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Efficacy of The Probiotic *Bacillus Subtilis* DG101 Against Intestinal Discomfort and Constipation in Healthy Adults: A Double-Blind, Placebo-Controlled Study

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Abstract

The incorporation of beneficial gut bacteria (i.e., probiotics) to a person's diet is recognized as a healthy and scientifically proved alternative to improve intestinal well-being. The spore-forming probiotic, Bacillus subtilis DG101 is incorporated into foods, beverages, and used in dietary supplements. To study the effects of this probiotic to prevent abdominal discomfort, quality and frequency of intestinal evacuations, we performed a randomized, double-blind, placebo-controlled, parallel clinical study of 180 healthy adults for 4 weeks. The participants were provided of two forms (Bristol Stool Scale, BSS and Gastrointestinal Symptom Rating Scale, GSRS) to complete once a week. Compared to placebo, 1 × 10^8 colony-forming units (CFU) of Bacillus subtilis DG101 per day improved the BSS score in ~ 22 % and ~24 % in men and female in the probiotic group, respectively. It is observed an improvement in the average frequency of stool deposition per week, reaching at the end of the study, 4.7 for men and 3.5 for female. These values represent a decrease in ITT of men and female allocated in the probiotic groups of ~ 12 h and ~ 19 h, respectively. Analysis of low intestinal discomfort measured by the abbreviate GSRS showed that the subjects who incorporated Bacillus subtilis DG101 have much less intestinal discomfort compared with the subjects in the placebo group. It was observed a decrease in the scores for low intestinal discomfort of individuals (men and female) in the probiotic groups of ~ 40.0 %, compared with the placebo groups. These results suggest that dietary supplementation of 1 × 10^8 CFU Bacillus subtilis DG101 is beneficial to prevent abdominal discomfort, and improve quality and frequency of intestinal evacuations in healthy adults.

Keywords: *Intestinal transit, stool consistency, constipation, diarrhea, probiotics, B. subtilis DG101.*

Introduction

An increasing number of healthy individuals experiment sporadic episodes of intestinal discomfort (e.g., low intestinal transit, diarrhea, abdominal pain, bloating) which seem to occur more frequently than before [1]. Intestinal discomfort in healthy individuals occur in the absence of organic abnormalities, and the different unpleasant gut sensations (e.g., constipation) can affect both mental and physical performance of the individual [2,3]. The possible causes of unpleasant gut feeling are multiple, probably related to the influence of the modern lifestyle (e.g., consume of ultra-processed foods, stress, urban pollution, and the accelerated life of each person day) [1,4,5]. Therefore, it is frequent that healthy people suffering of transitory or sporadic gut symptoms do not visit to the doctor and prefer consume laxatives, functional foods and dietary supplements because of their temporary and mild unpleasant intestinal sensations [6-8]. The intestine is not an aseptic organ; the gut lumen and mucosa are inhabited by trillions of microorganisms (i.e., bacteria, fungi, parasites, and viruses), recognized as the gut microbiota [9,7,8]. During the recent years, the role of the gut microbiota in health and disease has become unquestionable and indubitable [7,8]. The incorporation of beneficial gut bacteria (i.e., probiotics) to a person's diet is recognized as a healthy and scientifically-proved alternative to improve intestinal well-being [10-13]. Probiotics are defined as live microorganisms that, when administered in adequate quantities, and arriving alive at their site of action (for example, the intestine), produce a health benefit on the consumer [14]. Within probiotic bacteria, there are two broad categories: non-spore-forming probiotic bacteria (for example, lactic acid bacteria such as *Bifidobacterium*) and spore-forming probiotic bacteria of the *Bacillus* genus such as *B. subtilis* [11,15]. Probiotic *Bacillus* have a long and safe history of use and beneficial effects on human beings [15,13,16]. In this work, we present the results of a randomized, double-blind controlled-study on the effectiveness of the human probiotic *B. subtilis* DG101® [16,17,18-20] for improving the intestinal well-being of healthy individuals.

