**Bacillus coagulans GBI-30, 6086 Modulates Faecalibacterium prausnitzii in Older Men and Women**

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**Abstract**

**Background:** Advancing age is linked to a decrease in beneficial bacteria such as *Bifidobacterium* spp. and reduced aspects of innate immune function.

**Objectives:** We investigated whether daily consumption of a probiotic (*Bacillus coagulans* GBI-30, 6086 (BC30); GanedenBC30) could improve immune function and gut function in men and women aged 65–80 y, using a double-blind, placebo-controlled crossover design.

**Method:** Thirty-six volunteers were recruited and randomly assigned to receive either a placebo (microcrystalline cellulose) or the probiotic BC30 (1 × 10^9 colony-forming units/capsule). Volunteers consumed 1 treatment capsule per day for 28 d, followed by a 21-d washout period before switching to the other treatment. Blood and fecal samples were collected at the beginning and end of each treatment period. Fecal samples were used to enumerate bacterial groups and concentrations of calprotectin. Peripheral blood mononuclear cells (PBMCs) were extracted from whole blood to assess natural killer cell activity and lipopolysaccharide (LPS)-stimulated cytokine production. C-reactive protein concentrations were measured in plasma.

**Results:** Consumption of BC30 significantly increased populations of *Faecalibacterium prausnitzii* by 0.1 log10 cells/mL more than during consumption of the placebo (*P* = 0.03), whereas populations of *Bacillus* spp. increased significantly by 0.5 log10 cells/mL from baseline in volunteers who consumed BC30 (*P* = 0.007). LPS-stimulated PBMCs showed a 0.2 ng/mL increase in the anti-inflammatory cytokine IL-10 28 d after consumption of BC30 (*P* < 0.05), whereas the placebo did not affect IL-10, and no overall difference was found in the effect of the treatments.

**Conclusions:** Daily consumption of BC30 by adults aged 65–80 y can increase beneficial groups of bacteria in the human gut and potentially increase production of anti-inflammatory cytokines. This study shows the potential benefits of a probiotic to improve dysbiosis via modulation of the microbiota in older persons.

**Keywords:** BC30, *Bacillus coagulans*, probiotic, *Faecalibacterium prausnitzii*, interleukin-10

**Introduction**

The population of adults living longer has increased. Aging is met with many physiologic changes such as a decline in functionality of many tissues and organs, including the large gut microbiome. Bacterial numbers in the human colonic microbiota exceed that of total mammalian cells in the human body (1). As such, activities of these microorganisms significantly affect host health. With age >65 y, changes in the human gut microbiota occur with an increase in less favorable groups, for example, *Clostridium* spp. and *Bacteroides* spp., at the expense of beneficial bacteria such as *Bifidobacterium* spp. (2). Immunosenescence is the decline in immune function that occurs with advancing age, possibly leading to a higher predisposition to infections and other age-related diseases (3). It is also associated with increased production of cytokines, including IL-10, IL-6, IL-8, IL-1β, and TNF-α (4–7). TNF-α is a proinflammatory cytokine produced by activated macrophages. This, alongside other proinflammatory cytokines, may result in a state of fever, apoptosis, and inflammation, of which a low-grade state is seen in several situations, including obesity. Other proinflammatory cytokines, such as IL-1β and IL-8, initiate immune...
responses (8), whereas anti-inflammatory cytokines such as IL-10 suppress inflammatory cytokine activity (9).

Cytokines, originally classified as lymphokines and monokines, are small proteins (5–12 kDa) involved in cellular signaling. They can be activated by bacteria, viruses, and endotoxins (10, 11). The epithelial barrier consists of Peyer patches and the lamina propria. Peyer patches are areas of aggregated lymphoid tissue which contain B lymphocytes and macrophages and allow for the interaction of antigens with such immunomodulatory cells. The lamina propria is present in the mucosal layer of the gastrointestinal (GI) tract, specifically within the villi; they are rich in several cytokine-producing cells, including fibroblasts, macrophages, and lymphocytes. The presence of such cells indicates activity in the initial immune response, protecting intestinal tissue from interaction with pathogens (12).

Immunomodulation, via consumption of nondigestible carbohydrates (prebiotics) and live bacterial cultures (probiotics), has become the focus of several dietary intervention studies. This is based on evidence that shows their immunomodulatory effects, including down-regulation of proinflammatory cytokines (13). Studies have shown that ingestion of prebiotics reduces TNF-α and IL-6 (14–16) and up-regulates production of IL-10 (17, 18), providing a link between gut microbiota and modulation of the inflammatory response. Human studies have also shown the capability of prebiotics to improve cell-mediated immunity, via increased phagocytic activity, increased NK cell activity, and a reduction in expression of proinflammatory cytokines (6, 19). Studies that involve probiotics were also reported (20, 21), although the issue of strain survivability through the upper GI tract occurs.

