Probiotic *Bacillus subtilis* in Human Applications

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Bacillus subtilis has been used for more than 50 years in many different industrial applications, including farming, precision fermentation, and probiotic supplements. It is particularly attractive as a probiotic because of its ability to form shelf-stable, acid-resistant spores that lend to diverse applications in the food system. *B. subtilis* is the most ubiquitous species of the genus and can be isolated from a broad variety of environments including animal and human gastrointestinal (GI) tracts.

probiotic Bacillus subtilis gastrointestinal microbiota

1. *Bacillus subtilis* R0179

One of the earlier studies that included microbiota analysis [1] aimed to establish the oral dose–response tolerance and gastrointestinal viability of *B. subtilis* R0179 in human subjects (**Table 1**). The outcomes included daily questionnaire analysis (GI distress, cephalic, epidermal, ear-nose-throat, behavioral and emetic syndrome scores), survival of probiotic after gastrointestinal transit, and microbiota composition analysis. The study concluded that probiotic *B. subtilis* R0179 was well tolerated at all tested doses (0.1×10^9 to 10×10^9 CFU), did not persist in the human GI tract, and did not significantly affect local microbiota at the phylum level, though some changes occurred at lower taxonomic scales (i.e., increase in operational taxonomic units matching most closely to *Ruminococcus*). Interestingly, while *B. subtilis* R0179 did increase the prevalence of some taxa, it was more strongly associated with a decrease in other taxa. The authors speculate that supplementation might elicit some competitive inhibitory effects on the growth of undesired opportunistic pathogens.

Table 1. Overview of probiotic *B. subtilis* applications in human studies.

Reference	Study Design Subjects/Models	Probiotic Dose/Duration	Results
Patch et al. 2023 [2]	Randomized, double-blind, placebo-controlled, parallel-arm trial. Healthy adults (aged 18–75), *n* = 67, with self-reported diagnosis of functional gastrointestinal disorders (FGID).	B. subtilis BG01-4™ 5×10^9 CFU Daily dose for 4 weeks	Constipation in the probiotic group was significantly improved compared to placebo (33% vs.15%, respectively). Clusters for constipation (18% improvement), indigestion (11%), and dyspepsia (10%) were significantly improved in the probiotic group compared to the placebo.

Reference	Study Design Subjects/Models	Probiotic Dose/Duration	Results
Garvey et al. 2022 [3]	Randomized, double-blind, placebo-controlled, parallel-arm clinical trial. Healthy adults (aged 30–65), *n* = 76, with at least minimal complaints of abdominal bloating, burping, or flatulence.	B. subtilis BS50 2×10^9 CFU 1 capsule/day for 6 weeks	Improvement of 2 or more points in the 7-day, 3-item composite score according to GITQ (composite score for flatulence, bloating, and burping) between baseline and week 6 (47.4% vs. 22.2%). Compared to placebo, the proportion of participants with an improvement of 1 or more points in GITQ for burping (44.7% vs. 22.2%) and bloating (31.6% vs. 13.9%). There were no significant differences between groups for flatulence (47.4% vs. 44.4%). No change in bowel habits, sleep quality, respiratory infections, and blood markers for intestinal permeability, inflammation, and lipid profile.
Kordowski et al. 2022 [4]	Open-label, single-arm real-life exploratory study. Healthy adults, *n* = 192.	B. subtilis DSM32315 2×10^9 CFU (+290 mg L-Alanyl-L-Glutamine) 2 capsules/day for 4 weeks	Fasting glucose significantly decreased from pre- to post-treatment (96.92 ± 8.29 mg/dL vs. 94.58 ± 9.27 mg/dL, respectively). HbA1c significantly decreased from pre- to post-treatment (5.72% ± 0.27 vs. 5.65% ± 0.30). Postprandial glycemic response improved. Body weight (and BMI) significantly decreased. Relative abundance of Bacteroidetes significantly increased and Firmicutes decreased at post-treatment.
Dieck et al. 2021 [5]	Open-label, single-arm pilot study. Healthy men (aged 18–40), *n* = 18.	B. subtilis DSM32315 2×10^9 CFU (+290 mg L-Alanyl-L-Glutamine) Daily dose for 4 weeks	DSM32315 increased levels of butyrate and butyrate-producing taxa in gut microbiota. Plasma LDL-, total cholesterol, and LDL/HDL cholesterol ratio significantly decreased. Fasting levels of PYY (Peptide YY) and GLP-1 (Glucagon-like Peptide 1) significantly decreased.
Freedman et al. 2021 [6]	Randomized, double-blind, placebo-controlled, parallel-arm clinical trial. Healthy adults (aged 20–65), *n* = 44.	B. subtilis DE111 1×10^9 CFU 1 capsule/day for 4 weeks	Increase in anti-inflammatory immune cell populations in response to ex vivo LPS stimulation of PBMCs in the DE111 group. Overall perceived gastrointestinal health, microbiota, and circulating and fecal markers of inflammation (Il-6, sIgA) and gut barrier function (plasma zonulin) were largely unaffected by DE111 intervention.