Subjects and Methods

Two-hundred and fifty-eight (n = 258) healthy individuals, 18 – 75 years of age, were recruited by personnel of the Instituto de Nutrición Grupo Cardinali (INGC) over a 4-month period (September 2023 to December 2023) by letter, electronic email or WhatsApp. The inclusion criteria were normal body mass

index (BMI; between 18.0 and 25.0 kg/m^2); healthy determined by physical examination, clinical biochemistry, liver and kidney functional tests; no consumption of probiotics, postbiotics or prebiotics for 3 months before the start of the study (scheduled to start in February 2024). The exclusion criteria were pregnant and breast-feeding females; history of chronic diseases (i.e., diabetes, celiac disease, thyroid disorders, cancer); chronic inflammation; inflammatory bowel disease (IBD), irritable bowel syndrome (IBD); Crohn´s disease; small intestine bacterial/fungal/methanogenic overgrowth (SIBO/SIFO/IMO); gastrointestinal or duodenal ulcers; intestinal obstruction; colostomy or anal stenosis; gastrointestinal bleeding; infection; recent significant loss of weight; an any other condition that would affect the individual safety, ability or predisposition to follow and complete the study. The work consisted of a double-blind, placebo-controlled study of 4 weeks of duration between February 2024 and March 2024. After confirming eligibility, the selected participants (n = 180, Figure 1) were randomized to receive probiotic *B. subtilis* DG101® contained in 20 drops (~1 ml) of Kyojin Probiotic® (1 x 10^8 colony forming units (CFU) / ml of *B. subtilis* DG101®, 0.01 % lemon essence, 0.28 % citric acid –acidulant-, and 0.075 % potassium sorbate / 0.025 % sodium benzoate –preservatives, and 99.6 % distilled water; Register number RNPA 21-119482). The placebo group consumed 20 drops of the same solution than the probiotic group except that probiotic *B. subtilis* DG101® was excluded from this placebo formulation. The dose consisting of the indicated 20 drops corresponding to each group (probiotic and placebo) were diluted, and consumed, in ~ 100 – 200 ml of water, once a day in the morning. The participants were instructed to maintain unaltered physical activity and diet habits (suggested by the medical and nutritional staff of INGC) throughout the four weeks of the study. Participants were provided of two forms that had to be completed once a week. The forms consisted of the Bristol Stool Scale (BSS) [2, 12, 21] and Gastrointestinal Symptom Rating Scale (GSRS) [2,22,12,3,13]. The BSS helps to assess how long the stool has spent in the bowel, and the stool shape and appearance. The BSS comprises seven types or categories of stools (i.e., Types 1 to 7). Types 1 - 2 suggest constipation, Types 3 - 4 indicates ideal stools, easy to evacuate; and types 5 -7 indicate diarrhea [2,22,12, 3,13]. Physiological or desirable scores of BSS for a healthy person should be between 3 and 4 [2,22,12,3,13].

The GSRS comprises 15 questions with seven options each [2,22,12,3,13]. Therefore, the GSRS goes from a minimal value of 15 to a maximum value of 105 points (i.e., "not discomfort at all" to "very severe discomfort", respectively). A desirable score for each GSRS question should be between 1 and 3 points (i.e., "not discomfort at all" to "mild discomfort", respectively) and therefore a healthy individual should reach a global GSRS score not higher than 45 points. Six out of the 15 questions of the GSRS chart are related to lower intestinal discomforts (i.e., constipation, diarrhea, loose stools, hard stools, urgent need to have a bowel movement, and sensation of not completely emptying the bowel). In this work, focused on intestinal discomfort, the participants were provided with a list of the indicated 6 questions of the GSRS (i.e., abbreviate GSRS for low GI symptoms) referring to low intestinal discomfort. In this case, the minimal value of the abbreviate GSRS for low intestinal discomfort goes from a score of 6 (i.e., "not discomfort at all") to a maximum score of 42 (i.e., "very severe discomfort"). The values of abbreviate GSRS for low GI symptoms in healthy individuals should fit in the range of 6 to 18 (i.e., "not discomfort at all" and "mild discomfort", respectively).

The participant visiting to the nutritional clinic (i.e., INGC) were scheduled at the beginning of the study and its end (i.e., week zero and week four, respectively). The probiotic *B. subtilis* DG101® (Kyojin® Probiotic) and the placebo solutions were provided by Kyojin S.A. (www.kyojin.com.ar) in indistinguishable dropper cap bottles of 30 ml of content. The bottles (intervention and placebo) were labeled with a code (in accordance with the Good Clinical Practice Guidelines) by members of the staff of the INGC who were blinded in conducting any phase of the study. The sample size of this study was 180 healthy participants which were equally randomized in 2 groups of 90 participants in a double-blind manner (Figure 1). All participant completed the study, and not undesirable success or site effects were reported during the time study. Linear regression (ANOVA) was used to test differences in response to treatments (probiotic and placebo). The obtained values are presented as means ± S.D. unless otherwise specified. A p-value of < 0.05 was considered statistically significant. All analyzes were performed using the Statistical Analysis System (SAS 9.2; SAS Institute, Cary, NC, USA).

