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Potential Prebiotic and Anti-Obesity Effects of Codium fragile Extract

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Abstract: Polysaccharides from marine algae exhibit beneficial biological activities. In this study, we examined the effect of Codium fragile extract (CFE) on prebiotic and anti-obesity activity through in vitro experiments. CFE increases the growth of specific beneficial microbial populations with concomitant decrease in pathogenic microbes. Further, total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity (DPPH activity) after fermentation with CFE as the carbon source were higher than for glucose as the control. Moreover, CFE inhibited adipocyte differentiation by inducing differentiation-related factors when the induction of 3T3-L1 preadipocytes into adipocytes was induced. Therefore, we suggest that CFE can be used as a prebiotic material with an anti-obesity effect for human health.

Keywords: Codium fragile; marine algae; anti-obesity; prebiotics

1. Introduction

Marine algae are important sources of compounds with health-enhancing biological properties [1], including anti-inflammatory, anticancer, and immune enhancing functionalities [2–5]. Marine algae contain large amounts of sulfated polysaccharides that can be used as prebiotics [6]. These polysaccharides are structurally atypical compared to terrestrial glycans and are resistant to gastric acid, gastrointestinal absorption and host digestive enzymes [7]. In addition, enzymatic hydrolysis of seaweed-derived carbohydrates and proteins promotes changes in physicochemical properties and enhances the efficacy of bioactivities such as antioxidant activity [8,9]. Marine algae polysaccharides can be used as prebiotics for gut microbiota, which partially degrade them into other bioactive compounds, such as oligosaccharides and short chain fatty acids (SCFAs) acting as food sources for these organisms and allowing them to proliferate [10–14]. In addition to these polysaccharides, marine algae contain other bioactive compounds, such as polypeptides, polyphenols, and phytochemicals, which can be used as prebiotics. Despite their myriad of biologically active compounds, marine algae are predominantly consumed as food, processed with salt or seasoning. There is, therefore, scope for expanding the development of functional foods which contain various bioactive compounds derived from marine algae.

Codium fragile is a green alga used as a traditional food ingredient in Asia [15]. Green algae produce high amounts of sulfated polysaccharides including sulfated galactans, mannans, xylans, and ulvans. These sulfated polysaccharides are composed of galactose, rhamnose, xylose, glucose, glucuronic acid and sulfates (and more rarely, mannose and arabinose) [6,16]. Sulfated polysaccharides derived from green algae have shown various biological activities, including anti-viral, immune-stimulatory, and anticancer effects [17–19].

Obesity is a disorder involving excessive body fat, which increases the risk of many diseases, including diabetes, high blood pressure, and other cardiovascular complications.
Marine algae consumption is associated with an anti-obesity activity affected by various mechanisms including alterations of lipid metabolism, inhibition of inflammation, suppression of adipocyte differentiation and delay in gastric emptying [5]. Marine algae are rich in dietary fiber, so they can be used as an anti-obesity ingredient. In addition, there are many reports of potential therapeutic effects of marine algae consumption in weight and obesity management [20]. Ulvan from brown algae also lowers triglycerides, total cholesterol, and total lipid levels [21]. The anti-obesity activity of marine algae is associated with bioactive compounds, including polysaccharides, peptides, phlorotannins, and carotenoids [22]. Fucoxanthin is the most well studied marine algae bioactive compound displaying an anti-obesity effect [23]. Adipose tissue is a loose connective tissue composed mostly of adipocytes [24]. As an energy reservoir and endocrine organ, adipose tissue is known to play an important role in the regulation of energy metabolism for the human body through expression and secretion of an array of adipokines [25,26]. Adipocyte differentiation, also called adipogenesis, is the process during which fibroblast-like preadipocytes develop into mature adipocytes [27]. 3T3-L1 cells, originally derived from mouse embryos, have served as a useful in vitro model for adipocyte differentiation and function [28]. Phlorotannin has been shown to strongly inhibit lipid accumulation in 3T3-L1 adipocytes [29], as well as inhibit adipocyte differentiation. Fucoxsterol has been reported to decrease the expression level of the adipocyte marker proteins PPAR and C/EBP. Marine algae may also prevent obesity by modifying relative amounts of different gut bacteria and polysaccharides and have been shown to repair the intestinal barrier and reduce inflammation [30].

