The Use of Probiotics in Healthy Volunteers With Evacuation Disorders and Hard Stools

A Double-blind, Randomized, Placebo-controlled Study

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Background: Evacuation disorders and hard stools are common in industrialized countries, affecting on average 12% to 17% of the adult healthy population at any age. Dietary supplementation with probiotic microorganisms may be useful in reducing the disorder.

Methods: We performed a double-blind, randomized, placebo-controlled study to evaluate the effectiveness of 2 different probiotic blends, either mixed *Lactobacillus plantarum* LP01 (LMG P-21021) and *Bifidobacterium breve* BR03 (DSM 16604) or *Bifidobacterium animalis* subspecies *lactis* BS01 (LMG P-21384), in the management of evacuation disorders and intestinal discomfort. In a period of 5 years (2003 to 2008), the study involved 300 healthy volunteers (151 males and 149 females; age 24 to 71 y) with evacuation disorders and hard stools. In particular, subjects were divided into 3 groups: 80 subjects in the group A received placebo, 110 subjects in the group B received mixed *L. plantarum* LP01 and *B. breve* BR03 (2.5×10^9 colony-forming units/d of each strain), and 110 subjects in the group C received *B. animalis* subsp. *lactis* BS01 (5×10^9 colony-forming units/d) for 30 days. At the beginning of the observational study, the healthy status of volunteers was evaluated by a complete, laboratory and ultrasound study of the abdomen. The physical examination was repeated after 15 and 30 days. In particular, the main troubles typically associated with evacuation disorders and hard stools as well as abdominal bloating were considered as parameters of interest. Exclusion criteria were items of gastrointestinal diseases and antibiotics intake.

Results: Subjects treated with the mixed probiotic strains *L. plantarum* LP01 and *B. breve* BR03 or *B. animalis* subsp. *lactis* BS01 reported a significant improvement in the number of weekly bowel movements and in the main troubles associated with evacuations, particularly consistency of feces and ease of expulsion. Discomfort items such as abdominal bloating and anal itching, burning, or pain also registered a relevant improvement in the active groups receiving probiotics.

Conclusions: The intake of an effective amount of mixed *L. plantarum* LP01 and *B. breve* BR03 or *B. animalis* subsp. *lactis* BS01 for 30 days is able to significantly relieve the evacuation disorders and hard stools, thus providing a useful tool for the management of such condition, which is particularly widespread in industrialized countries at any age.

Key Words: intestinal motility, evacuation disorders, probiotic strain, intestinal microbiota, intestinal discomfort

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The number of bacterial cells in the intestinal lumen is approximately 10 times higher than the number of eukaryotic cells in the human body. Anaerobic bacteria are about 1000 times more numerous than aerobic bacteria. In addition, 10 to 40 genera, 100 to 500 species, and thousands of strains representing the bacterial microflora[1] are present in the intestinal lumen.

The small intestine contains some species that adhere to the epithelium, and others which only transit through the intestinal lumen. The scarcity of bacteria in the proximal tract of the intestine seems to be linked to the composition of material in transit (acid, bile, and pancreatic juice) which kills most of the ingested microorganisms and to the motor activity preventing stable bacterial colonization in the intestinal lumen. In contrast, the large intestine contains a complex and dynamic bacterial ecosystem with a high density of living microorganisms reaching concentrations of 10^{11} to 10^{12} cells per gram of intestinal content. These concentrations are similar to those that can be found in colonies growing in ideal conditions on the surface of laboratory cultural media. Moreover, 40% to 80% of the bacteria counted with the microscope cannot be cultivated. Molecular biology methods can currently be applied to study the bacterial ecosystem of the colon.[2]

Although recent data suggest that each individual has unique bacterial strains and that after the age of 2 years the bacterial flora remains considerably constant over time, it has been observed that the bacterial flora of elderly individuals contains less Bifidobacteria, whereas it is richer in fungi and Enterobacteria than that of young people and adults.[3–6] The likelihood of isolating *Clostridium difficile* is also higher in the elderly. Although this is partly related to hospitalization, it has been noted that considerably higher amounts of the *Clostridium* genus can be isolated in the feces of healthy elderly volunteers with respect to young volunteers. These alterations may be partially explained by the changes that the human body undergoes with ageing.