Results and Discussion

To evaluate the efficacy of *B. subtilis* DG101® (as a food supplement incorporated into the daily diet of a healthy person) to prevent intestinal discomfort, we asked each participant (total n = 180, Figure 1) to complete BSS and abbreviate GSRS forms at the beginning and the end of the study (weeks 0 and 4, respectively). The BSS allows the classification of stools into seven categories or types. Types 1 and 2 indicate constipation, types 6 and 7 diarrhea, and types 3 and 4 correspond to a healthy person whose stool is easy to evacuate and does not content liquid excess.

Figure 1

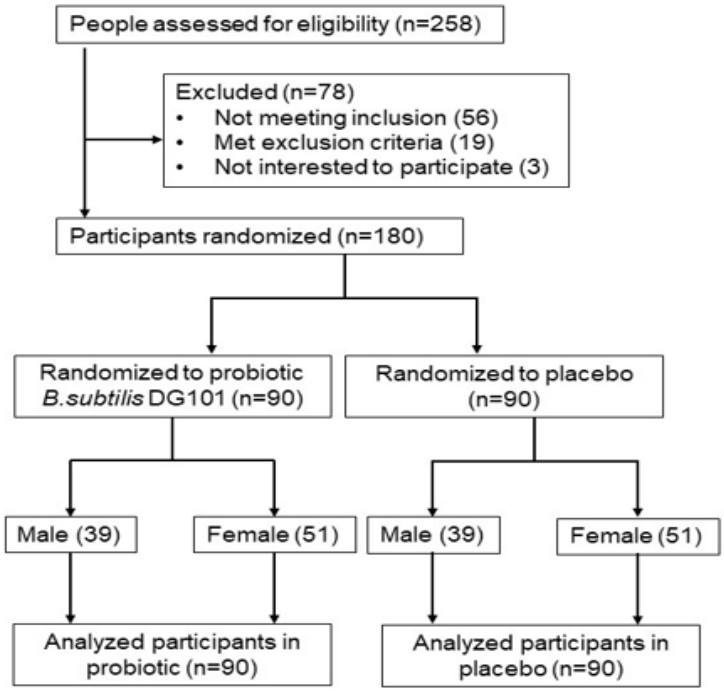

Figure 1: Study design.

As shown in Figure 2, healthy adults of both sexes who incorporated the probiotic *B. subtilis* DG101® into their diets (1 x 10^8 CFU/day) were able to maintain, and even improved, their values of BSS. The average mean in BSS of men in the intervention and placebo groups (Figure 2 A and B) after the 4 weeks of study was 3.12 ± 0.16 and 3.99 ± 0.20 ($p < 0.05$), respectively (yellow circles indicated in Figure 2 A and B, respectively). For women, the average mean in BSS, at the end of the study, was 3.39 ± 0.17 and 4.20 ± 0.21 ($p < 0.05$), respectively (yellow circles indicated in Figure 2 C and D). Overall, it was observed a decrease in the BSS score of men and female in the probiotic group (Figure 2 A and C) of ~ 21.8 % and ~ 23.9 %, respectively, compared with the BSS values observed in the male and female located in the placebo groups, respectively (Figure 2 B and D). The final BSS average values of 3.12 and 3.39 for male and female incorporating *B. subtilis* DG101® into their diets, indicate that subjects (of both sexes) in the intervention (i.e., probiotic) group produced less hard and more easily to evacuate stools compared to the subjects in the placebo group.

Figure 2

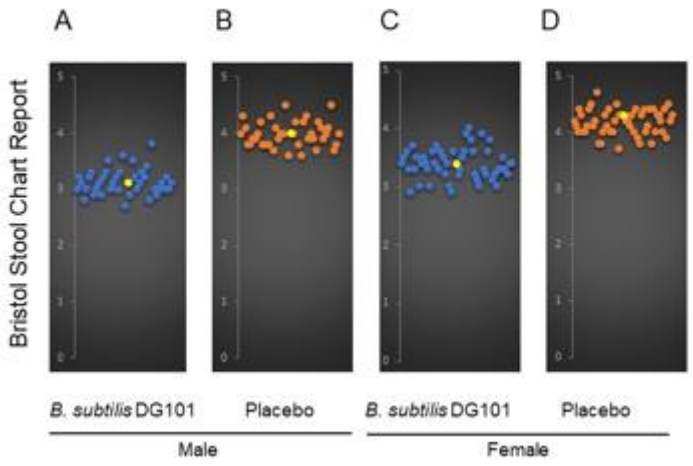

Figure 2: Effect of *B. subtilis* DG101® on the Bristol Stool Scale (BSS) in healthy individuals. Variation of BSS after the 4 weeks of the study in men (A-B) and women (C-D) with or without intervention with the probiotic *B. subtilis* DG101®. The blue and orange circles (participants who consumed the probiotic and participants who did not, respectively) indicate the final average value in BSS of each participant after the four weeks of the study. The yellow circles represent the global mean value of BSS of the participants in each group (placebo and intervention).