In this study, we hypothesized that *C. fragile* extract (CFE) are fermentation substrates for gut microbial populations that convert the CFEs into partially degraded bioactive compounds. A recent study showed that freeze dried *C. fragile* powder extracted with ethanol increases the abundance of SCFAs-producing bacteria, leading to an anti-obesity effect in mice [31]. Until now, studies on bioactive compounds from *C. fragile* have been conducted on samples extracted with toxic and expensive organic solvents, such as ethanol and n-hexane [5,31]. To the best of our knowledge, this is the first report determining the anti-obesity and prebiotic effects by extracting *C. fragile* with water in an environmentally friendly method.

2. Materials and Methods

2.1. Preparation of CFE

*C. fragile* was obtained from a seaweed farm in Wando, Jellanamdo, South Korea in September 2020. After removing salt by washing the *C. fragile* sample with distilled water for 10 min, the sample was dried at 47.5 ± 2.5 °C for 6 h. The dried *C. fragile* sample was ground to a powder. After passing through a 20-mesh, samples were extracted using 20 times the amount of distilled water at 100 °C for 3 h. Then, CFE was concentrated using a vacuum rotary evaporator, freeze-dried and stored at −20 °C for later use.

2.2. Chemical Composition of CFE

The sugar content of the CFE was determined using the phenol-sulphuric acid colorimetric assay [32]. The uronic acid content was quantified using the methods of Cesaretti, Luppi et al. [33]. The sulfate level was determined by hydrolyzing CFE with 1 M HCl for 12 h at 105 °C, followed by performing the Dodgson-Price assay [34]. The monosaccharide composition was determined using high-performance anion exchange chromatography (HPAEC). A Dionex™ CarboPac™ PA1 column (0.4 × 25 cm: Dionex Co., California, CA, USA) was used on a Dionex™ ICS-5000 (Dionex Co., California, CA, USA) for this analysis. The injection volume was 10 µL with a flow rate of 1.0 mL/min at 25 °C. The mobile phase was 18 mM NaOH in water.

2.3. Utilization of CFE by Individual Bacteria Species

Bacterial strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea)
and Chr. Hansen’s collection of dairy cultures (Chr. Hansen A/S, Hoersholm, Denmark). Evaluation of the growth of bacterial strains using CFE as a carbon source was performed according to a previously described method [35]. Briefly, each bacterial strain was grown separately in sugar-free brain heart infusion broth (BHI) containing 1% CFE. Glucose and galacto-oligosaccharide were used as controls [36]. Each bacterial strain was incubated for 24 h. The growth of each bacterial strain was determined by measuring optical density at 600 nm (OD
600
). Cells were harvested by centrifugation at 8000 × g for 10 min and diluted in phosphate-buffered saline (PBS) to an optical density (OD
600
) of approximately 0.5 before 0.2% was transferred for fermentation. After 24 h of incubation at 37 °C, OD
600
 readings were taken using an ELISA reader (BioTek Instruments, Winooski, VT, USA).

2.4. Stimulation of Probiotics Growth

Two probiotic strains, Lactobacillus plantarum subsp. plantarum (Lp) and Lactobacillus helveticus (Lh) were cultured in MRS broth, centrifuged and diluted in PBS to an optical density (OD
600
) of approximately 0.5. A 0.2% amount of the culture of each probiotic strain was added in MRS broth containing 1% CFE. Glucose was used as a control. Fermentations were monitored for 48 h at 37 °C, sampling every 8 h to determine the acidity and viable cell count of Lp and Lh. The acidity was determined by measuring the amount of 0.1 N NaOH required to adjust the pH to 8.2. The viable cell counts of Lp and Lh were determined by counting the colony forming units (CFU) after plating on MRS agar and incubating under aerobic condition at 37 °C for 48 h.