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Such changes include a reduction in gastric and pancreatic secretion, a reduction and alteration of bile secretion (particularly after a cholecystectomy), increased permeability of the intestinal mucosa, changes in the immune system, and the reduction of intestinal motility.[7]

Numerous studies[8–11] have analyzed the causes of the reduction of intestinal motility in elderly, which are related to:

About 37% decrease in myenteric and submucous plexus neurons in rats and humans primarily in the colon (calcitonin gene-related peptide and acetylcholine)
- Decreased cholinergic myenteric activity (fewer, smaller contractions)

Loss of nitric oxide-containing neurons
- Impaired relaxation and increased segmental contractions (impaired peristalsis)

Decreased neurotrophin production and release

Decreased calcium influx into neurons, less calcium released in smooth muscle
- Smaller contractions and impaired peristalsis

Increased connective tissue
- Increased intraluminal pressure and formation of diverticula.

Furthermore, psychologic, social, and economic factors should be taken into consideration and changes in the individual's health related to age, such as loneliness, reduced income, unhealthy diet, and the use of prescription drugs may worsen the individual's nutritional state and alter the intestinal bacterial flora.

Moreover, many people do not know what is "normal" bowel movement. The fact is that there is no definition of a normal bowel movement. There are gradations of normal, and every person is going to have different bowel habits. Many people believe that the definition of a normal bowel movement is to have 1 movement each day, but that is not true for everyone. There is no rule for frequency of bowel movements, but the general range is from 3 times a day to 3 times a week. Less than 3 movements a week may indicate an evacuation disorder.

Evacuation disorders and hard stools are indeed the most common disorders in industrialized countries and in a study on 1128 subjects[12–14] are associated with:

- Straining (52%)
- Hard stools (44%)
- Want to, but can not (34%)
- Infrequent stools (32%)
- Abdominal discomfort (20%)
- Have not finished (19%)
- Excess toilet time (11%).

Recent epidemiologic research, carried out on worldwide population, has shown that 12% of individuals suffer from evacuation disorders, with a higher incidence in America and in East Asia. In Europe, the incidence is approximately 9%.[15] The research has also highlighted the fact that a quarter of the individuals does not take any action to try to relieve their troubles.

Studies carried out on healthy elderly individuals suggest that integrating their diet with probiotics can improve their nutritional state, reduce immune deficiency related to age, and regulate the bowel movements, reducing evacuation disorders frequent in old age.[16] To verify this assertion, a group of elderly volunteers in good health with evacuation disorders and hard stools was followed after supplementation with 7 different probiotic strains.

MATERIALS AND METHODS

From November 2003 to November 2008, 300 healthy volunteers (151 males and 149 females; age 24 to 71 y) were enrolled after a complete physical examination, normal value of laboratory tests, and no evidence of gastrointestinal disease at plain abdomen x-ray and ultrasound studies. The use of antibiotics or food supplements containing probiotics or prebiotics was also an exclusion criterion. After the enrollment, the subjects were homogeneously, randomly distributed in 3 groups:

(A) Eighty subjects (38 males and 42 females; age 24 to 68 y) were used as a placebo-control group and received upon awakening a 3 g dose of maltodextrin dissolved in a half glass of water;
(B) One hundred and ten subjects (50 males and 60 females; age 24 to 70 y) were given mixed *Lactobacillus plantarum* PROBIAL LP01 (LMG P-21021) and *Bifidobacterium breve* PROBIAL BR03 (DSM 16604) at a concentration of 2.5×10^9 colony-forming units per day of each strain;
(C) One hundred and ten subjects (63 males and 47 females; age 26 to 71 y) received *Bifidobacterium animalis* subspecies *lactis* PROBIAL BS01 (LMG P-21384) at a concentration of 5×10^9 colony-forming units per day.

The composition of the probiotic preparations was 2.95 g maltodextrin and 50 mg of lyophilized probiotics, either a mix of 2 strains or a single microorganism, equivalent to a total of 5 billion bacteria.