The BSS is very sensitive and its value reflects very well the intestinal transit time (ITT) of an individual [1,22]. A physiological ITT goes from 30 h to 40 h, with an upper limit of ~72 h (). This ITT, for a healthy person, equals to an average stool frequency of one complete bowel movement every ~ 1.5 days (~ 36 h), with an upper limit of one complete deposition each ~ 3 days (72 h). In individuals with functional or chronic constipation, the defecation frequency rise to one event every ~ 4.5 days (~ 110 h), and it is usually accompanied by sensations of uncompleted evacuation and pain [2,1,22,13]. As shown in Figure 3, male and females reported an average stool frequency per week of 3.5 (one complete bowel evacuation every 48 h approximately) and 2.5 (one complete bowel evacuation approximately every 67 h), respectively (Figure 3, baseline values at week zero) at the beginning of the study (week zero). In the placebo groups, the average stool deposition per week during the duration time of the study did not change significantly (Figure 3, black symbols). Interestingly, in the probiotic groups (Figure 3, white symbols), it is observed an improvement in the average frequency of stool deposition starting to be significant after the first week of the study and continue to improving until reach a final stool frequency value, at week four, of ~ 4.7 (one complete bowel evacuation every 36 h approximately) for men and ~ 3.5 (one complete bowel evacuation approximately every 48 h) for female (Figure 3 3 white circles and white squares, respectively). These stool frequency values represent a decrease in ITT of men and female allocated in the probiotic groups of ~ 12 h and ~ 19 h, respectively.

Figure 3

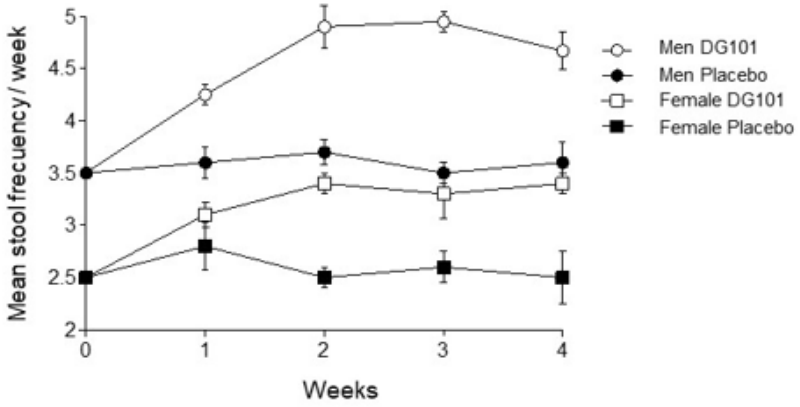

Figure 3: Effect of *B. subtilis* DG101® on the time course of spontaneous complete bowel movement in healthy adults. It is shown the weekly variation of the defecation frequency in men (circles) and women (squares) with or without intervention with the probiotic *B. subtilis* DG101® (white and black symbols, respectively).

The GSRS combines 15 items or questions clustered into five gastrointestinal symptoms: acid reflux, abdominal pain, indigestion, diarrhea, and constipation [2,22,12,3,13]. Six out of the fifteen GSRS items are specifically related to low intestinal discomfort (i.e., sensations of -constipation / diarrhea / loose stools / hard stools / urgent need to have a bowel movement / sensation of not completely emptying the bowel- experienced by the person during the past week), and were considered in this study (see Subjects and Methods for details). As shown in Figure 4, healthy adults of both sexes who incorporated the probiotic *B. subtilis* DG101® into their diets (1 x 10^8 CFU/day) significantly improved their abbreviate GSRS for low intestinal discomfort scores. The average mean in abbreviate GSRS for low intestinal discomfort of men in the intervention and placebo groups (Figure 4 A and B) after the 4 weeks of study was 9.15 ± 0.46 and 15.17 ± 0.76 ($p < 0.05$), respectively (yellow circles indicated in Figure 4 A and B, respectively). For women, the average value of abbreviate GSRS for low intestinal discomfort in the probiotic and placebo groups, at the end of the study, was 9.13 ± 0.46 and 15.24 ± 0.76 ($p < 0.05$), respectively (yellow circles indicated in Figure 4 C and D). Overall, it is observed a decrease in the abbreviate GSRS for low intestinal discomfort value of men and female in the probiotic group (Figure 4 A and C) of ~ 40.0 %, compared with the values observed in the male and female placebo groups (Figure 4 B and D). The final abbreviate GSRS for low intestinal discomfort values of 9.15 and 9.13 for male and female incorporating *B. subtilis* DG101® into their diets (probiotic groups, Figure 4 A and C, respectively), indicate that subjects (of both sexes) in the intervention (i.e., probiotic) group have much less intestinal discomfort (less problems of ITT, better stool consistency and stool appearance, and better sensation of complete bowel evacuation) compared to the subjects (of both sexes) in the placebo group (see Subjects and Methods for details).

