Synergistic effect of partially hydrolyzed guar gum on *Clostridium butyricum* in a synbiotic combination for enhanced butyrate production during in-vitro fermentation

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ABSTRACT

Background: *Clostridium butyricum* is a butyrate-producing beneficial bacterium and is generally recognized as a significant indicator of appropriate gut microbial metabolism in human health.

Objective: The synergistic effects of commercially available prebiotic partially hydrolyzed guar gum (PHGG) dietary fiber as a carbon source on a butyrate-producing bacterial strain were evaluated during in-vitro fermentation with *Clostridium butyricum* bacterial strain in a basal medium. Additionally, their prebiotic activities were compared to those of other dietary fibers.

Methods: The examined functional dietary fiber substrates (PHGG, LMW-PHGG, indigestible dextrin, and inulin) demonstrated selective prebiotic effects on pH variation of a basal medium, leading to enhanced bacterial growth and butyrate production with *Clostridium butyricum* bacterial strains during in-vitro fermentation.

Results: Prebiotic PHGG supplementation had the highest fermentability among dietary fibers, resulting in greater bacterial growth (OD_{660}: 1.93 ± 0.01) of the *Clostridium butyricum* strain and enhanced butyrate generation (4.52 ± 2.09 mM) after cultivation in a basal medium. A significant difference in promoting bacterial growth ($p \leq 0.05$), pH reduction ($p < 0.05$), and butyrate production ($p < 0.05$) compared to indigestible dextrin and inulin was observed. Mannose demonstrated the strongest butyrogenic effect and improved fermentability on the *Clostridium butyricum*, among the studied prebiotic monosaccharides (galactose, glucose, and starch). The order of bacterial growth and butyrate synthesis was mannose > galactose > glucose > starch. The PHGG with a relatively lower molecular weight (LMW-PHGG) exhibited the improved bacterial growth of *Clostridium butyricum* and demonstrated the highest butyrate production after cultivation in a basal medium. A similar trend was observed when *Clostridium butyricum* was cultivated in-vitro using PHGG-supplemented artificial intestinal fluid containing MRS-agar medium.

Conclusion: These findings suggest that the symbiotic combination of prebiotic PHGG and probiotic *Clostridium butyricum* could have major industrial applications as a therapeutic adjuvant for improved gastrointestinal health.

Keywords*:* Partially hydrolyzed guar gum, synbiotic, prebiotic, probiotic, *Clostridium butyricum,* butyrate production

Schematic illustration of symbiotic combination of prebiotic PHGG and probiotic *Clostridium butyricum* for health benefits:
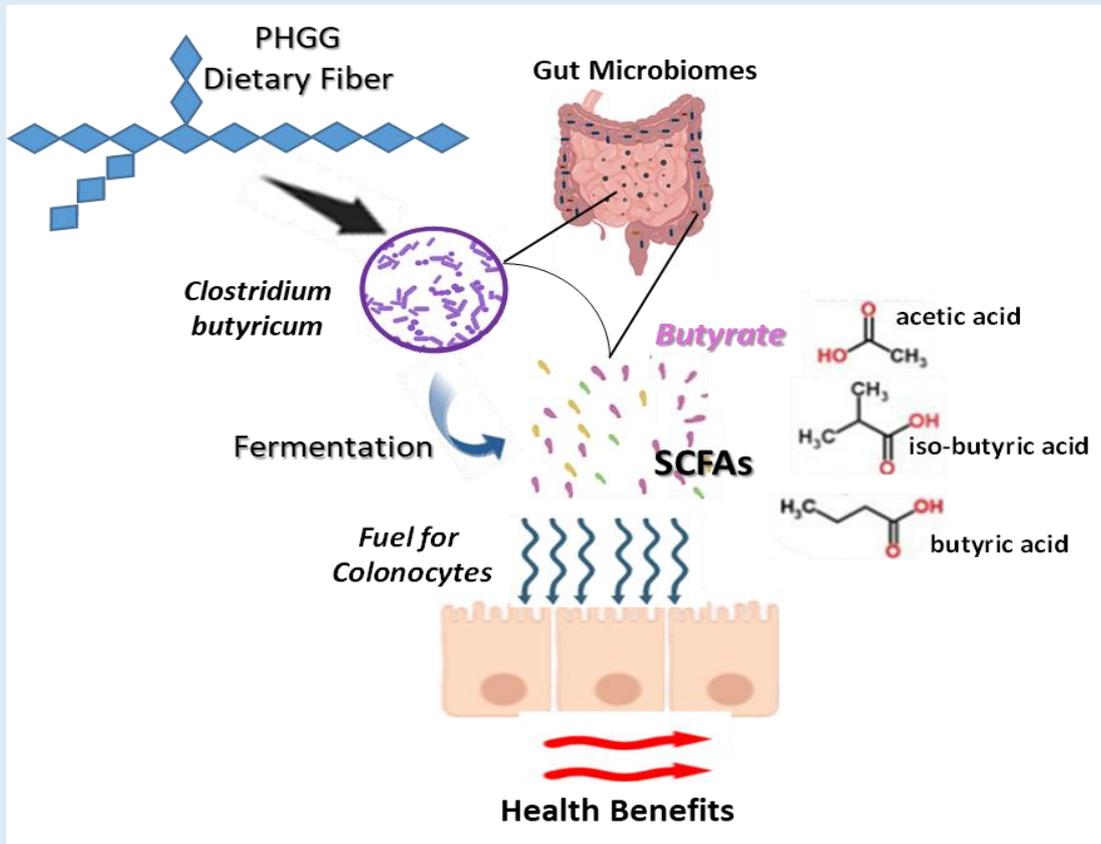
INTRODUCTION

A complex ecology on microbes colonizes the human intestine, and the variety of intestinal microbial flora is linked to different physiological phenomena in both health and disease. The robustness of the healthy microbial flora protects us against impairment of health and metabolic disorders [1–5]. In the bacterial population of the human gut, the genus *Clostridium butyricum* is defined as a butyrate-producing beneficial bacterium, which produces one of the most dominant fermentation end-products produced via the butyrate kinase (buk) pathway [6]. The majority of Firmicutes are butyrate-producing commensal bacteria, primarily anaerobes. Certain *Clostridium butyricum* strains,

notably the commercially available MIYAIRI 588, have been routinely used as probiotics in Japan, Korea, and China for decades [7]. The studies revealed that MIYAIRI 588 administration increased the beneficial microorganisms' abundance. As a result, butyrate- and butyrate-producing bacteria are generally recognized as significant indicators of appropriate gut microbial metabolic homeostasis in human health.

Prebiotics are substrates that are indigestible by the host but are utilized by gut microorganisms to confer some health benefits [8]. They comprise mainly polyols, oligosaccharides, and soluble fiber. Partially hydrolyzed guar gum (PHGG) is a soluble prebiotic dietary fiber derived from the enzymatic degradation

of the endosperm of guar beans [9-10]. It has been extensively researched in recent decades due to its numerous beneficial activities, associated with the reduction of constipation and diarrhea symptoms, irritable bowel syndrome (IBS), small intestinal bacterial overgrowth, pediatric functional gastrointestinal disorders, a role in satiety, and metabolic syndrome-related functions like aberrant lipid and glucose metabolism [11–17]. Previous research using human and experimental animal models has revealed that PHGG dietary fiber enhances the proliferation of beneficial bacteria as well as the formation of short-chain fatty acids (SCFAs), because of their ameliorative influence on intestinal health [18-19]. Prebiotic PHGG serves as food for colonic bacteria, which play an important role in the metabolism of dietary carbohydrates and polysaccharides that human enzymes are unable to completely digest. Short-chain fatty acids (SCFAs) are the primary metabolites produced in the intestinal lumen by anaerobic bacterial fermentation of predominantly undigested dietary carbohydrates. The SCFAs in the intestine are mostly composed of acetate, propionate, and butyrate, with a mean molar ratio in the colon of about 60:20:20 [20-21]. SCFAs are major anions in the colon that are rapidly absorbed and accelerate water and sodium absorption while decreasing colonic pH, stimulating intestinal peristalsis, improving the intestinal microenvironment, and regulating the colon's micro-ecological equilibrium [22].