The probiotics and placebo formulations were kindly manufactured and provided by Probiotical, Novara. Each formulation was analyzed for the number of viable cells and the absence of microbiologic contaminants. The strains used were characterized at species level using species-specific polymerase chain reaction according to what reported by Walter et al[17] for *L. plantarum*, Matsuki et al[18] for *B. breve*, and Ventura et al[19] for *B. animalis* subsp. *lactis*. With reference to the strains biotype, the molecular fingerprinting was performed by pulsed-field gel electrophoresis for *L. plantarum* LP01 (LMG P-21021)[20] and *B. breve* BR03 (DSM 16604)[21] or by multi-locus sequence typing[22] and genome sequencing (by BRM Genomics-Padova, IT) for *B. animalis* subsp. *lactis* BS01 (LMG P-21384).[23]

Each dose was taken upon awakening, diluted in a half glass of water. All volunteers received the probiotics or the placebo for 30 days and were then clinically followed for 15 days. After the enrollment, other visits were conducted at T_0, T_{15}, and T_{30}. At enrollment, the subjects were asked to record each parameter in a specific case report form throughout the week before each following visit (T_0: the week before the beginning of each treatment; T_{15}: the second week of treatment; and T_{30}: the fourth and last week of treatment).

In addition to the filled case report forms, subject's general condition and vital status were assessed during each visit. In detail, the parameters recorded in the forms were:

(A) number of weekly defecations;
(B) feces consistency;
(C) ease of expulsion;
(D) sensation of complete emptying;
(E) anal itching, burning, or pain during or after defecation;
(F) abdominal bloating.

Although the determination of the number of defecations seemed simple and direct, the other parameters were noted and recorded using the easiest method for the subjects, that is identifying the lowest value with + and

the highest one with + + +, after explaining the method and agreeing upon it with the subjects. The value scale was explained and agreed upon with the volunteers as follows:

(A) number of weekly defecations (arabic numeral)
(B) consistency of feces (+ + + soft, + + solid, and + hard)
(C) ease of expulsion (+ + + complete, + + satisfactory, and + difficult)
(D) sensation of complete emptying (+ + + complete, + + satisfactory, and + incomplete)
(E) itching, burning, or pain during or after defecation (– no symptom, + itching, burning, or pain);
(F) abdominal bloating (+ + + light, + + moderate, and + severe).

Subjects who voluntarily discontinued the management or forgot to take powder formulations for at least 3 days were considered drop-outs.

The number of weekly defecations is expressed as mean ± SD. All values relative to the other parameters are expressed as mean ± SD after conversion of the symbol "–," "+," "+ +," and "+ + +" in the numbers "0," "1," "2," and "3," respectively. Student *t* test statistical analysis was used to compare the results. In detail, Student *t* test for paired data was chosen to calculate *P* values for each parameter at T_{15} compared with T_0 and at T_{30} compared with T_0 in each active group and in the placebo. Furthermore, Student *t* test for independent data was used to calculate *P* values for each parameter at T_0, T_{15}, and T_{30} in the 3 groups. Differences were considered significant at $P \leq 0.05$.

RESULTS

The molecular fingerprinting of the 3 strains used in the active formulations is reported in Figure 1 and Table 1.

Furthermore, the resistance profiles for 20 different antibiotics were assessed to verify the complete safety of the strains (data not reported).

No statistically significant differences were recorded at time 0 in any of the 2 active groups compared with placebo, thus confirming the homogeneous distribution of the enrolled subjects into the groups.

The results obtained are reported in Table 2.

An approximate 10% drop-out rate was noted, which can be considered physiological in this type of research. In detail, 6 drop-outs were recorded in group A, 10 in group B, and 7 in group C.

The results show that there is a significant increase in the number of bowel movements and an improvement in the majority of the parameters recorded (consistency of feces, ease of expulsion, sensation of complete emptying, and abdominal bloating) in the 2 active groups versus placebo. In the same active groups, a significant reduction in itching, burning, or pain during or after defecation was also noted especially after 30 days. No volunteer manifested any disorder, adverse, or side effects throughout the study.