Figure 4

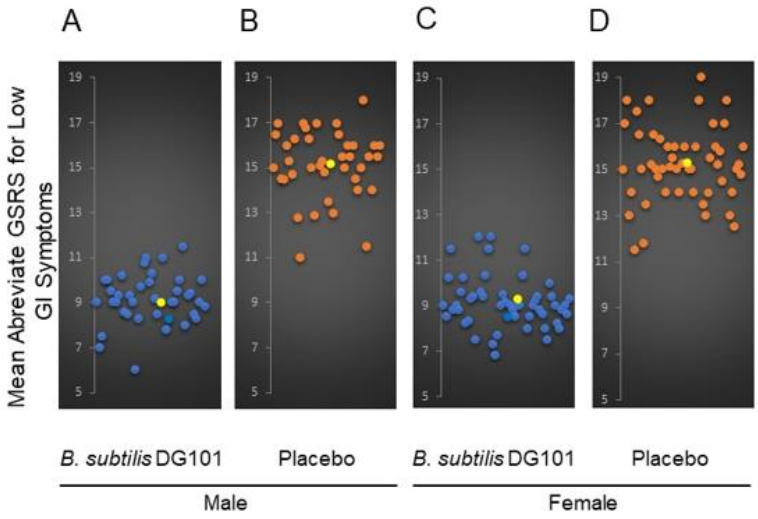

Figure 4: Effect of *B. subtilis* DG101® on the Gastrointestinal Symptom Rating Scale (GSRS) restricted to low intestinal discomfort in healthy individuals. Variation of abbreviate GSRS for low intestinal discomfort after the 4 weeks of the study in men (A-B) and women (C-D) with or without intervention with the probiotic *B. subtilis* DG101®. The blue and orange circles (participants who consumed the probiotic and participants who did not, respectively) indicate the average value in abbreviate GSRS for low intestinal discomfort of each participant after the four weeks of the study. The yellow circles represent the global mean value of abbreviate GSRS for low intestinal discomfort of the participants in each group (placebo and intervention).

Conclusions

The results presented in this double-blind, placebo-controlled study performed on healthy adults, demonstrate the effectiveness of the probiotic *B. subtilis* DG101® (incorporated in the commercial product Kyojin Probiotic®) to significantly improve physiological parameters related to intestinal well-being (i.e., BSS. ITT and GRSR). There is a significant amount of scientific evidence supporting the existence of a physiological relationship between the intestinal microbiota and the correct functioning of the intestine (Olle, 2013; Cuevas Sierra et al, 2019; Zmora et al, 2019). This cause – effect association, places probiotics as a potential tool of interest for the prevention of intestinal discomfort in healthy people, and also for the treatment with probiotics of functional or chronic constipation. The fact that *B. subtilis* DG101® significantly improved the intestinal functioning and performance in healthy adults, points to this probiotic bacterium out as an interesting and useful food supplement to improve the individual´s life quality.

Author Contributions

NC: conceptualization, methodology, data collection, and interpretation of the results.
FRA and CL: methodology, data collection and statistical analysis.
OP: conceptualization, methodology, and interpretation.
RG: conceptualization, methodology, interpretation and writing of the manuscript.
All authors read and approved the final manuscript.

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Conflict of Interest Statement

NC, OP, and RG declare that they have no conflict of interest regarding the publication of this article. FRA and CL are employees of Kyojin S.A.

Funding Information

This study was funded by Kyojin S.A.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

This study was conducted in accordance with the ethical principles indicated in the Declaration of Helsinki, its subsequent amendments, and the ethical recommended guidelines of the National University of Rosario and the Hospital Provincial del Centenario, Rosario - Santa Fe, Argentina.

Consent

Written informed consent was obtained from each participant of the present study in accordance with the journal's patient consent policy.

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