Different intestinal flora produces different SCFAs, which are engaged in metabolism and play an essential role in host health. In particular, butyrate plays a crucial role as a primary energy source for colonocytes, enterocytes, and epithelial cells, while also regulating various cellular functions to maintain colonic health [16–19]. PHGG is fermented by intestinal microorganisms, which results in the formation of SCFAs, particularly butyrate [23-24]. A previous clinical investigation revealed that PHGG consumption stimulated the proliferation of SCFAs-producing bacteria in the intestine, particularly Bifidobacterium

and butyrate-producing bacteria [10]. As a result, PHGG has proven beneficial for improving the intestinal microbial flora and the production of SCFAs.

Research on the *Clostridium butyricum* probiotic has primarily focused on its potential benefits to intestinal health and overall well-being [25]. Studies have shown that *Clostridium butyricum*, a spore-forming gram-positive butyrate-producing anaerobic bacteria found in the human intestine, is classified mostly as a Firmicutes [26–29] and is crucial in maintaining gut homeostasis. *Clostridium butyricum* supplementation has been linked to improved gut barrier function and enhanced epithelial barrier integrity by modulating the immune response and inhibiting inflammation in the gut [30–31]. Furthermore, *Clostridium butyricum* has been studied for its ability to protect the human gut from a variety of intestinal conditions, including intestinal injuries and infections, as well as its potential to treat gastrointestinal disorders like inflammatory bowel disease and irritable bowel syndrome [32]. *Clostridium butyricum* -containing diets could potentially improve growth performance, SCFAs production, and microbial community stability in the human gut. Further, the combination of specific strains of probiotics and fibrous prebiotics that function synergistically is described as a synbiotic. They are generally more effective than probiotics or prebiotics when administered alone regarding intestinal health and function [8, 33]. Synbiotics have shown promising outcomes, despite the modest amount of research conducted to date. Several isolated *Clostridium butyricum* strains have been identified as mannanase producers [26, 34]. As a result, the dietary fiber PHGG was hypothesized as a suitable synergistic carbon source for *Clostridium butyricum*. The combination of *Clostridium butyricum* and PHGG may be employed as a viable and successful synbiotic, but their potential synergistic effects in the human gut still need to be determined. Until date, limited research has investigated the efficacy of

synbiotics including *Clostridium butyricum* and PHGG in promoting enhanced bacterial growth and intestinal health, or the underlying mechanisms.

In this study, we conducted a thorough examination of the synergistic effects of a commercially available PHGG (Sunfiber®) on *Clostridium butyricum* to increase butyrate production. We compared the utilization preferences and metabolic variations of two PHGG dietary fibers of different molecular weights with indigestible dextrin and inulin, as well as galactosyl and mannosyl oligosaccharides (non-digestible carbohydrates), glucose, and starch, during in-vitro fermentation by intestinal microorganisms. *Clostridium butyricum* was selected as the target strain to evaluate its growth and metabolism in the presence of these prebiotics. Although the effects depend on each host's unique indigenous microbial flora, the combination of prebiotic dietary fiber PHGG and *Clostridium butyricum* probiotics is effective and can provide useful information for their further application as prebiotics. This would be a promising direction for the development of synbiotics in the area of functional food ingredients.

MATERIALS AND METHODS

Bacterial strain and partially hydrolyzed guar gum:

The spore-forming bacterial strain *Clostridium butyricum* TO-A was provided by Toa Pharmaceutical Co., Ltd. The commercially available partially hydrolyzed guar gum (PHGG; proprietary name Sunfiber®; average molecular weight ~20,000 Dalton; produced by Taiyo Kagaku Co. Ltd., Japan) and modified PHGG with low molecular weight (LMW-PHGG; Mw: ~6000 Dalton) were investigated as primary carbon sources in this study, along with indigestible dextrin and inulin dietary fibers. In addition, the monosaccharides mannose, galactose, glucose, and starch were also probed as carbon sources under identical conditions, as they are the primary components of dietary fibers examined in this study.

Culture conditions and in-vitro fermentation:

The compositional description of MRS-agar (De Man-Rogsa-Sharpe agar; Difco Laboratories, Detroit, MI, USA) used in this study was as follows: proteose peptone No. 3 (10 g); beef extract (10 g); yeast extract (5 g); dextrose (20 g); polysorbate 80 (1 g), ammonium citrate (2 g); sodium acetate (5 g); magnesium Sulfate (0.1 g); manganese Sulfate (0.05 g); dipotassium phosphate (2 g); agar (15 g) per litter of deionized water. *Clostridium butyricum* TO-A strain stored at -80 °C was pre-cultured using MRS-agar medium (10 mL), modified according to guar medium reported elsewhere [30], infilled, firmly capped vials under anaerobic ambient conditions (H_2:CO_2:N_2, 10:10:80, v/v/v) at 37 °C for 48 h. One mL of the pre-culture was inoculated into 10 mL of basal medium comprising 1.0% carbon sources (PHGG, LMW-PHGG, indigestible dextrin, inulin, starch, glucose, mannose, or galactose) and 0.01% yeast extract (Difco Laboratories, Detroit, MI, USA) in per-liter basal medium at pH 7.0. The basal medium contained $(NH_4)_2HPO_4$ (1.0 g); KH_2PO_4 (0.2 g); K_2HPO_4 (1.6 g); $MgSO_4·7H_2O$ (0.2 g); NaCl (0.1 g); $CaCl_2·2H_2O$ (0.02 g); $FeSO_4·7H_2O$ (0.01 g); $Na_2MoO_4.·2H_2O$ (0.5 mg); $Na_2WO_4·2H_2O$ (0.5 mg); $MnSO_4$ (0.5 mg); L-cysteine HCl·H_2O (0.25 g); and $Na_2S·9H_2O$ (0.25 g) in 1 L of distilled water at pH 7.0. The in-vitro fermentation was carried out in 10 ml firmly capped vials (sterilized at 110 °C for 15 min before use) of the basal medium at 37°C for 120 h with continuous shaking. Then, the effects of different carbon sources on bacterial growth, pH alteration, and butyrate production were investigated. The experimental protocols are detailed in Figure 1 (see Protocol A).
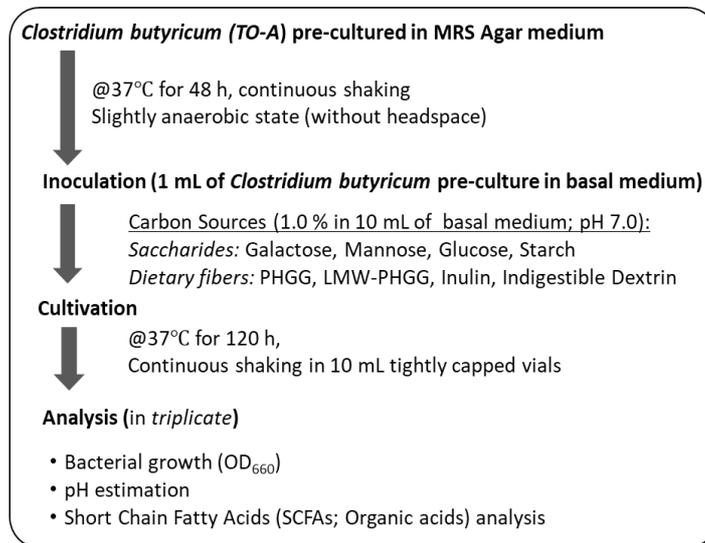
In another experiment referred to as protocol-B, the artificial intestinal fluid contained MRS-agar medium (Difco, MI, USA) comprising 0.2% gall powder (Wako Chemicals, Tokyo, Japan), which was mixed with either 1.0% PHGG or LMW-PHGG at a spore concentration of 1 x 10^8 CFU/g. *Clostridium butyricum* TO-A (3×10^6 spores/10 ml) were inoculated into the above growth medium in firmly capped vials (sterilized