Bacillus coagulans GBI-30, 6086 (BC30; Ganeden Biotech) is a commercially available spore-forming probiotic. The use of a spore former helps survivability through gastric and bile acids as shown in vitro (22, 23), and studies have shown beneficial effects on postprandial gas-related symptoms (24), improvement in irritable bowel syndrome–related symptoms (25), and increase in immune function (26).

This study investigated the potential effects of daily administration of BC30 on immune and gut function in asymptomatic elderly persons.

Methods

Subjects. Volunteers aged 65–80 y were recruited. They had BMI 18–31 kg/m², were in general good health, and were free from any chronic diseases, including GI illnesses. They were required to refrain from consuming any pharmaceuticals, including immunosuppressive drugs and anti-inflammatory agents. Volunteers were also asked to avoid consumption of any probiotic or prebiotic supplements for the duration of the study and for at least 6 mo before the study and to discontinue the use of any laxatives or any foods with a laxation effect. Volunteers with a history of drug and alcohol abuse were also excluded from the study.

Twenty-five women and 17 men were recruited from the local Reading (UK) area, 3 volunteers dropped out during the first treatment period, one because of antibiotic treatment, one who could not keep the required visits, and one because of an allergic reaction from prescribed medication unrelated to the study. A further 3 volunteers dropped out during the second treatment period because of antibiotic treatment unrelated to the study and other personal reasons. For statistical analysis, 36 volunteers completed the study, and their data points were included; all other data points were omitted. Written consent was gained from all volunteers, and all were screened to ensure they were in good health. The research ethics committee at the University of Reading approved the study (Project Ref: UREC 12/24).

Study design. The study was conducted as a randomized, double-blind, placebo-controlled crossover that contained 2 treatment periods consisting of 28 d separated by a 21-d washout period. Volunteers consumed both interventions (probiotic and placebo) and were randomly allocated into group A or B. Group A consumed BC30 first for 28 d while group B consumed the placebo, both the placebo (microcrystalline cellulose) and BC30 were supplied by Ganeden Biotech and Schill Nutrition (Supplemental Figure 1). Neither the volunteers nor investigators were aware of which intervention was being taken. This also applied to the study statistician. Samples of feces and blood were collected at the beginning of each treatment (baseline) and after the 28-d treatment to assess comparative effects.

Probiotic BC30 and the placebo were both consumed once a day as capsules, each capsule of BC30 contained 1 × 10⁷ CFUs. They looked and tasted identical.

Volunteer diaries. During the study, volunteers were asked to maintain diaries that recorded stool frequency and consistency, abdominal pain, bloating, and flatulence, alongside mood diaries that monitored energy levels and emotional state. Concomitant medication was also noted.

Sample collection and preparation. Fresh fecal samples were collected and processed as described by Vulevic et al. (6).

Enumeration of fecal microbiota. Fluorescence in situ hybridization was performed according to Rycroft et al. (27). DNA oligonucleotide probes used were Bif164 (targeting most of the Bifidobacterium spp.) (28), LAB158 (targeting most of the Lactobacillus spp. and Enterococcus spp.), EREC482 [which targets Clostridium cocoides, Eubacterium rectale group, (Clostridium cluster XIVa and XIVb), CLIT135 [targeting the Clostridium lituseburense group (Clostridium cluster XI)] (29), BAC303 (targeting most of the Bacteroidaceae spp., Prevotellaceae spp., and some of the Porphyromonadaceae spp.) (30), Fpbrae645 (targeting Faecalibacterium prausnitzii and relatives), and Bcoa191 (targeting B. coagulans, including B. coagulans) (31, 32). The fluorescent DNA stain 4′,6-diamidino-2-phenylindole dihydrochloride (50 ng/μL) was used to detect total bacteria (31). All counts are presented as log₁₀ cells/mL sample.

Fecal FA analysis. SCFAs were converted into tertbutyldimethylsilyl derivatives, and analysis was performed with the extraction procedure described by Richards et al. (33). Quantification was performed with a Hewlett Packard GC (Agilent Technologies Ltd.) 5890 series II GC system with a volume of 1 μL injected at 220°C with a split of 100:1 onto a Rtx-1 (10 m × 0.18 mm with a 0.20-μm coating). Injection and detector temperatures were set at 275°C with initial column temperature being held at 63°C for 3 min then increasing by 10°C/min to 190°C; carrier gas helium was delivered at a flow rate of 0.7 mL/min.