Reference	Study Design Subjects/Models	Probiotic Dose/Duration	Results
Penet et at. 2021 [7]	Randomized, double-blind, placebo-controlled, parallel-arm clinical trial. Healthy adults (aged 18–75), *n* = 100, with self-reported symptoms of bloating, abdominal discomfort, and gas.	B. subtilis MB40 5×10^9 CFU 1 capsule/day for 4 weeks	No significant differences in bloating intensity, number of days with and duration of bloating, abdominal discomfort, and gas between MB40 and placebo groups. Physical limitation, vitality, and social functioning were significantly improved from baseline to week 4 in the MB40 group. At 2 weeks, physical functioning significantly improved in the MB40 group versus placebo. Clinical, but not statistically significant (10%), reductions in bloating intensity, number of days with abdominal discomfort, gas, bloating, and duration of gas, and 10% improvement in general health score in male sub-group receiving MB40 compared to placebo.
Trotter et al. 2020 [8]	Randomized, double-blind, placebo-controlled, parallel-arm clinical trial. Healthy adults (aged 18–65), *n* = 88.	B. subtilis DE111 1×10^9 CFU 1 capsule/day for 4 weeks	Significant reduction in total cholesterol and non-high-density lipoprotein cholesterol in DE111 group. Improvements in endothelial function and in low-density lipoprotein cholesterol.
Paytuvi-Gallart et al. 2020 [9]	Randomized, double-blind, placebo-controlled, parallel arm study. Healthy children (aged 2–6), *n* = 101, attending daycare.	B. subtilis DE111 1×10^9 CFU 1 capsule/day for 8 weeks	Microbiome composition analysis: alpha diversity increased in probiotic group; no significant changes in the overall microbiome equilibrium; six taxa (at the genus level) significantly increased after probiotic intake, and three taxa significantly decreased.
Hatanaka et al. 2020 [10]	Randomized, double-blind, placebo-controlled, parallel-arm study. Healthy adults, *n* = 44.	B. subtilis C-3102 4.8×10^{10} CFU Daily dose for 4 weeks	Body fat percentage was significantly lower in the C-3102 group than in the placebo group at 2 weeks after probiotic. Mean corpuscular hemoglobin level was significantly higher, and cholinesterase, total cholesterol, and triglyceride levels were significantly lower 2 weeks after intake in the C-3102 group than in the placebo group. Direct bilirubin was significantly higher and total cholesterol significantly lower 4 weeks after intake in the C-3102 group than in the placebo group. No significant changes in other measured parameters.
Townsend et al.	Randomized, double-blind, placebo-controlled,	B. subtilis DE111 1×10^9 CFU	Supplementation with DE111 does not affect plasma amino acid response