To determine SCFAs concentration, fresh feces samples were obtained from healthy seven-week-old Balb/c male mice that had not been treated with antibiotics. Feces were homogenized with 0.1 M anaerobic phosphate-buffered saline (pH 7.0) using a Bead Ruptor Homogenizer (Omni International, NW Kennesaw, GA, USA) to make 10% (w/v) slurries. Samples with or without 1% CFE were fermented in an anaerobic chamber at 37 °C for 24 h. After fermentation, SCFAs were determined using HPLC analysis. Two shim-pack SCR-102H (300 × 8.0 mm, Shimadzu, Kyoto, Japan) were used in series together with a shim-pack guard column SCR-102H (50 × 6.0 mm, Shimadzu, Kyoto, Japan) on the HPLC system (Shimadzu, Kyoto, Japan). The injection volume was 20 µL with a flow rate of 0.8 mL/min at 40 °C. The mobile phase was 4 mM p-toluenesulfonic acid in water.

Additionally, total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging activity (as determined by a DPPH assay) were determined before and after fermentation. TPC was determined according to the Folin-Ciocalteu procedure [37]. Briefly, samples (200 µL) were mixed with 2% Na
2
CO
3
(2.0 mL) and Folin-Ciocalteu’s reagent (200 µL). After 15 min, the absorbance values at 750 nm were measured using a microplate reader. Gallic acid was used as a standard and TPC was expressed as mg of gallic acid equivalents (GAE)/g of the fermented broth. TFC was estimated using the protocol established by Chang et al. [38]. Briefly, 150 µL of each fermented broth sample was mixed with 280 µL of de-ionized water, 10 µL of 10% aluminum chloride, and 10 µL of 1 M potassium acetate. After incubation for 30 min, the absorbance at 420 nm was measured using a microplate reader. The TFC content was presented as mg of quercetin equivalents (QAE)/g of the fermented broth. The radical scavenging activity of the fermented broth was determined using a DPPH assay [39]. Briefly, the fermented broth was prepared with different concentrations diluted with methanol. A mixture of 20 µL sample and 200 µL of 0.1 mM DPPH-methanol solution was taken and incubated in the dark for 30 min. The absorbance at 517 nm was measured. The fermented broth was replaced with methanol to serve as the negative control, and ascorbic acid was used as a positive control.

2.5. Cell Viability

The 3T3-L1 cells were seeded at 5 × 10³ per well in 96-well plates in culture medium. Cells were incubated with CFE (0.5–6mg/mL) for 24 h at 37 °C in a humidified 5% CO₂ atmosphere. MTT solution (5 mg/mL) was added to each well followed by incubation for
2 h. The medium was removed. 0.1 mL of buffered dimethyl sulfoxide was added to each well, and the plate was shaken to dissolve the formazan. Absorbance at 540 was measured.

### 2.6. Oil Red O staining Assay

An Oil Red O staining assay was used according to the method described by Lee et al. [40]. Briefly, the 3T3-L1 preadipocytes were seeded at $5 \times 10^5$ cells/mL in a 24-well plate and adipocyte differentiation was induced by treatment with CFE (1, 2, and 4 mg/mL). N-Acetyl-cysteine (NAC; 5 mM) was used as a positive control. 3T3-L1 cells were fixed with 3.7% formaldehyde for 30 min and washed with PBS and ethanol. The cells were then stained with Oil Red O in isopropyl alcohol/distilled water for 30 min and washed with PBS and ethanol. Lipid droplets were observed with an Eclipse TE2000-U microscope (Nikon, Tokyo, Japan) and dissolved in isopropyl alcohol containing 4% Nonidet™ P-40 (Sigma-Aldrich, St. Louis, MO, USA) before quantification with a microplate reader (SpectraMax 190PC; Molecular Devices, Sunnyvale, CA, USA) at 510 nm.