at 110 °C for 15 min before use). In-vitro fermentation was carried out at 37 °C for 120 h and performed in triplicate. Aliquots of the incubated artificial intestinal fluid were collected at each time point at 8, 24, 48, and 120 h of fermentation. Similarly, the synergistic effects of prebiotic PHGG dietary fiber supplementation on bacterial growth, pH alteration, and enhanced butyrate production were investigated. The procedures adopted above are described in Protocol B (see Figure 1).

Sample analysis: Bacterial (cell) growth was measured by detecting the culture medium's absorbance at 660 nm (OD_{660}) during in-vitro fermentation. The pH and butyrate concentrations were also measured. To analyze the concentration of short-chain fatty acids (SCFAs), the collected samples were centrifuged at 10,000 x g for 3 min at 4 °C, and the supernatant was then filtered through a cellulose-acetate membrane filter (pore size, 0.45 µm). The yield of organic acids (lactate, formate, acetate, iso-butyrate, and butyrate) was determined by ion-exclusion high-performance liquid chromatography (HPLC; GC2010 Plus system; Shimadzu, Kyoto, Japan) equipped with a DB-FFAP column (AgilentTechnologies, Santa Clara, CA, USA) as described elsewhere [35].

Protocol 1: Comparison of bacterial growth and butyrate production with different carbon sources in a synbiotic combination *Clostridium butyricum* (TO-A) culture.

Clostridium butyricum (TO-A) **pre-cultured in MRS Agar medium**

> @37°C for 48 h, continuous shaking
> Slightly anaerobic state (without headspace)

Inoculation (1 mL of *Clostridium butyricum* pre-culture in basal medium)

> Carbon Sources (1.0 % in 10 mL of basal medium; pH 7.0):
> *Saccharides:* Galactose, Mannose, Glucose, Starch
> *Dietary fibers:* PHGG, LMW-PHGG, Inulin, Indigestible Dextrin

Cultivation

> @37°C for 120 h,
> Continuous shaking in 10 mL tightly capped vials

Analysis (in *triplicate***)**

- Bacterial growth (OD_{660})
- pH estimation
- Short Chain Fatty Acids (SCFAs; Organic acids) analysis

Protocol-2: Synergistic effect of prebiotic PHGG dietary fiber supplementation on *Clostridium butyricum* (TO-A) culture for enhanced bacterial growth and butyrate production in artificial intestinal fluid.
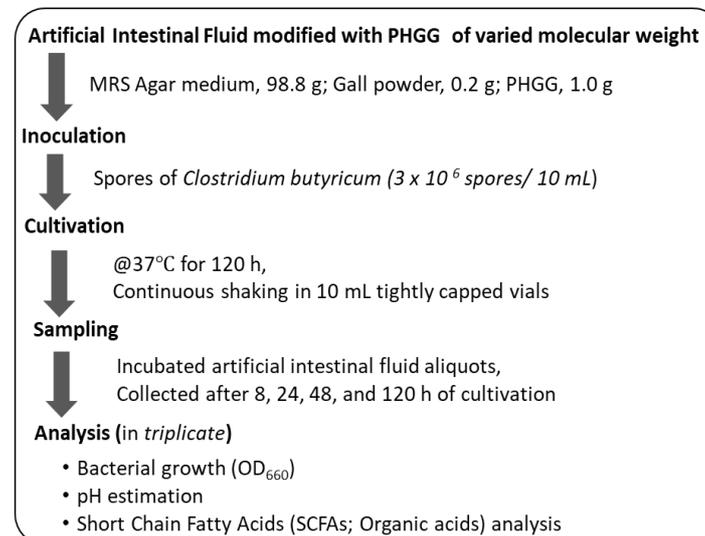
Artificial Intestinal Fluid modified with PHGG of varied molecular weight

> MRS Agar medium, 98.8 g; Gall powder, 0.2 g; PHGG, 1.0 g

Inoculation

> Spores of *Clostridium butyricum (3 x 10^6 spores/ 10 mL)*

Cultivation

> @37°C for 120 h,
> Continuous shaking in 10 mL tightly capped vials

Sampling

> Incubated artificial intestinal fluid aliquots,
> Collected after 8, 24, 48, and 120 h of cultivation

Analysis (in *triplicate***)**

- Bacterial growth (OD_{660})
- pH estimation
- Short Chain Fatty Acids (SCFAs; Organic acids) analysis

Figure 1. Protocols of experimental procedures adopted for the utilization of prebiotics were examined in the present study for enhanced butyrate production.

Statistical analysis: The results were expressed as the mean (M) ± standard deviation (SD), and the differences were considered statistically significant at $p \leq 0.05$. Significant differences in the pH of basal medium, bacterial growth OD_{660}, and butyrate production were assessed between the samples collected from two groups of dietary fiber as carbon sources using the Mann-Whitney U test. While the significance difference in the time course of pH of basal medium, bacterial growth OD_{660}, and butyrate production was assessed either by a paired student t-test or a non-parametric Wilcoxon signed rank test, statistical analysis was performed using either Excel Spreadsheet Software or JMP 14.0 Software (SPSS Inc., Chicago, IL, USA).

RESULTS

Effect of dietary fiber supplementation as a carbon source on Clostridium butyricum: To investigate the synergistic effect of dietary fibers on *Clostridium butyricum* for enhanced butyrate productivity, the dietary fibers PHGG, LMW-PHGG, indigestible dextrin, and inulin as effective carbon sources were investigated, with bacterial growth, change in pH of the basal medium, and organic acid production were compared. Short-chain fatty acids (SCFAs) production can be accompanied by bacterial growth; thus, pH and concentrations of various organic acids were measured in the culture medium. The primary fermentation end products were lactate, formate, acetates, iso-butyrate, and butyrate. *Clostridium butyricum* bacteria grew relatively slowly in the absence of dietary fiber supplementation. Incubation with 1.0% PHGG as a carbon source resulted in a significant ($p \leq 0.05$) higher growth of *Clostridium butyricum* compared to

indigestible dextrin and inulin, as indicated by higher OD_{600} values (Figure 2a). However, inulin was discovered to have the least bacterial growth of *Clostridium butyricum* among the dietary fibers examined. Figure 2b shows that the pH of PHGG-supplemented culture media declined significantly ($p < 0.001$) compared to those supplemented with indigestible dextrin and inulin. In the same culture medium (Figure 2c), *Clostridium butyricum* produced considerably more butyrate ($p \leq 0.05$) with PHGG addition compared to other dietary fibers.