Reference	Study Design Subjects/Models	Probiotic Dose/Duration	Results
2020 [11]	parallel-arm study. Recreationally active adults, *n* = 22.	1 capsule/day for 28 days	following acute whey protein ingestion.
Toohey et al. 2020 [12]	Randomized, double-blind, placebo-controlled, parallel-arm study. Division I college female athletes, *n* = 23.	B. subtilis DE111 1×10^9 CFU 1 capsule/day for 10 weeks	Significant reduction in body fat % in DE111 supplementation group (−2.05 ± 1.38%) compared with placebo (0.2 ± 1.6%). No other differences between probiotic and placebo groups were observed.
Townsend et al. 2018 [13]	Randomized, double-blind, placebo-controlled, parallel-arm study. Division I college male athletes, *n* = 25.	B. subtilis DE111 1×10^9 CFU 1 capsule/day for 12 weeks	TNF-α concentrations were significantly lower after DE111 compared to placebo. No significant group differences in any other measured biochemical markers. No effect on body composition, performance, hormonal status, or gut permeability.
Takimoto et al. 2018 [14]	Randomized, double-blind, placebo-controlled, parallel-arm study. Healthy postmenopausal Japanese women (aged 50–69), *n* = 76.	B. subtilis C-3102 3.4×10^9 CFU Daily dose for 24 weeks	Significant increase in total hip BMD in probiotic group (placebo = 0.83 ± 0.63%, C-3102 = 2.53 ± 0.52%). Significantly lower uNTx probiotic vs. placebo group at 12 weeks of treatment. A trend of a decrease in the bone resorption marker TRACP-5b when compared with the placebo group at 12 weeks of treatment. No change in markers of bone formation, BAP and iPTH. Changes in microbiota composition after C-3102 supplementation.
Hatanaka et al. 2018 [15]	Randomized, double-blind, placebo-controlled, parallel-arm study. Healthy adults (aged 20–79), *n* = 82, with loose stools.	B. subtilis C-3102 2.2×10^9 CFU Daily dose for 8 weeks	Stool frequency per day significantly decreased after C-3102 treatment. Stool quality (measured by BBC scores) significantly improved. Abdominal sound symptoms (reported by GSRS) significantly decreased. Change in microbiota composition following C-3102 treatment.
Cuentas et al. 2017 [16]	Randomized, double-blind, placebo-controlled, parallel-arm clinical trial. Healthy adults (aged 18–65), *n* = 50, with occasional constipation and/or diarrhea.	B. subtilis DE111 1×10^9 CFU 1 capsule/day for 90 days	By day 90, the proportion of normal stools (43.1%) to non-normal stools (6.13%) in the DE111 group differed significantly from placebo group (evaluated by BSC). The proportion of normal stools increased from week 1 to the last week in DE111 group (37.36% to 43.1%) vs. no change in placebo (33.77% to 35.43%).

Reference	Study Design Subjects/Models	Probiotic Dose/Duration	Results
Lefevre et al. 2015 [17]	Randomized, double-blind, placebo-controlled, parallel-arm study. Healthy elderly (aged 60–74), *n* = 100.	B. subtilis CU1 2×10^9 CFU 1 capsule/day for 10 days, intermittent with 18 days, no ingestion, for 4 months	No significant decrease in mean number of days of reported for CID symptoms over the 4 months of study. B. subtilis CU1 significantly increased fecal and salivary secretory IgA concentrations compared to the placebo. No statistically significant differences in the plasma concentrations of cytokines (IL-1beta, IL-4, IL-6, IL-8, IL-10, IL-12p70, IgA, and TNF-alpha) between the probiotic and the placebo groups from pre- to post-supplementation.
Hanifi et al. 2015 [1]	Randomized, double-blind, placebo-controlled, parallel-arm clinical trial. Healthy adults, *n* = 81.	B. subtilis R0179 0.1×10^9, 1.0×10^9, or 10×10^9 CFU 1 capsule/day for 4 weeks	The scores of the GI distress syndrome between placebo, 0.1, 1.0, and 10×10^9 CFU groups were equivalent. The 0.1×10^9 CFU (0.3 ± 0.1) group was not equivalent to the 1×10^9 (0.6 ± 0.1). The abdominal pain, reflux, diarrhea, indigestion, and constipation syndrome were equivalent across all periods by treatment comparisons. Microbiota composition was affected by probiotic treatment.