### 2.7. Western Blotting

Western Blotting was determined using the methods described by Lee et al. [40]. The 3T3-L1 preadipocytes were seeded at $5 \times 10^5$ cells/mL in a 24-well plate and adipocyte differentiation was induced by treatment with CFE (1, 2, and 4 mg/mL). 3T3-L1 cells were lysed whilst kept on ice for 30 min in RIPA lysis buffer (ForBioKorea, Korea) containing a protease inhibitor cocktail (Sigma-Aldrich). The cells were centrifuged at 13,000 $\times$ g for 30 min at 4 °C to obtain the supernatant. The cell lysates (50 µg) were separated on an 8% SDS polyacrylamide gel and transferred onto nitrocellulose membranes (Whatman, St. Louis, MO, USA). The membrane was blocked for 2 h by treatment with Tris-buffered saline containing 0.1% (v/v) Tween-20 (TBST) and 5% skim milk, after which antibodies against the following were applied at 4 °C overnight: Peroxisome proliferator-activated receptors PPARγ, C/EBPα, Fas, and β-actin (Cell Signaling Technology, Danvers, MA, USA). The blot was probed by anti-rabbit secondary antibodies (Cell Signaling Technology, Danvers, MA, USA) for 2 h at 4 °C. The proteins were detected with SuperSignal® West Pico chemiluminescent substrate (Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the Amersharm Imager 600 (GE Healthcare Life Sciences, Chicago, IL, USA).

### 2.8. Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). Data were analyzed with SPSS 22.0 software by Student’s t-test and ANOVA (nonparametric) analysis for comparison of two groups, followed by the post hoc Tukey’s multiple comparison test for more than two groups.

### 3. Results

#### 3.1. Physiochemical Properties of CFE

The chemical composition of CFE is shown in Table 1. The sugar, uronic acid, and sulfate content are 34.9%, 18.6%, and 11.4%, respectively. Galactose and glucose are major monosaccharides and a minor amount of mannose, arabinose, fucose, rhamnose and xylose are also present.

<table>
<thead>
<tr>
<th>Neutral sugars (%)</th>
<th>Uronic Acid (%)</th>
<th>Sulfate (%)</th>
<th>Monosaccharide Composition (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucose</td>
<td>Rhamnose</td>
<td>Arabinose</td>
<td>Galactose</td>
</tr>
<tr>
<td>34.9 ± 0.1</td>
<td>18.6 ± 0.1</td>
<td>11.4 ± 0.1</td>
<td>5.69 ± 0.10</td>
</tr>
<tr>
<td>5.12 ± 0.18</td>
<td>11.30 ± 0.16</td>
<td>81.08 ± 0.71</td>
<td>77.45 ± 1.71</td>
</tr>
<tr>
<td>28.48 ± 0.66</td>
<td>4.49 ± 0.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD of three independent experiments.

#### 3.2. Utilization of CFE by Individual Bacteria

Culture media containing glucose and galacto-oligosaccharide (GOS) was used as a positive control. CFE was added to culture medium to evaluate the proliferation of
probiotics and food-born pathogenic bacteria compared to the positive control. The growth of nine microbial strains in these media was individually confirmed after 24 h incubation (Table 2). CFE promoted the growth of all probiotic strains, with *B. bifidum* showing the highest amount of growth. CFE also had enhanced prebiotics effects on *Lh* and *Lp* probiotic strains compared to both the glucose and GOS positive controls. By contrast, the growth of the food born pathogenic bacteria was not promoted by CFE.

**Table 2.** Growth (OD600) of probiotics and food-born pathogenic bacteria grown in brain heart infusion (BHI) medium supplemented with CFE.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Absorbance of Culture (OD600)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em> KTCT 3202</td>
<td>1.45 ± 0.05 g</td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em> subsp. <em>lactis</em> Bb-12</td>
<td>1.26 ± 0.07 e</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> ATCC 53103</td>
<td>1.26 ± 0.05 e</td>
</tr>
<tr>
<td><em>Lactobacillus helveticus</em> ATCC 15807</td>
<td>1.18 ± 0.01 d</td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em> subsp. <em>Plantarum</em> ATCC 14917</td>
<td>1.26 ± 0.01 e</td>
</tr>
<tr>
<td><em>Lactobacillus brevis</em> KTCT 3498</td>
<td>1.41 ± 0.01 g</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> ATCC 14579</td>
<td>1.04 ± 0.04 c</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 25922</td>
<td>0.98 ± 0.04 c</td>
</tr>
<tr>
<td><em>Salmonella Typhii</em> ATCC 19430</td>
<td>1.05 ± 0.06 c</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three independent experiments. Sample means with different lower-case letters for the same index are significantly different at *p* < 0.05.