To further support the aforementioned finding, the monosaccharides mannose, galactose, glucose, and starch were also probed as carbon sources under identical conditions, as these are the primary components of dietary fibers examined in this study. *Clostridium butyricum* thrived well on mannose and galactose, as evidenced by higher OD_{660} values (Figure 3a), which are the primary components of PHGG dietary fiber, outperforming glucose, and starch (the components of indigestible dextrin and inulin). While the pH of the basal medium did not change significantly during the in-vitro fermentation (Figure 3b), mannose and galactose were also found to demonstrate suitability for *Clostridium butyricum* utilization, resulting in a significantly higher level of butyrate (Figure 3c) without causing a decrease in the fermentation pH. Figure 4 illustrates the profile of organic acid (short-chain fatty acids; SCFAs) production with each carbon source investigated in this study utilized by *Clostridium butyricum* bacterial stain during in-vitro fermentation for 120 h. The prebiotic PHGG dietary fiber produced increased butyric acid compared to inulin or digestible dextrin.
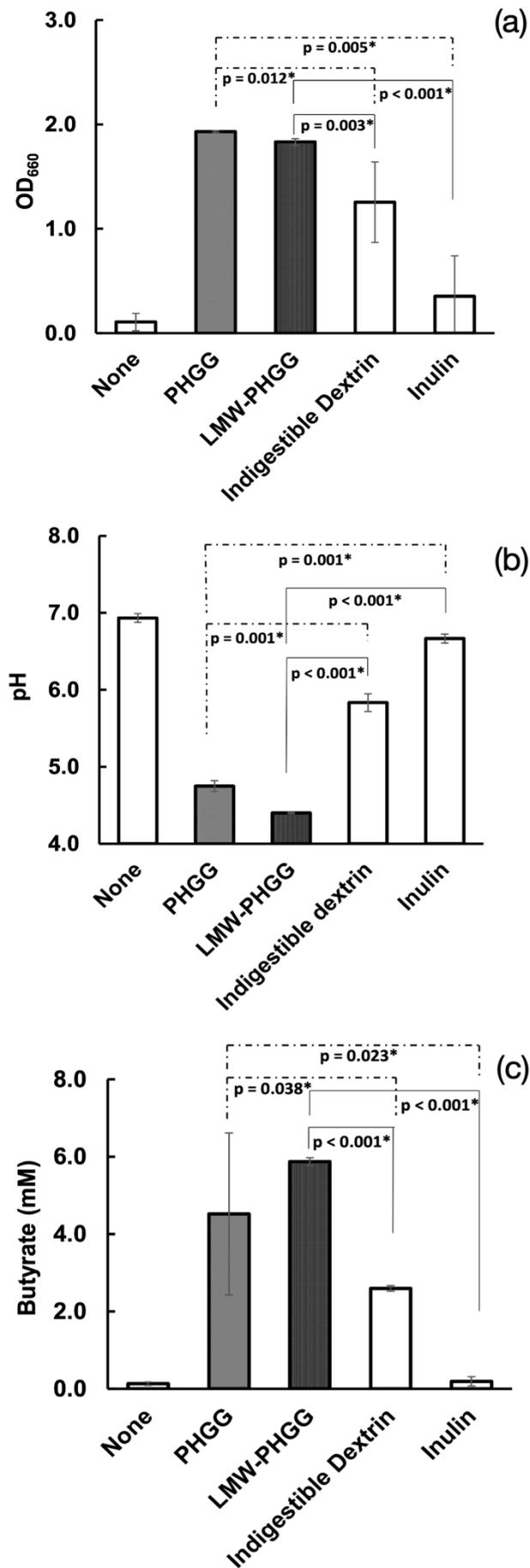
Figure 2. Synergistic effect of different prebiotic dietary fiber supplementation as a carbon source on *Clostridium butyricum* bacterial stain for enhanced butyrate production during in-vitro fermentation. (a) bacterial growth (OD_{660}), (b) variation in pH of basal medium, and (c) butyrate production. [Statistical significance: $p \leq 0.05$]
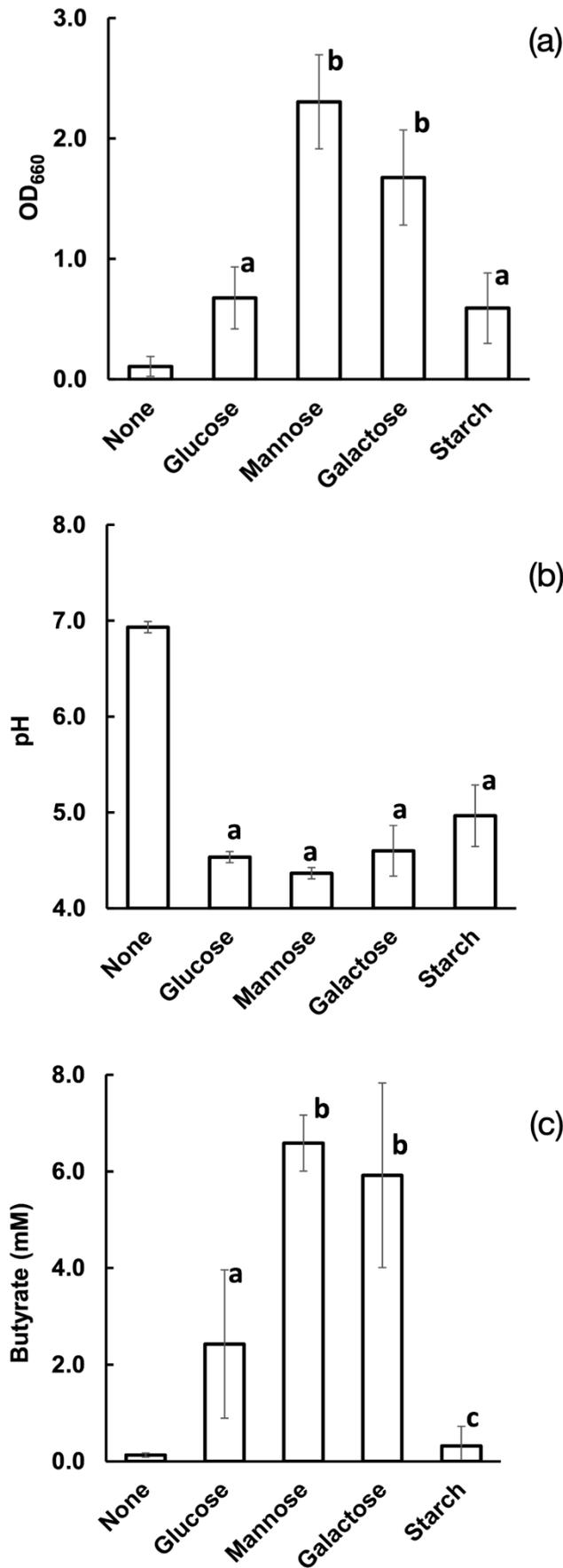
Figure 3. Utilization of prebiotic monosaccharides (non-digestible carbohydrates) by *Clostridium butyricum* bacterial stain during in-vitro fermentation. (a) bacterial growth (OD$_{660}$), (b) variation in pH of basal medium, and (c) butyrate production. [Statistical significance: p ≤ 0.05*; different letters indicate significantly different results]
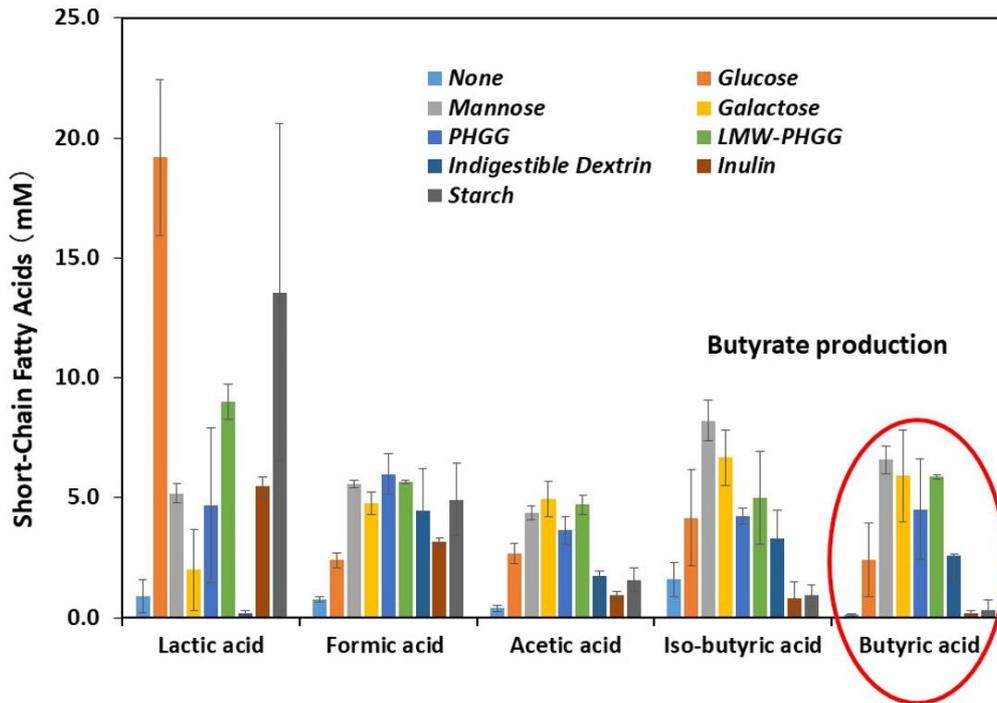
Figure 4. Profile of organic acid (short-chain fatty acids; SCFAs) production with each carbon source examined in this study utilized by *Clostridium butyricum* bacterial stain during in-vitro fermentation for 120 h. Prebiotic PHGG dietary fiber showed enhanced production of butyric acid compared to inulin and digestible dextrin.