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conditions, but increased ability to respond to inflammatory stimuli. Other cell types were unchanged, as were circulating inflammatory markers and markers of intestinal permeability. These data suggest a possible effect of probiotic *B. subtilis* DE111 on the immune system of the intestinal tube, which warrants further exploration.

A microbiota composition analysis following 8 weeks of *B. subtilis* DE111 supplementation was performed on stool samples of healthy children attending daycare (**Table 1**) [9]. At the phylum level, the intake of *B. subtilis* DE111 significantly increased microbial community diversity (Shannon and Simpson indices) without globally shifting the equilibrium of the microbiome. However, there were changes in the differential abundance of some taxa at the genus level that could be considered beneficial. Members of *Alistipes*, *Bacteroides*, *Parabacteroides*, *Odoribacter*, and Rikenellaceae increased in the probiotic group. According to the authors' statements, representatives of these taxa are implicated in immune regulation and reduction of inflammation, while the decreased taxa that included *Eisenbergiella*, *Lactobacillales*, and *Streptococcaceae* may be considered pro-inflammatory. Thus, the increased diversity and specific taxa changes suggest a shift towards a healthier microbiota composition following probiotic supplementation. A decrease in the *Bacillota/Bacteriodota* (formerly referred to as *Firmicutes/Bacteroidetes*) ratio following probiotic supplementation was also observed. Gastrointestinal health, or any other characteristics, were not evaluated in this study.

Notation: CFU—colony-forming units. GSRS—Gastrointestinal Tolerance Questionnaire. HbA1c—glycated hemoglobin (a stable indicator of glucose status and indicator for diabetes). Caco-2—cultured human intestinal epithelial cells. LPS—lipopolysaccharides. PBMC—peripheral blood mononuclear cells. BMD—bone mineral density. uNTX—urinary type I collagen cross-linked N-telopeptide. TRACP 5b—tartrate-resistant acid phosphatase isoform 5b. BAP—bone alkaline phosphatase. iPTH—intact parathyroid hormone. BSC—Bristol stool chart. GSRS—gastrointestinal symptom rating scale. CID—common infectious disease.

The effects of *B. subtilis* DE111 on stool profiles were investigated by Cuentas et al. [16] in healthy adults suffering from occasional constipation and/or diarrhea (**Table 1**). The study evaluated GI health using the Bristol stool chart and digestive health questionnaires. Blood samples were collected at three timepoints during the study and analyzed for C-reactive protein, lipid profiles, and comprehensive metabolic panels. The authors reported

improvements in stool type (normal vs. abnormal) in the probiotic group. No other effects of supplementation on GI health were reported. All blood markers stayed within normal refence ranges for both probiotic and placebo groups, and no changes were recorded for biomarkers throughout the study. According to the authors' conclusions, *B. subtilis* DE111 can help to maintain gastrointestinal health by improving occasional constipation and/or diarrhea.