### 3.3. Effect of Fermentation on Cell Viability Count and Acidity of CFE

CFE fermentation was performed, and cell viability count and acidity observed over time. The changes to cell viability during fermentation are shown in Figure 1. The viable cell counts of *Lp* in CFE supplemented media were 9.26 and 7.39 log CFU/mL at 24 and 48 h, respectively. For *Lh*, the viability counts in CFE supplemented medium were 9.35 and 9.41 log CFU/mL at 24 h and were 8.06 and 8.89 log CFU/mL at 48 h, respectively.

![Figure 1](image_url) Changes in cell viability counts of *Lp* (a) and *Lh* (b) with glucose or CFE. Values are expressed as mean ± SD.

The changes in acidity of the CFE fermentation solution are shown in Figure 2. For both *Lp* and *Lh*, in CFE supplemented media, fermentation led to gradual increases in acidity. For these lactic acid bacteria, growth results in lactic acid production, which explains the increase in acidity from 0.2 % to 2.0 % after 48 h fermentation. For *Lp*, the
acidity level was similar between the CFE and glucose-supplemented media, with an increase from 0.2% to 2.0% over the course of fermentation. For \( Lh \), the acidity level was lower after fermentation in CFE-supplemented medium when compared with that of glucose-supplemented medium.

**Figure 2.** Changes in acidity of \( Lp \) (a) and \( Lh \) (b) with glucose or CFE. Values are expressed as mean ± SD.

Table 3 shows SCFAs content after in vitro fermentation of CFE by mouse feces. When CFE was employed as the carbon source, acetic acid concentration was significantly higher than that of the mouse fecal-fermented sample. Butyric acid and propionic acid were not detected.

**Table 3.** SCFAs contents after in vitro fermentation of CFE by mouse feces.

<table>
<thead>
<tr>
<th>Sample</th>
<th>SCFAs (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetic Acid</td>
</tr>
<tr>
<td>Mouse feces</td>
<td>17.52 ± 4.08\textsuperscript{a}</td>
</tr>
<tr>
<td>Mouse feces with CFE</td>
<td>60.38 ± 1.10\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three independent experiments. -, not detected. Sample means with different lower-case letters for the same index are significantly different at \( p < 0.05 \).

Table 4 shows TPC, TFC, and DPPH activity in glucose or CFE supplemented media before and after fermentation by probiotics. Fermentation cultures grown in CFE-supplemented medium showed higher TPC, TFC, and DPPH activity compared to cultures grown in glucose-supplemented medium. After fermentation, TPC, TFC, and DPPH activity had significantly increased compared to non-fermentation.

**Table 4.** TPC, TFC, and DPPH activity of glucose and CFE during fermentation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg QAE/g)</th>
<th>DPPH Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NF</td>
<td>AF</td>
<td>NF</td>
</tr>
<tr>
<td>( Lp ) + glucose</td>
<td>37.57 ± 1.19</td>
<td>42.97 ± 1.35</td>
<td>10.92 ± 1.85</td>
</tr>
<tr>
<td>( Lp ) + CFE</td>
<td>48.14 ± 0.26</td>
<td>67.72 ± 2.35</td>
<td>80.35 ± 1.90</td>
</tr>
<tr>
<td>( Lh ) + glucose</td>
<td>37.75 ± 1.87</td>
<td>60.15 ± 0.54</td>
<td>18.12 ± 4.63</td>
</tr>
<tr>
<td>( Lh ) + CFE</td>
<td>58.50 ± 1.54</td>
<td>81.50 ± 2.45</td>
<td>85.92 ± 6.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three independent experiments. -, not detected. NF = non-fermentation, AF = after fermentation.
3.4. Inhibition of Lipid Accumulation in 3T3-L1 Cells of CFE

To evaluate the cytotoxicity of CFE on 3T3-L1 preadipocytes, we performed an MTT assay. As shown in Figure 3a, CFE (0.5–6 mg/mL) did not affect cell growth; therefore, 1, 2, and 4 mg/mL of CFE were used in further studies.