Synergistic effect of PHGG on *Clostridium butyricum* utilization in artificial intestinal fluid: Figures 5 and 6 revealed the findings of assessing the PHGG function in the intestine. The pH of the *Clostridium butyricum*-inoculated artificial intestinal fluid with growth nutrients (MRS-agar medium) decreased with and without supplementation of PHGG. Figure 5a depicts an image of bacterial growth with and without prebiotic PHGG, demonstrating the synergistic effect of PHGG dietary fiber supplementation on *Clostridium butyricum* ricum (TO-A) spores in artificial intestinal fluid. The *Clostridium butyricum* bacteria in the examined samples consumed the PHGG (carbohydrates), lowering the pH of the medium that accompanied bacterial growth and was attributable to the formation of organic acids. During the in-vitro fermentation process, the pH of the medium gradually decreased, and the incubated artificial intestinal fluids slowly turned blurred (see Figure 5b).

Despite significant pH shifts throughout the first 8 hours, butyrate generation was minimal. However, LMW-PHGG produced substantial amounts of butyrate after 24 hours of cultivation. Following that, a drastic and considerable reduction in pH was accompanied by a significant increase in butyrate production, indicating the rapid utilization of the PHGG to generate SCFAs, particularly butyrate (Figure 6a). After 48 hours of cultivation, the rate of butyrate production was extremely low in the absence of PHGG dietary fiber supplementation. Furthermore, LMW-PHGG had a considerably different rate of pH lowering over time, as well as the highest yield over time for butyrate generation. Thus, butyrate production was remarkably improved after 48 h of cultivation ($p \leq 0.05$) by 2.11 and 2.61 folds when PHGG and LMW-PHGG, respectively, were supplemented to the *Clostridium butyricum* growth medium. For all samples examined, the pH was in the range of 5.2 and 5.3, and butyrate production was consistent between 7.1 and 7.8 mM after 120 h of cultivation (see Figure 6b). These findings further suggest that *Clostridium butyricum* may thrive well even in artificial intestinal fluid, where the *Clostridium butyricum* bacterial strain preferentially utilizes the prebiotic PHGG.
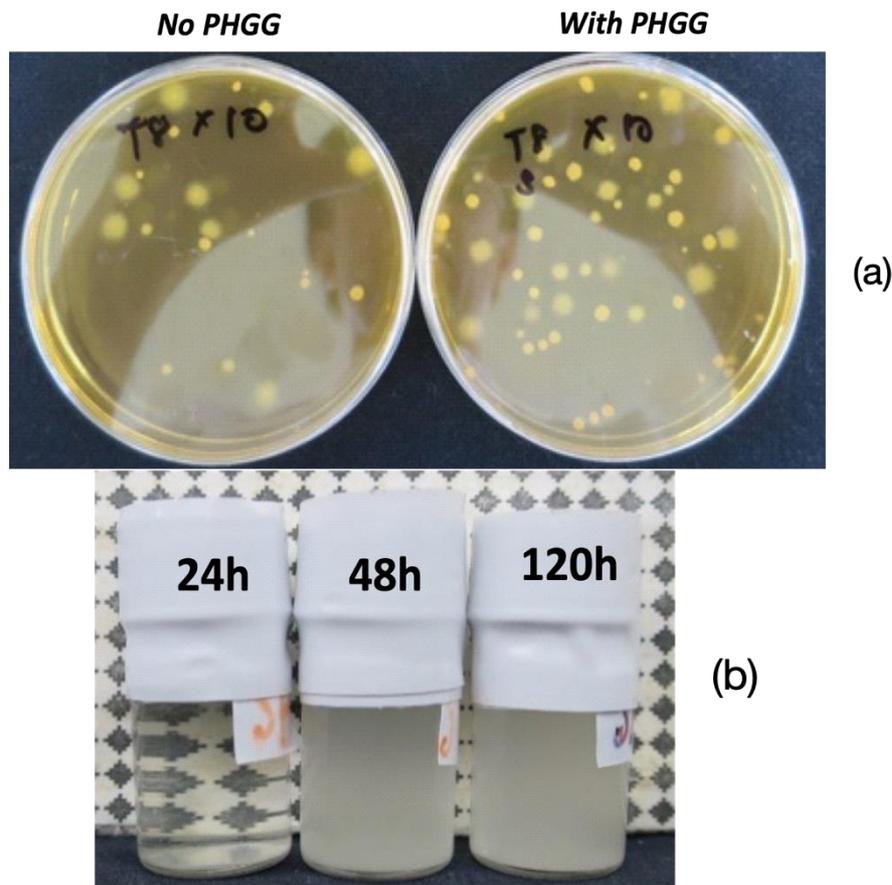
No PHGG   **With PHGG**