An exploratory study on the use of *B. subtilis* DE111 supplementation in college athletes and physically active adults was completed at the Human Performance Laboratory at Lipscomb University. A study by Townsend et al. [13] was the first to examine the potential benefits of probiotic *B. subtilis* DE111 supplementation in male college athletes during offseason training (**Table 1**). Body composition was evaluated as an indicator of athletic status pre- and post-training season. Dynamic strength, ten-yard sprint, pro-agility test, and standing long jump were primary outcomes for testing athletic performance. Biochemical analyses included measurements of salivary immunoglobulins SIgA and SIgM, which were used as indicators of mucosal immunity. Blood samples were analyzed for the following markers: TNF-α, IL-10, zonulin, testosterone, and cortisol. Though, *B. subtilis* DE111 supplementation was well tolerated by athletes, it did not affect body composition, performance, hormonal concentrations, and gut permeability, but it did result in lowering blood concentrations of TNF-α. According to the authors' conclusions, attenuating circulating TNF-α concentrations in college athletes following offseason training may be beneficial; however, the relevance of this effect on overall health is still unexplored. A follow-up study [12] examined the effects of the similar *B. subtilis* DE111 supplementation on female college athletes during their offseason resistance training. The measured outcomes were limited to body composition and resistance performance. No analysis of biological samples was conducted in this study. Probiotic supplementation did not affect athletic performance, but it improved body composition (**Table 1**). The next study conducted in the same Human Performance Lab [11] determined if probiotic *B. subtilis* DE111 supplementation influenced plasma amino acid (AA) response to acute whey protein ingestion in physically active adults. Fasting blood samples were collected at baseline and post-treatment visits from time zero at 15 min intervals for 2 h after ingestion of 25 g of whey protein dissolved in 400 mL of water. The following amino acids were quantified in blood plasma: leucine, branched-chain AA, essential AA, and total AA. The study did not find any significant differences between treatment and placebo groups and concluded that DE111 supplementation does not affect protein utilization in exercising adults.

Trotter et al. [8] explored the potential health effects of probiotics administered alone or concurrently with bacteriophages. One of the probiotics tested was *B. subtilis* DE111, which was administered as a single strain as one arm of this study. A pilot exploration aimed to determine whether the four-week consumption of (1) maltodextrin placebo; (2) *Bifidobacterium lactis* alone, or (3) *Bifidobacterium lactis* in combination with a cocktail of *E. coli*-targeting bacteriophages; and (4) *Bacillus subtilis* DE111 altered risk factors for CVD. The primary outcome measures included blood pressure, endothelial function, and plasma lipid profiles. Researchers hypothesized that probiotic consumption would improve one or more measures of cardiovascular function in a healthy adult population, and that simultaneous supplementation with *E. coli*-targeting bacteriophages might further enhance these beneficial cardiovascular effects [8]. Interestingly, the authors did not find any significant changes in measured CVD parameters among individuals consuming *Bifidobacterium lactis* with or without bacteriophages. However, supplementation with *B. subtilis* DE111 resulted in a significant reduction in total cholesterol and non-

high-density lipoprotein cholesterol relative to baseline measures. There were also modest, but clinically relevant, improvements in endothelial function and low-density lipoprotein cholesterol following the consumption of *B. subtilis* supplements. The authors concluded that *B. subtilis* DE111 supplementation may be beneficial for improving risk factors associated with CVD (**Table 1**).

3. *Bacillus subtilis* C-3102

Three human studies have looked at the various health impacts of consuming *B. subtilis* C-3102 in Japanese cohorts. Takimoto et al. investigated the effect of this probiotic on bone health in post-menopausal women [14] (**Table 1**). The outcomes included bone mineral density (BMD) measured at the lumbar spine and hip using dual-energy X-ray absorptiometry and markers of bone turnover. Markers of bone resorption included urinary type I collagen cross-linked N-telopeptide (uNTx) and serum tartrateresistant acid phosphatase isoform 5b (TRACP-5b). The markers of bone formation included serum bone alkaline phosphatase (BAP) and intact parathyroid hormone (iPTH). Also, microbiota composition analysis was performed on fecal samples. The measurements and sample collection were performed at baseline and at 12-week and 24-week treatment periods. After 24 weeks of probiotic supplementation, total hip BMD significantly increased; however, there was no significant difference between the probiotic and placebo groups for lumbar spine BMD. Both markers of bone resorption were decreased in the probiotic group after 12 weeks of supplementation; however, there was no significant difference between the placebo and C-3102 groups in these two bone resorption markers at 24 weeks of treatment. No significant changes were recorded for the bone formation markers at either timepoints. Gut microbiota analysis showed a decrease in Chao1 and Shannon indices of alpha-diversity after 24 weeks of probiotic treatment. The differential abundance analysis at the genus level showed a relative increase in *Bifidobacterium* in the C-3102 group at 12 weeks of treatment when compared with the baseline, and genus *Fusobacterium* significantly decreased in the C-3102 group at 12 and 24 weeks of treatment when compared with the baseline. The authors concluded that the results were suggestive of the positive effects of probiotic strain *B. subtilis* C-3102 on bone health in post-menopausal women. Also, they are suggestive that *B. subtilis* C-3102 modulates host-gut microbiota; however, specific microbiota modifications did not significantly correlate with BMD or bone turnover markers.