Analysis of plasma for C-reactive protein concentration. Heparinized blood was centrifuged at 1600 × g at 4°C for 10 min, and plasma was removed and stored at −20°C until analysis. Samples were measured with an ILab 600 biochemical analyzer and a Quantex C-reactive protein ultrasensitive kit (Instrument Laboratories).

Preparation of PBMCs. Blood samples were drawn from fasted volunteers in sodium heparin tubes (Greiner Bio-One Ltd.), and cell extraction of peripheral blood mononuclear cells (PBMCs) was performed with the method detailed by Dong et al. (34).

Measurement of NK cell activity. NK cell activity of PBMCs was determined as detailed by Dong et al. (34). All samples were analyzed...
with a BC Accuri C6 flow cytometer (BD Biosciences), and results were processed with CFlow plus 1.0.265.264.15 (BD Biosciences). Results were obtained and expressed as percentage of cell death.

Measurement of cytokine production by PBMC cultures. Cytokine analyses were performed as described by Vulevic et al. (6). These included IL-6, IL-10, TNF-α, and IL-1β. All samples were analyzed with a BC Accuri C6 flow cytometer (BD Biosciences), and data processing was performed with FCAP Array 3.0 (BD Biosciences). Results were reported in pg/mL.

Analysis of calprotectin. Fecal samples were mixed with an appropriate volume of extraction buffer and mixed with a vortex mixer at 600 × g for 30 min. A 1.5-mL sample was centrifuged, and supernatant fluid was used for calprotectin analysis by using a calorimic, calprotectin-antigen detection ELISA kit according to the manufacturer’s protocol (Alpha Laboratories).

Statistical analysis. Statistical analysis was performed with GraphPad Prism 5 for Windows. Two-factor repeated-measures ANOVA was performed to test time, treatment, and treatment × time, with a CI of 95%. Bonferroni post-tests were performed to determine significant differences within treatments for the changes from baseline results. Stool and mood diaries were analyzed with chi-square tests at a CI of 95%. All data were analyzed to investigate the effect of consumption of BC30 over the whole treatment period on fecal microbiota and aspects of innate immune function compared with consumption of the placebo. Changes from baseline within a treatment were also analyzed.

Results

Effect on NK cell activity. Effector cells used to determine NK cell activity were mononuclear cells that were separated from heparinized blood; target cells were K562 cells. NK cell activity measured at each ratio of effector to target cells showed no significant differences between consumption of BC30 and placebo or significant changes between baseline and 28 d of treatment (Supplemental Table 1).

Effect on markers of systemic and intestinal inflammation. Stimulation of PBMCs by LPS initiated a significant increase from baseline in IL-10 after consumption of BC30 (P < 0.05) (Figure 1, Table 1). TNF-α significantly increased from baseline after consumption of the placebo (P < 0.05); this was not observed during BC30 consumption (Table 1). However, no significant differences were found in the overall effect of BC30 treatment compared with the placebo. Neither the results that analyzed C-reactive protein in plasma nor calprotectin in fecal samples were significantly different between the 2 treatments nor did they significantly change from baseline (Supplemental Table 2).

Effect on bacterial populations and SCFA production. F. prausnitzii populations were significantly greater in volunteers who consumed BC30 than volunteers who consumed the placebo (P = 0.02). In response to the consumption of BC30, Bacillus spp. showed a significant increase population from baseline (P < 0.007); however, the overall effect of BC30 consumption was not significantly greater than that seen during consumption of the placebo. No other bacterial groups showed any changes (Table 2). Fecal SCFA significantly increased from baseline in response to both treatments during the study; however, neither treatment elicited a greater response than the other (Table 3).

Analysis of gas-related symptoms during both treatments showed no differences between the 2 interventions (Supplemental Table 3). In addition, no effects on mood or consistency of stool samples were found throughout the study.

Discussion

This double-blind, placebo-controlled crossover study was undertaken to investigate fecal bacteriology and potential immunomodulatory effects of BC30. Aging encompasses many changes that have a detrimental effect on host health, such as a decrease in bifidobacteria, increase in clostridia, and altered gut permeability (2). Probiotic intervention was investigated in younger and seemingly healthier cohorts. Here, the interaction between probiotic use and dysbiosis was looked at in older persons in whom increased immunosenescence occurs. The probiotic BC30 was previously shown not only to survive adverse conditions associated with the upper GI tract but also to improve competitive exclusion of pathogens in the colon (23).