Figure 5. Prebiotic partially hydrolyzed guar gum (PHGG) dietary fiber supplementation promotes the bacterial growth of the spores of *Clostridium butyricum* (TO-A) culture during in-vitro fermentation in artificial intestinal fluid containing MRS-agar medium. (a) Image of bacterial growth with and without PHGG, and (b) Incubated artificial intestinal fluids turned cloudy after 8, 24, and 48 h of cultivation.
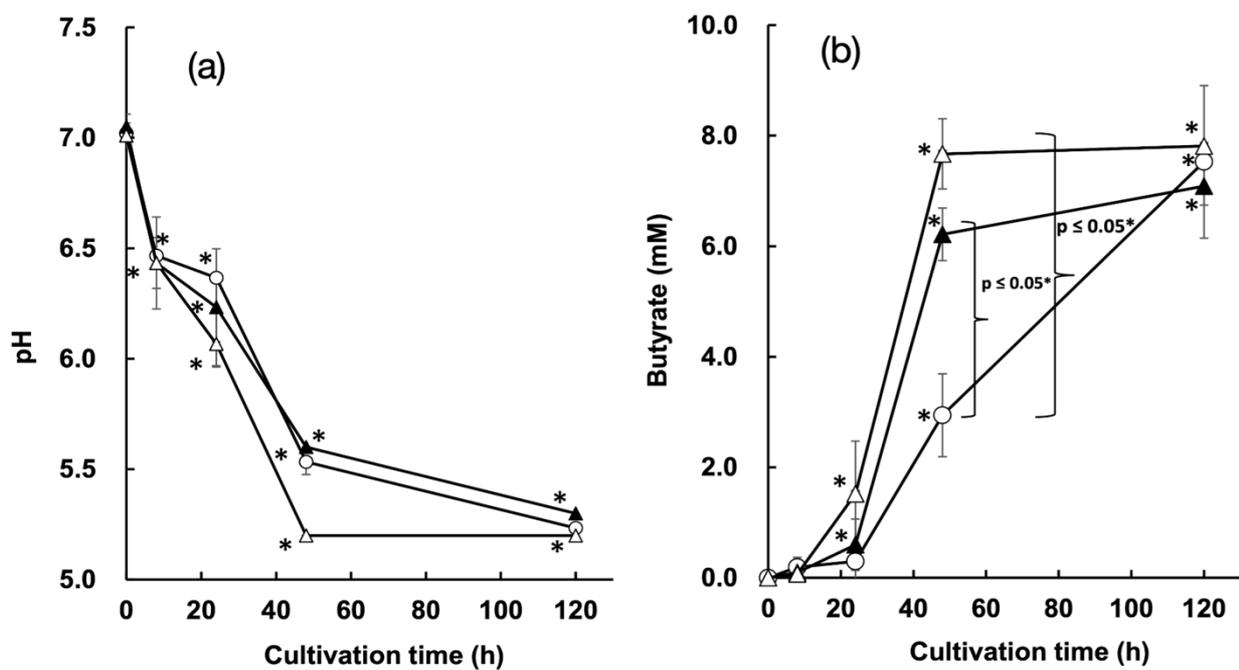

Figure 6. (a) Change in pH of the artificial intestinal fluid containing MRS-agar medium, and (b) the yield of butyrate production, with time on course cultivation during the utilization of prebiotic PHGG dietary fibers with varied molecular weight by *Clostridium butyricum* bacterial stain. [Artificial intestinal juice containing MRS-agar growth medium: ○ without PHGG; ▲ with PHGG; and Δ with LMW-PHGG].

DISCUSSION

Partially hydrolyzed guar gum (PHGG) is a promising prebiotic dietary fiber with monomeric compositions such as galacto-oligosaccharides (composed of β-(1, 4)-linked galactose) and manno-oligosaccharides (composed of β-(1, 4)-linked D-mannoses) for increasing of intestinal butyrate production and improving intestinal absorption functions [36-37]. The structural composition of non-digestible carbohydrates, the type of glycosidic bonding, degree of polymerization and branching, and molecular weight have shown a significant influence on their in-vitro fermentation characteristics. Thus, the in-vitro fermentation of dietary fibers and monosaccharides investigated in this study was typically associated with a varying production of short-chain fatty acids and concomitant decreases in the pH of the medium. Supplementation with PHGG dietary fiber and oligosaccharides, including galactose or mannose on the *Clostridium butyricum* bacterial strain, resulted in a substantial proliferation-promoting impact and delivered a specific pattern of SCFAs with predominant butyrate production as the primary constituent. This may be attributed to their common β (1→4) glycosidic bond structure [38]. In contrast, indigestible dextrin, inulin, oligosaccharides containing glucose, or starch exhibited a lower proliferation-promoting impact. This suggested that the prolific consumption characteristics of the non-digestible carbohydrates examined in this study with varying structural components during bacterial stain fermentation could differ significantly. Considering the resulting production of butyrate from all dietary fibers examined as a carbon source in this study, the prebiotic PHGG dietary fibers were typically beneficial for *Clostridium butyricum* in terms of bacterial growth, and the pH was concomitantly reduced, which can be attributed to the establishment of a healthy microbial community for improved intestinal health. Because oligosaccharides containing mannose or galactose are known to be effective butyrogenic substrates [39], the PHGG dietary fiber (a galactomannosyl non-digestible carbohydrate) has strong prospective and promising potential as a butyrogenic prebiotic.

Furthermore, when PHGG and LMW-PHGG were compared, although there were no significant differences between the groups, LMW-PHGG was more likely to increase *Clostridium butyricum* growth and butyric acid synthesis than PHGG. The findings demonstrate that *Clostridium butyricum* utilized LMW-PHGG relatively quickly, and the rate of utilization corresponded to the bacterial growth results, as evidenced by enhanced butyrate production. Non-digestible carbohydrates with a lower polymer degree have been reported to be preferentially consumed by bacterial stains [40]. Previously, it was reported that the lower molecular weight exopolysaccharide from the medicinal fungus was utilized more rapidly by the fecal microbiota, resulting in a beneficial fecal microbial modification when compared to the original exopolysaccharide [41]. In another study, the lower molecular weight blackberry polysaccharide was shown to be more easily degraded by gut bacteria, which affected its fermentation capabilities [42]. The above inference is corroborated by results obtained with oligosaccharides constituted of galactose, or mannose, which exhibited increased butyrate-producing related bacteria and relatively higher butyrate production when compared to PHGG prebiotic dietary fibers (see Figures 2c and 3c). Mannose and galactose prebiotics have a lower molecular weight than PHGG. Therefore, they were easily utilized by the *Clostridium butyricum* bacterial strain. On the other hand, the presence of the polymeric degree of the chain of galactomannan in PHGG dietary fiber, which may also be influenced by the galactose side chain branches, may have restricted the rapid degradation of the polysaccharide backbone structure by *Clostridium butyricum* during the in vitro fermentation process. However, considering that the actual gut ecosystem contains a variety of bacteria, different carbohydrates, and other nutrients, these factors need to be considered. In an in vitro culture

system using human feces, which includes various bacterial species, a study comparing the fermentability of PHGG with average molecular weights of 20 kDa and 5 kDa observed minor differences in the bacterial species each promoted and the extent of short-chain fatty acid production. Nonetheless, no significant differences were discovered, and the fermentation rates were comparable. It is concluded that PHGG will have identical prebiotic effects regardless of molecular weight [43]. Therefore, it is necessary to investigate the cross-feeding interactions between *Clostridium butyricum* and other gut bacteria to better understand the synbiotic effects of *Clostridium butyricum* and PHGG.

In clinical research, Ohashi and colleagues revealed that consuming PHGG dietary fiber dramatically raised copy numbers of the butyryl-CoA-transferase gene and the 16S rRNA gene of butyrate-producing bacterium strain SS2/1 [10]. The butyryl-CoA-transferase pathway in Clostridium strains is responsible for the majority of intestinal butyrate synthesis [10, 44–45]. *Clostridium butyricum* is symbiotic with its host and thrives by digesting PHGG dietary fiber, mostly producing butyrate via the buk pathway [6]. However, the enhancement of butyrate production is dependent on the intestinal microbial flora of individuals. As a result, the synergistic combination of prebiotic PHGG dietary fiber and probiotic *Clostridium butyricum* (synbiotics) could be extremely effective for preserving human intestinal health. Thus, considering the observed specific *Clostridium butyricum* bacterial growth and metabolism patterns, the prebiotic PHGG dietary fiber having galactomannosyl structure (i.e., a constituent with a directional combination of galactosyl and mannosyl carbohydrates) appears to be suitable for butyrate production by *Clostridium butyricum* and could show great potential for industrial applications.

