsule or placebo daily for 8 wk. Thereafter, a subject's feces was collected and analyzed by next-generation sequencing for the relative abundance (%) of (A) *Bifidobacterium*, (B) *Eubacterium*, (C) *Ruminococcaceae*, and (D) *Sellimonas*. *, compared with baseline (week 0) ($P < 0.05$, **$P < 0.01$). $^{\#}$, compared with the placebo group ($P < 0.05$).

with our results, subjects supplemented with BCT for 8 wk may be able to reduce fatty liver. We also found that the increased relative abundance of *Bifidobacterium*, *Eubacterium*, *Ruminococcaceae*, and *Sellimonas* after TCI711 supplementation. *Bifidobacterium* mainly reduced liver lipid metabolism and intestinal permeability by increasing the content of propionic acid and butyric acid in feces and ultimately inhibited liver inflammation and fat accumulation to alleviate NAFLD [43]. *Eubacterium* regulates liver inflammation, and *Sellimonas* is an important microbe regarding intestinal metabolic balance because of its ability to utilize glucose and the fermentation intermediates acetate and lactate to form butyrate and hydrogen [44]. The abundance of bacteria from the *Ruminococcaceae* family seems negatively associated with hepatic markers including liver weight, serum transaminase levels, HS, and inflammation degree [45]. However, the detailed underlying therapeutic mechanism of BCT in regulating intestinal flora needs to be explored. It is currently speculated that BCT may affect the intestinal flora metabolites short-chain fatty acids, such as acetic acid (acetic acid), propionic acid, and butyric acid, to improve the fat liver [46]. Apart from various strengths, our study also includes a few limitations. Exercise increases fatty acid metabolism, thereby preventing mitochondrial and hepatocellular damage. Therefore, subject conditions should exclude exercise. Furthermore, the number of females in the placebo group is more than twice that of men, leading to a bias in the BMI criterion between the 2 groups. Because men generally have a higher muscle mass, this can artificially elevate the BMI values in the intervention group, potentially distorting the observed effect of the intervention. Exclusion criteria did not address the consumption of alcohol before the trial. Cessation of alcohol consumption, as required for eligibility in the study, might have more impact or less impact on NALFD abatement depending upon a patient's history of alcohol consumption.

In conclusion, BCT may indirectly improve NAFLD by regulating gut microbiota. Our gut microbiota profile results indicate that some identified beneficial bacteria could be developed into pharmaceuticals or health products, such as probiotics, postbiotics (metabolites of probiotics), and prebiotics (food for probiotics).

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Author contributions

The authors' responsibilities were as follows – CF-C, M-TY: designed the study; W-YL, M-TY: reviewed the design; Y-JC,

W-YL, M-TY: collected data; Y-KL, Y-HL, M-TY: analyzed the data; C-FC: prepared the first draft of the manuscript; C-FC, M-TY: contributed to manuscript writing; M-TY, R-HH: finalized the manuscript; and all authors: read and approved the final manuscript.

Conflict of interest

M-TY reports that financial support was provided by TCI Co., Ltd. Y-HL and C-FC report a relationship with TCI Co., Ltd. that includes funding grants. The other authors declared no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Data availability

Data described in the manuscript, code book, and analytic code will not be made available because approval has not been granted by participants.

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