The second Japanese study evaluated the possible preventive effects of the ingestion of *B. subtilis* C-3102 on chronic diarrhea in healthy volunteers with loose stools [15]. The study utilized gastrointestinal health and quality of life questionnaires, Bristol stool chart, determination of fecal water content, and microbiota analysis. Several parameters were significantly improved via C-3102 treatment (**Table 1**). Gut microbiota analysis revealed no changes in alpha-diversity after 8 weeks of C-3102 ingestion. However, the relative abundance of two genera in the gut microbiota (*Lachnospira* and *Actinomyces*) was significantly changed: *Lachnospira* increased in relative abundance, and *Actinomyces* decreased post treatment. The authors concluded that improvement in bowel habits may be related to the modifications in gut microbiota in response to C-3102 ingestion.

Finally, the third study evaluated the safety of excessive consumption of *B. subtilis* C-3102 in healthy volunteers [10]. The outcomes were based on anthropometric parameters, blood hematological tests (including complete white blood cell count), very extensive blood biochemical analyses, urinalyses, and measurements of bone mineral

density. Subjects also completed a medical questionnaire to determine their health status at three assessment points (baseline, 2-week, and 4-week). The major findings are presented in **Table 1**. In addition, some differences were observed between males and females: the cholinesterase levels were significantly higher in female subjects in the C-3102 group than in the placebo group after 2 weeks of probiotic intake, while there were no changes in the blood parameters in males. No changes were reported for medical questionnaire reports, urinalysis, and BMD. Moreover, all the reported changes remained within clinical reference ranges and did not indicate any medical conditions or complications. Therefore, the consumption of excessive amounts of probiotic *B. subtilis* C-3102 was determined to be safe by the investigators.

4. *Bacillus subtilis* BS50

A unique strain *B. subtilis* BS50 that was isolated from soil and showed promise as a probiotic was evaluated by Brutscher et al. [18] in preclinical trials. Before any clinical trial of a new strain can occur, the safety profile should be evaluated in preclinical testing. This study screened the genome for genes encoding virulence factors, *Bacillus* toxins, and antibiotic resistance. Cultured human intestinal epithelial cells (Caco-2) were used to perform viability and permeability assays. Several gene clusters were identified that are involved in the biosynthesis of secondary antimicrobial metabolites that do not present any harm to the intestinal cells. The study concluded that BS50 was unlikely to negatively affect human enterocytes or disrupt gut barrier integrity. A follow-up clinical trial investigated the safety and efficacy of the daily supplementation of *B. subtilis* BS50 for 6 weeks in healthy adults who had mild gastrointestinal distress before the start of supplementation [3]. Intestinal distress was defined as having a combined score of 3 or more for abdominal bloating, burping, and flatulence by assessment using the Gastrointestinal Tolerance Questionnaire (GITQ) (**Table 1**). Besides GI symptoms assessment using the GITQ, the other outcomes included a bowel habit diary, sleep quality and respiratory infection questionnaire, and blood sample analysis. Fasting blood samples were analyzed for markers of intestinal permeability (zonulin, occludin, and LBP), inflammatory markers (CRP, IL-8, IL-6, IL-10, IFN-γ, and TNF-α), and lipid profiles (TG, total-C, HDLC, LDL-C). Six weeks of *B. subtilis* BS50 supplementation significantly improved GI symptoms (bloating, burping, and flatulence) as assessed using the GITQ (**Table 1**). Bowel habits did not significantly change with the intervention. The number of bowel movements slightly increased during the weeks of supplementation but was not significantly different from placebo. No changes were recorded for the symptoms of discomfort during bowel movement, straining, or feeling of incomplete evacuation. There was no effect of supplementation on the quality of sleep and respiratory infections. The markers of intestinal permeability, lipid profiles, and most inflammatory markers were not affected by the treatment. There was a slight increase in circulating anti-inflammatory cytokine, IL-10, in the *B. subtilis* BS50 group vs. placebo, but it failed to achieve statistical significance. The authors concluded that daily oral supplementation with probiotic *B. subtilis* BS50 was safe and well tolerated and improved the composite score for bloating, burping, and flatulence, compared to placebo. Supplementation may be recommended to alleviate gas-related gastrointestinal symptoms in a generally healthy population.