CONCLUSION

Health promotion is the primary benefit of functional foods [46]. The present research is novel because it explores an innovative approach that showcases the evolving understanding of how functional dietary fiber and its components can interact with beneficial gut microbiota, which in turn promotes gut health and improves gut barrier function. The study addresses the synergistic effects of prebiotic partially hydrolyzed guar gum (PHGG) dietary fiber and *Clostridium butyricum* (a butyrate-producing bacteria) in a synbiotic combination on increased bacterial growth and enhanced butyrate production. The variation in pH of basal medium, bacterial growth, SCFAs production, and prebiotic non-digestible carbohydrate utilization were investigated, and the examined monosaccharides and dietary fiber substrates exhibited distinctive prebiotic effects for *Clostridium butyricum* bacterial stain. The fermentation characteristics of *Clostridium butyricum* with prebiotics PHGG investigated in this study reflect the extent to which this functional dietary fiber would promote bacterial growth with a rapid rate of utilization by *Clostridium butyricum* for enhanced butyrate production during the in-vitro fermentation. Also, the butyrate production when utilizing prebiotic PHGG dietary fiber by *Clostridium butyricum* was discovered to be insignificantly related to the varying molecular weights of these non-digestible carbohydrates. Although additional well-designed human clinical studies are necessary, the use of PHGG dietary fiber as a prebiotic supplement in conjunction with *Clostridium butyricum* has shown promise and provided a rational basis for establishing the industrial development of novel synbiotics with *Clostridium butyricum* to maintain gut health and promote and regulate a balanced intestinal microecology.

REFERENCES

1. Lescheid DW. Probiotics as regulators of inflammation: A review. Functional Foods in Health and Disease 2014; 4(7): 299-311.
   DOI: https://ffhdj.com/index.php/ffhd/article/view/2

2. F, Anderson JM, Bharti R, Raes J, Rosenstiel P. The resilience of the intestinal microbiota influences health and disease. Nature Reviews Microbiology 2017, 15(10): 630-638. DOI: https://doi.org/10.1038/nrmicro.2017.58

3. Shimizu K, Ogura H, Asahara T, Nomoto K, Morotomi M, Tasaki O, Matsushima A, Kuwagata Y, Shimazu T, Sugimoto H. Probiotic/synbiotic therapy for treating critically ill patients from a gut microbiota perspective. Digestive Diseases and Sciences 2013; 58:23-32.
   DOI: https://doi.org/10.1007/s10620-012-2334-x

4. Ettinger G, MacDonald K, Reid G, Burton JP. The influence of the human microbiome and probiotics on cardiovascular health. Gut Microbes 2014; 5(6): 719-728.
   DOI: https://doi.org/10.4161/19490976.2014.983775

5. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M, Colecchia A. Gut microbiota and metabolic syndrome. World Journal of Gastroenterology: WJG 2014; 20 (43): 16079. DOI: https://doi:10.3748/wjg.v20.i43.16079

6. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: short-Chain Fatty Acids as Key Bacterial Metabolites. Cell 2016, 165: 1332–1345. DOI: https://doi:10.1016/j.cell.2016.05.041.

7. Seki H, Shiohara M, Matsumura T, Miyagawa N, Tanaka M, Komiyama A, Kurata S. Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYAIRI. Pediatrics International 2003; 45(1): 86-90.
   DOI: https://doi.org/10.1046/j.1442-200X.2003.01671.x

8. Zhang XF, Guan XX, Tang YJ, Sun JF, Wang XK, Wang WD, Fan JM. Clinical effects and gut microbiota changes of using probiotics, prebiotics or synbiotics in inflammatory bowel disease: A systematic review and meta-analysis. European Journal of Nutrition 2021; 60: 2855-2875.
   DOI: https://doi.org/10.1007/s00394-021-02503-5.

9. Kapoor MP, Ishihara N, Okubo T. Soluble dietary fibre partially hydrolysed guar gum markedly impacts on postprandial hyperglycaemia, hyperlipidaemia and incretins metabolic hormones over time in healthy and glucose intolerant subjects. Journal of Functional Foods 2016; 24: 207-220.
   DOI: https://doi.org/10.1016/j.jff.2016.04.008

10. Ohashi Y, Sumitani K, Tokunaga M, Ishihara N, Okubo T, Fujisawa T. Consumption of partially hydrolysed guar gum stimulates Bifidobacteria and butyrate-producing bacteria in the human large intestine. Beneficial Microbes 2015; 6(4): 451-455.
    DOI: https://doi.org/10.3920/BM2014.0118

11. Naito Y, Ichikawa H, Akagiri S, Uchiyama K, Takagi T, Handa O, Yasukawa Z, Tokunaga M, Ishihara N, Okubo T, Mukai J. Identification of cysteinylated transthyretin, a predictive biomarker of treatment response to partially hydrolyzed guar gum in type 2 diabetes rats, by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. Journal of Clinical Biochemistry and Nutrition 2016; 58(1): 23-33.
    DOI: https://doi.org/10.3164/jcbn.15-98

12. Furnari M, Parodi A, Gemignani L, Giannini EG, Marenco S, Savarino E, Assandri L, Fazio V, Bonfanti D, Inferrera S, Savarino V. Clinical trial: the combination of rifaximin with partially hydrolysed guar gum is more effective than rifaximin alone in eradicating small intestinal bacterial overgrowth. Alimentary Pharmacology & Therapeutics 2010; 32(8): 1000-1006.
    DOI: https://doi.org/10.1111/j.1365-2036.2010.04436.x

13. Kapoor MP, Sugita M, Fukuzawa Y, Okubo T. Impact of partially hydrolyzed guar gum (PHGG) on constipation prevention: A systematic review and meta-analysis. Journal of Functional Foods 2017; 33: 52-66.
    DOI: https://doi.org/10.1016/j.jff.2017.03.028

14. Kapoor MP, Koido M, Kawaguchi M, Timm D, Ozeki M, Yamada M, Mitsuya T, Okubo T. Lifestyle related changes with partially hydrolyzed guar gum dietary fiber in healthy athlete individuals–A randomized, double-blind, crossover, placebo-controlled gut microbiome clinical study. Journal of Functional Foods 2020; 72: 104067.
    DOI: https://doi.org/10.1016/j.jff.2020.104067

15. Romano C, Comito D, Famiani A, Calamarà S, Loddo I. Partially hydrolyzed guar gum in pediatric functional abdominal pain. World Journal of Gastroenterology: WJG 2013, 19(2): 235. DOI: https://doi:10.3748/wjg.v19.i2.235

16. Yasukawa Z, Naito Y, Takagi T, Mizushima K, Tokunaga M, Ishihara N, Juneja LR, Yoshikawa T. Partially hydrolyzed guar gum affects the expression of genes involved in host

defense functions and cholesterol absorption in colonic mucosa of db/db male mice. Journal of Clinical Biochemistry and Nutrition 2012; 51(1): 33-38. DOI: https://doi.org/10.3164/jcbn.11-104

17. Dall'Alba V, Silva FM, Antonio JP, Steemburgo T, Royer CP, Almeida JC, Gross JL, Azevedo MJ. Improvement of the metabolic syndrome profile by soluble fibre–guar gum–in patients with type 2 diabetes: a randomised clinical trial. British Journal of Nutrition 2013; 110(9): 1601-1610. DOI: https://doi:10.1017/S0007114513001025

18. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nature Communications 2013; 4(1): 1829. DOI: https://doi.org/10.1038/ncomms2852

19. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 2013; 341(6145): 569-573. DOI: https://doi:10.1126/science.1241165

20. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews 2001; 81(3): 1031-1064. DOI: https://doi.org/10.1152/physrev.2001.81.3.1031

21. Hallert C, Björck I, Nyman M, Pousette A, Grännö C, Svensson H. Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. Inflammatory Bowel Diseases 2003;9(2): 116-121. DOI: https://doi.org/10.1097/00054725-200303000-00005

22. Ma N, Guo P, Zhang J, He T, Kim SW, Zhang G, Ma X. Nutrients mediate intestinal bacteria–mucosal immune crosstalk. Frontiers in Immunology 2018; 9: 5. DOI: https://doi: 10.3389/fimmu.2018.00005

23. Ohashi Y, Harada K, Tokunaga M, Ishihara N, Okubo T, Ogasawara Y, Juneja LR, Fujisawa T. Faecal fermentation of partially hydrolyzed guar gum. Journal of Functional Foods 2012; 4(1): 398-402. DOI: https://doi.org/10.1016/j.jff.2011.09.007

24. Takagi T, Naito Y, Higashimura Y, Ushiroda C, Mizushima K, Ohashi Y, Yasukawa Z, Ozeki M, Tokunaga M, Okubo T, Katada K. Partially hydrolysed guar gum ameliorates murine intestinal inflammation in association with modulating luminal microbiota and SCFAs. British Journal of Nutrition 2016; 116(7): 1199-1205. DOI: https://doi:10.1017/S0007114516003068

25. Markowiak-Kopeć P, Śliżewska K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients 2020; 12(4): 1107. DOI: https://doi.org/10.3390/nu12041107

26. Nakajima N, Matsuura Y. Purification and Characterization of Konjac Glucomannan Degrading Enzyme from Anaerobic Human Intestinal Bacterium, *Clostridium butyricum* –Clostvidium beijerinckii Group. Bioscience, Biotechnology, and Biochemistry 1997; 61(10):1739-42. DOI: https://doi.org/10.1271/bbb.61.1739

27. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nature Reviews Microbiology 2014; 12(10): 661-672. DOI: https://doi.org/10.1038/nrmicro3344.

28. Rey FE, Faith JJ, Bain J, Muehlbauer MJ, Stevens RD, Newgard CB, Gordon JI. Dissecting the in vivo metabolic potential of two human gut acetogens. Journal of Biological Chemistry 2010; 285(29): 22082-22090. DOI: https://doi.org/10.1074/jbc.M110.117713.

29. Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. Proceedings of the Nutrition Society 2015; 74(1):13-22. DOI: https://doi.org/10.1017/S0029665114001463.

30. Kataoka N, Tokiwa Y. Isolation and characterization of an active mannanase-producing anaerobic bacterium, Clostridium tertium KT-5A, from lotus soil. Journal of Applied Microbiology 1998; 84(3): 357-367. DOI: https://doi.org/10.1046/j.1365-2672.1998.00349.x

31. Hamer HM, Jonkers DM, Venema K, Vanhoutvin SA, Troost FJ, Brummer RJ. The role of butyrate on colonic function. Alimentary Pharmacology & Therapeutics 2008; 27(2): 104-119. DOI: https://doi.org/10.1111/j.1365-2036.2007.03562.x

32. Stoeva MK, Garcia-So J, Justice N, Myers J, Tyagi S, Nemchek M, McMurdie PJ, Kolterman O, Eid J. Butyrate-producing human gut symbiont, *Clostridium butyricum*, and its role in health and disease. Gut Microbes 2021;13(1): 1907272. DOI: https://doi.org/10.1080/19490976.2021.1907272

33. Roselli M, Finamore A. Use of synbiotics for ulcerative colitis treatment. Current Clinical Pharmacology 2020; 15(3): 174-182. DOI: https://doi.org/10.2174/1574884715666191226120322

34. Xiuzhu D, Schyns PJ, Stams AJ. Degradation of galactomannan by a *Clostridium butyricum* strain. Antonie van Leeuwenhoek 1991; 60: 109-114. DOI: https://doi.org/10.1007/BF00572700

35. Ushida K, Sakata T. Effect of pH on oligosaccharide fermentation by porcine cecal digesta. Animal Science and Technology. 1998; 69: 100-107.

36. Saville BA, Saville SH. Functional attributes and health benefits of novel prebiotic oligosaccharides derived from xylan, arabinan, and mannan. Prebiotics and Probiotics-Potential Benefits in Nutrition and Health 2019. DOI: https://doi:10.5772/intechopen.89484

37. Wu C, Liu J, Tang Y, Li Y, Yan Q, Jiang Z. Hepatoprotective potential of partially hydrolyzed guar gum against acute alcohol-induced liver injury in vitro and vivo. Nutrients 2019; 11(5): 963. DOI: https://doi.org/10.3390/nu11050963

38. Xu J, Wang R, Liu W, Yin Z, Wu J, Yu X, Wang W, Zhang H, Li Z, Gao M, Zhu L. The specificity of ten non-digestible carbohydrates to enhance butyrate-producing bacteria and butyrate production in vitro fermentation. Food Science and Human Wellness 2023; 12(6): 2344-2354. DOI: https://doi.org/10.1016/j.fshw.2023.03.038

39. Tiwari U, Jha R. PSIII-22 Fermentation characteristics of xylo-and manno-oligosaccharides and soluble and insoluble arabinoxylan studied using an in vitro model of swine. Journal of Animal Science 2018; 96 (Suppl 3): 309. DOI: https://doi:10.1093/jas/sky404.679

40. Cardelle-Cobas A, Corzo N, Olano A, Peláez C, Requena T, Ávila M. Galactooligosaccharides derived from lactose and lactulose: influence of structure on Lactobacillus, Streptococcus and Bifidobacterium growth. International Journal of Food Microbiology. 2011; 149(1): 81-87. DOI: https://doi.org/10.1016/j.ijfoodmicro.2011.05.026

41. Mao YH, Song AX, Li LQ, Yang Y, Yao ZP, Wu JY. A high-molecular weight exopolysaccharide from the Cs-HK1 fungus: Ultrasonic degradation, characterization and in vitro fecal fermentation. Carbohydrate Polymers. 2020; 246: 116636. DOI: https://doi.org/10.1016/j.carbpol.2020.116636.

42. Dou Z, Chen C, Fu X. Digestive property and bioactivity of blackberry polysaccharides with different molecular weights. Journal of Agricultural and Food Chemistry 2019; 67(45): 12428-12440. DOI: https://doi.org/10.1021/acs.jafc.9b03505.

43. Sasaki D, Sasaki K, Abe A, Ozeki M, Kondo A. Effects of partially hydrolyzed guar gums of different molecular weights on a human intestinal in vitro fermentation model. J Biosci Bioeng 2023;136(1): 67-73. DOI: https://doi:10.1016/j.jbiosc.2023.04.002.

44. Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. Journal of Bacteriology 2004; 186(7): 2099-2106. DOI: https://doi.org/10.1128/jb.186.7.2099-2106.2004

45. Louis P, Flint HJ. Development of a semiquantitative degenerate real-time pcr-based assay for estimation of numbers of butyryl-coenzyme A (CoA) CoA transferase genes in complex bacterial samples. Applied and Environmental Microbiology 2007; 73(6): 2009-2012. DOI: https://doi.org/10.1128/AEM.02561-06

46. Martirosyan D, Lampert T, Lee M. A comprehensive review on the role of food bioactive compounds in functional food science. Functional Food Science-Online ISSN: 2767-3146. 2022, 2(3): 64-78. DOI: https://doi.org/10.31989/ffs.v2i3.906