In the present study, populations of Bacillus spp. significantly increased over time, during consumption of BC30, as expected. Populations of F. prausnitzii were also significantly higher during BC30 supplementation. This could be due possibly to the production of lactic acid by B. coagulans which then influenced F. prausnitzii numbers (35). This is viewed as a positive effect because F. prausnitzii was shown to induce production of anti-inflammatory cytokines, specifically IL-10 (35). LPS-stimulated PBMCs showed a significant increase in IL-10 from baseline after consumption of BC30 (P < 0.05); however, this was not
significantly different from the placebo group ($P = 0.10$). All fecal FAs analyzed significantly increased in response to consumption of both BC30 and the placebo. However, levels in feces are not reflective of in vivo production because most is absorbed from the colon after production by bacteria. *F. prausnitzii* was the subject of studies that were based on health-promoting effects seen in patients with Crohn disease (35); this is likely to be because of its ability to use acetate, a major metabolite of fermentation of sugars, and to produce butyrate, leading to the possibility of cross feeding between bacteria. Sokol et al. (35) showed populations of *F. prausnitzii* in healthy volunteers to be $\sim 10.4 \times 10^9$ cells/g, much higher than populations seen during the baseline levels of this study, and more comparable with populations in persons with Crohn disease. This decline with age might be related to several aspects, including the increase in oxidative stress with age (36) or the reduced gut motility later in life, possibly associated with reduced fiber intake and therefore reduction of riboflavin in the diet, an important redox mediator for bacterial extracellular electron transfer and growth in the gut (37).

Jensen et al. (26) did not characterize the metabolites in the culture supernatant fluid of BC30; however, many beneficial effects were seen as previously mentioned. Difficulties in analysis of FAs in human studies are such that the body readily absorbs those that are needed, resulting in the inability to detect changes due to some dietary interventions. However, analysis of fermentative metabolites of several strains of *Bacillus subtilis* revealed higher levels of lactate and acetate (38), a substrate used by *F. prausnitzii*.

This study showed that acetate increased significantly over time; however, this did occur in both groups ($P < 0.0001$). The increase after consumption of BC30 was slightly more, although not significantly higher than the placebo group. This amount of acetate may be capable of adding, in some small part, to the increase in populations of *F. prausnitzii* by providing increasing available substrates.

Concentrations of IL-4, IL-6, and IL-10 increased after incubation with cell wall fractions of BC30 and metabolites after a 5-d lymphocyte proliferation culture (26). Similarly, a previous study showed that *B. coagulans* had the ability to stimulate the production of major anti-inflammatory cytokines (26). IL-10 is one of the more important anti-inflammatory cytokines, produced primarily from CD4$^+$ T helper type 2 cells and monocytes. It is an effective inhibitor of T helper type 1 cytokines, thereby inhibiting the production of proinflammatory cytokines (9). Suggested specific anti-inflammatory effects of IL-10 were shown to be due partially to down-regulation of inflammatory cytokines IL-1β and TNF-α, because IL-10 may modulate the innate immune responses critical for controlling infection (39). As previously mentioned, low-grade inflammation is associated with several diseases, including diabetes and obesity (40). Experimental animal models of endotoxemia have shown an increase in survival rates after IL-10 injections, and reduced systemic symptoms and neutrophil responses were also seen in human volunteers given IL-10 after endotoxin challenge (41, 42).

Among changes seen during immunosenesence is a reduction in the number of mature CD3$^+$ lymphocytes in circulation, poor immature CD45RA$^+$ T lymphocytes, and a decrease in the ability of peripheral blood lymphocytes to initiate apoptosis (43). NK cell activity acts as surveillance for viral infections and other pathogens and is a major contributor to cell-mediated immunity. A fall in NK cell activity with age was documented (44, 45). It is suggested that reduction in NK cell

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Placebo, ng/mL</th>
<th>BC30, ng/mL</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 28 d</td>
<td>Baseline 28 d</td>
<td>Time</td>
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<tr>
<td>IL-1β</td>
<td>1.9 ± 0.9</td>
<td>2.0 ± 0.7</td>
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<tr>
<td>IL-6</td>
<td>38 ± 24</td>
<td>38 ± 16</td>
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<tr>
<td>IL-10</td>
<td>0.4 ± 0.3</td>
<td>0.3 ± 0.9</td>
<td>0.62</td>
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<tr>
<td>TNF-α</td>
<td>0.5 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>0.15</td>
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1 Values are means ± SDs. $n = 36$. BC30, *Bacillus coagulans* GBI-30, 6086; PBMC, peripheral blood mononuclear cell. *Different from baseline, $P < 0.05$.**