To evaluate the inhibition effect of CFE on adipocyte differentiation, 3T3-L1 cells were cultured in adipocyte differentiation media at different concentrations of CFE. As shown in Figure 3b, CFE significantly reduced adipocyte differentiation in a dose-dependent manner without inducing cytotoxicity. Lipid droplet accumulation in 3T3-L1 cells treated with 1, 2, and 4 mg/mL CFE decreased to 13.20, 36.20, and 78.81%, respectively, relative to untreated cells.

As shown in Figure 3c, treatment with CFE inhibited PPARγ, C/EBPα, and Fas protein expression during 3T3-L1 differentiation.
Figure 3. CFE inhibits lipid accumulation in 3T3-L1 cells. (a) Confirmation of cell viability on adipocyte differentiation using the MTT assay. (b) Confirmation of the inhibitory effect on adipocyte differentiation using oil red O staining. (c) Confirmation of CFE-induced changes in the expression of protein related to adipocyte differentiation using Western blot analysis. Data are representative of more than three separated experiments and are presented as mean ± SD. Significant differences are noted as * p < 0.05, ** p < 0.01 as compared to differentiated 3T3-L1 cells without CFE treatment. MDI: methylisobutylxanthine, dexamethasone, and insulin, NAC: N-Acetyl-cysteine, ## p < 0.05 as compared to normal cells.

4. Discussion

The cultivated green algae, C. fragile, contains large amounts of structural sulfated polysaccharides. Sulfated galactan from C. fragile consists of a large amount of galactose residues with trace arabinose and the presence of pyruvate and sulfate as substituents [41,42]. In addition, the polysaccharide composed of β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3,6)-β-D-Galp-(1 → 3,4)-Galp-(1 → residues and sulfates are mainly linked at O-4 of 3)-Galp-(1 → and → 3,6)-Galp-(1 → residues [15]. Partial degradation products of sulfated structural polysaccharides, produced during fermentation by the human gut microbiota, have been shown to have bioactivity [6,16,18,19]. Furthermore, the gut microbiome is well known for its role in regulating obesity and other metabolic disease.

CFEs extracted with water comprise many galactose residues with traces of arabinose, and the presence of sulfate as substituents. In accordance with these results, Yuko et al. [15] reported that sulfated polysaccharides isolated from C. fragile were mainly composed of galactose, arabinose, and glucose. Therefore, our results indicate that CFEs belongs to the galactan/arabinan group (or arabinogalactan). Moreover, Afonso et al. [43] reported that the elemental composition and bioaccessibility of marine algae were different depending on pretreatment such as seaweed rehydration. Therefore, further research on the processing of seaweed for use as a functional material is required.

To evaluate the growth effect of CFE on probiotics, CFE was used as the single carbon source. CFE was able to promote growth of all the probiotic bacterial strains used in this study, while there was no increase in growth of pathogenic bacteria. CFE enhanced the growth of Lp and Lh to a greater extent than GOS. These results are consistent with a previ-
ous report that sulfated galactan can promote the proliferation of lactic acid bacteria [44]. Therefore, fermentation involving of \( Lp \) and \( Lh \) with CFE as a substrate was further studied.

The fermentation of CFE with \( Lp \) and \( Lh \) resulted in an increase in acidity, which correlated with the drop in pH. These results suggest that CFE can be employed as carbon source for lactic acid bacteria and affected medium acidity and secondary metabolite production, such as SCFA. The main SCFAs produced during lactic fermentation are acetate, propionate and butyrate [45,46]. SCFAs can also inhibit the invasion and colonization of pathogens by lowering the intestinal pH [47–49]. Gupta et al. [50], reported that accumulation of acids during fermentation can decrease growth rate. CFE fermented with probiotics showed the highest viable cell count with 24 h of fermentation, following which the viable cell count decreased. An inflection point was identified at 24 h of fermentation in CFE supplemented medium and a sigmoidal curve was observed that correlated with a decrease in the substrate and adaptation of the cells to the second carbohydrate. Compared with the glucose supplemented medium, the growth and cell viability of \( Lp \) and \( Lh \) fermented in CFE supplemented medium was similar up to 24 h, but then decreased until 48 h.