5. *Bacillus subtilis* MB4

A subset of gastrointestinal distress symptoms, i.e., bloating, abdominal discomfort, and gas, were the primary outcomes in Penet et al.'s study examining the effects of *B. subtilis* MB40 supplementation (**Table 1**) [7]. During the 4 weeks of treatment, participants completed three questionnaires daily: a modified Abdominal Discomfort, Gas, and Bloating (mADGB) questionnaire, a modified Gastrointestinal Symptoms Rating Scale (mGSRS), and a Bowel Habits Diary (BHD). For the quality-of-life and general health assessment, a modified RAND SF-36 questionnaire was filled out at baseline and weeks 2 and 4. Blood samples were analyzed for hematologic and chemical parameters to evaluate the safety of probiotic administration. All blood parameters were within the normal clinical ranges before and after the treatment. At the end of 4 weeks, the study did not find any significant differences between MB40 and placebo groups in the average weekly number of days with bloating, bloating intensity, or abdominal discomfort and gas. However, the male sub-group in MB40 showed clinically relevant improvements in some of those parameters and also reported improvements in some quality-of-life characteristics and the general health score (**Table 1**). The authors concluded that *B. subtilis* MB40 supplementation was safe and well-tolerated but did not significantly improve major outcome parameters in the MB40 group versus the placebo.

6. *Bacillus subtilis* CU1

Lefevre et al. investigated the effect of probiotic strain *B. subtilis* CU1 intake on resistance to common infectious diseases (CIDs) in healthy, free-living seniors [17]. The primary outcome was the mean cumulative number of days with CID in participants. The secondary outcomes determined the effect of *B. subtilis* CU1 intake on the stimulation of the mucosal and systemic immune response by measuring intestinal and salivary sIgA levels and serum cytokine levels in a subset of 44 subjects out of 100 subjects completing the trial (**Table 1**). The primary outcomes did not show any statistically significant difference between the probiotic and the placebo groups in mean duration, intensity, and frequency of CID during the observation period. In the subset of 44 individuals, the frequency of respiratory infections was significantly lower in the probiotic group compared to the placebo group. The significant increases were also reflected in intestinal and salivary SIgA levels in the probiotic group compared to the placebo group. Also, IFN-gamma concentrations significantly increased in the probiotic group after 10 days of probiotic consumption, while no change was observed for the placebo. The results of the study indicate that *B. subtilis* CU1 can modulate the immune response in the elderly population; however, no definite conclusion can be made about the effect of *B. subtilis* CU1 supplementation on CID.

7. *Bacillus subtilis*-Containing Synbiotic Products

Synbiotics are a combination of a probiotic with a prebiotic which can act either synergistically, where the prebiotic specifically supports the growth and survival of the probiotic strain, or in a complementary manner, where each component exerts independent beneficial effects on the GI tract. A novel complementary synbiotic formulation of the strain *B. subtilis* DSM32315 was tested on human subjects in a single-arm study (**Table 1**) [5]. The synbiotic formulation was developed in the laboratory in Germany and included *B. subtilis* DSM32315 and L-Alanyl-L-Glutamine as main ingredients, plus plant extracts, minerals, and vitamins (SAMANA® Force, Evonik, Darmstadt, Germany). By using a probiotic in combination with an amino acid, the researchers were targeting butyrate-