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Placebo, log$_{10}$ cells/mL</th>
<th>BC30, log$_{10}$ cells/mL</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 28 d</td>
<td>Baseline 28 d</td>
<td>Time</td>
</tr>
<tr>
<td>Faecalibacterium prausnitzii</td>
<td>8.4 ± 0.3</td>
<td>8.3 ± 0.5</td>
<td>0.25</td>
</tr>
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<td>Bifidobacteria spp.</td>
<td>8.1 ± 0.4</td>
<td>8.3 ± 0.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>10.7 ± 0.2</td>
<td>10.6 ± 0.3</td>
<td>0.098</td>
</tr>
<tr>
<td><em>Bacillus</em> spp.</td>
<td>7.1 ± 0.6</td>
<td>7.3 ± 0.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Eubacterium rectale</td>
<td>9.1 ± 0.4</td>
<td>9.3 ± 0.5</td>
<td>0.007</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>8.8 ± 0.4</td>
<td>8.8 ± 0.7</td>
<td>0.23</td>
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<tr>
<td>Clostridium lituseburensi</td>
<td>6.6 ± 0.4</td>
<td>6.9 ± 0.4</td>
<td>0.52</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>8.1 ± 0.4</td>
<td>8.0 ± 0.6</td>
<td>0.07</td>
</tr>
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</table>

1 Values are means ± SDs. $n = 36$. BC30, *Bacillus coagulans* GBI-30, 6086; Placebo, microcrystalline cellulose. *Different from baseline, $P < 0.01$.**
activity in the older population is primarily affected by the population of circulating cells as opposed to their ability to initiate apoptosis in viral, tumor, and other pathogenic cells (46). This investigation showed that, at a ratio of effector to target cells of 100:1, there was a nonsignificant fall in NK cell activity in PBMCs after 3-wk consumption of unsupplemented milk (47). The same strain of B. coagulans sporogenes showed enhanced NK cell activity in a study with similar design after 3-wk consumption of unsupplemented milk, which returned to baseline after 3-wk consumption of unsupplemented milk (47). The same strain of L. rhamnosus HN001 increased leukocyte phagocytic activity in older volunteers after a 3-wk dose of 10^8 CFU/mL in milk, which returned to baseline after 3-wk consumption of unsupplemented milk (47). The latter strain of L. rhamnosus with Bifidobacterium lactis HN019 also showed enhanced NK cell activity in a study with similar design (45). This latter study showed an increase in CD56+ cells in PBMCs, no apparent trend was found between NK-like cells were seen to significantly increase as did NK cell activity of PBMCs, no apparent trend was found between NK activity and suppression of inflammatory cytokines (48). Low-grade inflammation occurs with advancing age, thus leading to an elevated level of inflammatory cytokines (48). Low-grade inflammation occurs with advancing age, thus leading to an elevated level of inflammatory cytokines.

Calprotectin was used to determine inflammatory status in individuals with GI disorders such as ulcerative colitis and inflammatory bowel disease, often in conjunction with C-reactive protein in plasma (49–52). Costa et al. (50) found positive correlations between fecal calprotectin and disease index for Crohn disease and ulcerative colitis (P < 0.01, r = 0.44 and P < 0.001, r = 0.6, respectively). Therefore, with recruitment of a healthy population of older volunteers, as was the case here, it is unlikely that such a correlation would be found in a low-grade inflammatory status. Indeed, C-reactive protein and calprotectin were not significantly different when comparing treatments (Supplemental Table 2).

Production of SCFAs in the large gut is seen as largely beneficial. A large proportion of SCFAs is absorbed by the host with only ~5% being excreted (53–55). Given that only a minority of SCFAs is excreted, it is not surprising that no significant differences were seen in feces between BC30 and the placebo. However, previous work in vitro showed a 3-fold increase in SCFAs produced from the fecal microbiota of volunteers on BC30, after a 28-d treatment period (56).

In conclusion, this study showed that BC30 administration to older volunteers led to a significant increase in beneficial bacterium F. prausnitzii (P = 0.03). We also showed a positive effect on the immune response, denoted by an increase in secretion of anti-inflammatory cytokine IL-10 after 28 d of supplementation by stimulated PBMCs (P < 0.05), and maintenance of NK cell activity compared with placebo which fell compared with baseline. This shows that dietary inclusion of probiotics such as BC30 may provide a beneficial option for enhancing markers of GI health in older persons. Further investigation could more definitively identify influences on systemic inflammation associated with aging.

Acknowledgments

SF, HAC, DK, DC, and GRG designed the research; EPN, SF, HAC, DK, and GRG drafted the paper. All authors read and approved the final manuscript.

References