The prebiotic potential of polysaccharides and SCFAs from algae has mainly been studied [7,51,52]. There is scope to determine the prebiotic potential of other phytochemical components, such as polyphenols and carotenoids [53]. Fermentation via lactic acid bacteria may convert various phytochemicals to more bioactive aglycone forms. Ye et al. [54] reported that lactic acid bacteria fermentation significantly increased the level of TPC in vegetables. In the fermentation of CFE by mouse feces, when feces containing CFE were fermented, acetate acid production was about 3.4 times higher than that of control fermented with mouse feces only. TPC, TFC, and DPH activity after fermentation with CFE as the carbon source were higher than in the non-fermentation control. Huang et al. [55] reported that antioxidant activity was increased by promoting digestion of marine algae, mimicking the gastrointestinal and upper intestinal environment. Therefore, the findings of this study may be similar in the human digestive environment, and further studies are needed. Fermentation disrupts the marine algal cell wall, resulting in increased polyphenol content in the fermented samples. Therefore, could be possible to utilize CFE, not only as a prebiotic material, but also as a bioactive material for health promotion by increasing antioxidant activity during fermentation.

Everard et al. [56] reported that prebiotics improve glucose homeostasis and leptin sensitivity; furthermore, enteronocrine cell activity is improved by modulating specific gut microbiota in obesity and diabetes. Therefore, indigestible polysaccharides derived from \( C. \ fragile \) can be used not only as a probiotics, but also as excellent anti-obesity functional materials. No cytotoxicity of CFE was found in 3T3-L1 at any concentration. The morphological characteristics of cells and stained adipocytes were quantitatively evaluated using Oil Red O staining. In this study, it was confirmed that the number and size of adipocytes and the degree of Oil Red O staining decreased according to the concentration of CFE. Using Western blotting, CFE was also found to block expression of PPAR\( \gamma \), C/EBP\( \alpha \), and Fas during 3T3-L1 differentiation. Adipogenesis is regulated by transcriptional activators, such as PPAR\( \gamma \), C/EBP\( \alpha \), and Fas [57]. PPAR\( \gamma \) activation can enhance C/EBP\( \alpha \) activity, regulate differentiation, promote adipogenesis and lipogenesis [58], and increase body weight gain and hepatic TG levels in mice [59]. The differentiation of preadipocytes into adipocytes involves exposure of a confluent, quiescent population of cells to a variety of effectors that activate a cascade of transcription factors. This cascade begins with the CCAAT/enhancer-binding protein (C/EBP) \( \beta \) and C/EBP\( \delta \), which induces the expression of C/EBP\( \alpha \) and the peroxisome proliferator-activated receptor (PPAR) \( \gamma \) [60,61]. Furthermore, there are studies showing that the expression and/or activity of lipogenic enzymes, such as fatty acid synthase (FAS) and lipid droplet-associated proteins such as perilipin A, are required for adipocyte differentiation [62–64]. These transcription factors coordinate the expression of genes involved in creating and maintaining the adipocyte phenotype, including the
genes for adipocyte fatty acid binding protein, glucose transporter 4, lipoprotein lipase, and leptin [65–67]. Moreover, Kim et al. [68] reported that fucoidan, a macromolecule derived from brown algae, inhibits adipogenesis in 3T3-L1 preadipocytes, due to inhibition of the mitogen-activated protein kinase signaling pathway that involves adipogenic transcription factors. Taken together, the present authors found that CFE inhibits adipocytes differentiation by inducing differentiation-related factors when the induction of 3T3-L1 preadipocytes into adipocytes is induced. However, further research on the role of CFE in regulating lipid metabolism is required.

In conclusion, these results suggest that CFE promotes the growth of specific beneficial microbial populations with a concomitant decrease in pathogenic microbes. Furthermore, TPC, TFC, and DPPH activity after fermentation with CFE as the carbon source were higher compared to glucose as a carbon source. Finally, CFE inhibited adipocyte differentiation by suppressing differentiation-related factors during the transformation of 3T3-L1 preadipocytes into adipocytes. Therefore, we suggest that CFE may be used as a prebiotic material, with anti-obesity effects on human health.


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