producing commensal microbes that are able to process peptides and amino acids as substrates in the pyruvate/acetyl-CoA pathway. The study was based on the presumption that probiotic *B. subtilis* can modulate the human colonic microbiota towards an increase in pro-butyrogenic species that can selectively use a stable, non-fiber substrate L-alanyl-L-glutamine for butyrate production. Healthy males with "health unconscious eating patterns" consumed the formulation daily for 4 weeks. The blood and fecal samples were collected at baseline, 2-week, and 4-week timepoints. The microbiota analysis and quantification of short-chain fatty acids (SCFAs) were performed on the fecal samples. Blood was analyzed for lipid profiles, fasting glucose, and the hormones PYY (Peptide YY) and GLP-1 (Glucagon-like Peptide 1).

A follow-up exploratory human study used the same synbiotic supplementation [4]. A cohort of men and women consumed two capsules of synbiotic formulation (SAMANA® Force, Evonik, Darmstadt, Germany) daily for 4 weeks. Blood and fecal samples were collected at baseline and post-treatment. Participants filled out several questionnaires throughout the intervention, which captured the supplement's effects on digestive parameters, frequency and consistency of bowel movements, feelings of hunger and satiety, well-being, and physical performance. The main results for the total cohort are presented in **Table 1**. Briefly, investigators showed that the supplement use was associated with significant decreases in fasting blood glucose and glycated hemoglobin, HbA1c, a stable marker of blood glucose and indicator of the diabetic status of an individual. However, based on the baseline fasting glucose levels and HbA1c values, participants were divided into two subgroups: prediabetic (*n* = 62) and non-prediabetic (healthy). The subgroup analysis revealed that improvements in fasting glucose, HbA1c values, and glycemic response were driven by the prediabetic subgroup. Significant weight loss was recorded for both subgroups, and the overall average was 1.07±2.30 kg; specifically, the differences before and after the study were 1.47 ± 2.82 kg and 0.87 ± 1.97 kg, respectively, for prediabetic and healthy participants. Questionnaires did not record major changes during supplementation, except for the feeling of hunger, which was reduced significantly towards the end of the study. Microbiome analysis also reflected different reactions between subgroups. Shannon's index of alpha-diversity was increased significantly post-treatment in the healthy subgroup but did not change in the prediabetic subgroup. In the total population, *Bacteroidota* (formerly *Bacteroidetes*) significantly increased throughout the observation, and the abundance of *Bacillota* (formerly *Firmicutes)* decreased, and these changes were largely driven by the prediabetic population. In healthy participants, no significant differences were observed at the phylum levels between the start and end of the observation. The authors suggested that the tested supplement may be recommended for the management of hyperglycemia and metabolic syndrome.

8. *Bacillus subtilis* as a Postbiotic

A new proprietary strain of inactivated *B. subtilis* BG01-4™ high in branched-chain fatty acids (BCFA) was used to treat participants with self-reported diagnoses of functional gastrointestinal disorders (FGIDs) [2]. The effects were evaluated based on the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire filled out at baseline, 2-week, and 4-week timepoints. Three primary outcomes included Total GSRS score, GSRS-constipation, and GSRS-diarrhea, while secondary outcomes were indigestion, dyspepsia, and abdominal pain syndrome. Based on

the results of the study (**Table 1**), the authors concluded that postbiotic *B. subtilis* BG01-4™ can improve specific symptoms of constipation and related GI dysfunction in people with a FGID.

A summary of the results of the above-presented studies in terms of *B. subtilis*'s possible positive effects on gastrointestinal health shows improvements in constipation, indigestion, and dyspepsia [2]; bloating and burping [3]; quality of life and physical functioning related to gastrointestinal conditions [7]; stool frequency and quality [15]; abdominal sound symptoms [15]; and the proportion of normal stools [16]. Many studies reported shifts in microbiota composition following *B. subtilis* administration [1][5][9][14][15]; however, these modifications warrant further analyses in terms of their overall effects on gastrointestinal health.

References

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