Number of Weekly Evacuations

A statistically significant difference was recorded at T_{15} and T_{30} versus T_0 both in group B (T_{15} vs. T_0 $P < 0.001$; T_{30} vs. T_0 $P < 0.001$) and in group C (T_{15} vs. T_0 $P < 0.001$; T_{30} vs. T_0 $P < 0.001$). No differences have been registered in the placebo-control group (T_{15} vs. T_0 $P = 0.148$; T_{30} vs. T_0 $P = 0.507$).

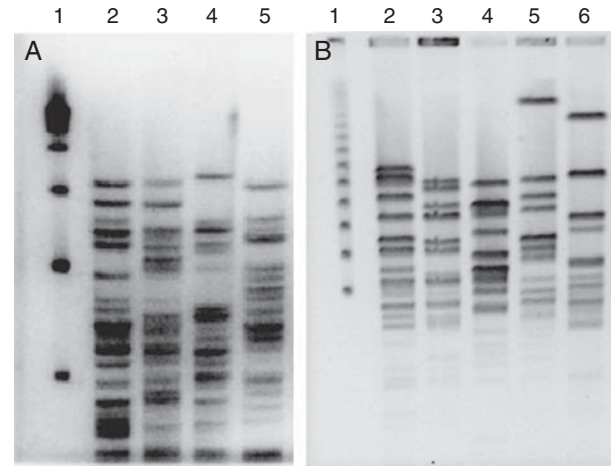

FIGURE 1. Fingerprinting profile of the strains *Lactobacillus plantarum* LP01 (LMG P-21021), *Bifidobacterium breve* BR03 (DSM 16604). A, PFGE: *L. plantarum* LP01 (enzyme *Not*I). (1) Electrophoretic Marker, Sigma 50 to 1000 kb; (2) Sample strain, *L. plantarum* LP 01 (ID 1171) LMG P-21021; (3) Comparison strain, *L. plantarum* ID 091 LMG P-21020; (4) Comparison strain, *L. plantarum* ID 1393. B, PFGE: *B. breve* BR03 (enzyme *Sfi*I). (1) Electrophoretic Marker, Sigma 50 to 1000 kb; (2) Sample strain, *B. breve* BR03 (ID 1270) DSM 16604; (3) Comparison strain, *B. breve* ID 1274; (4) Comparison strain, *B. breve* ID 1413; (5) Comparison strain, *B. breve* DSM 16596. PFGE indicates pulsed-field gel electrophoresis.

Consistency of Feces

At T_{15} and T_{30}, the administration of either mixed *L. plantarum* LP01 and *B. breve* BR03 or *B. animalis* subsp. *lactis* BS01 was able to significantly improve the consistency of feces compared with placebo ($P = 0.003$ at T_{15} and $P = 0.001$ at T_{30} in group B, $P = 0.001$ at T_{15} and $P < 0.001$ at T_{30} in group C).

No statistically significant differences have been registered at T_0 (group B vs. placebo: $P = 0.107$; group C vs. placebo: $P = 0.909$), thus confirming the proper randomization of the enrolled volunteers.

Ease of Expulsion

The strain *B. lactis* BS01 induced a significant result both at T_{15} and at T_{30} versus placebo ($P = 0.044$ and $P < 0.001$, respectively). *B. breve* BR03 + *L. plantarum* LP01 significantly ameliorated the score at T_{15} and T_{30} versus placebo ($P = 0.002$ and $P < 0.001$, respectively).

No statistically significant differences have been registered at T_0 neither between the 2 active groups nor between each active group and placebo (group B vs. placebo: $P = 0.164$; group C vs. placebo: $P = 0.818$; and group B vs. group C: $P = 0.085$).

TABLE 1. Profile of *Bifidobacterium animalis* Subspecies *lactis* BS01

MLST: Profile: rpoB-1; rplB1; purF-1; ileS-1; gyrB-1; fusA-1; clpC-1
Genome sequence (by 454 methodology): in progress

MLST indicates multi-locus sequence typing.

TABLE 2. Assessment of the Main Parameters Associated to Evacuation Disorders (Mean±SD) Before and After the Administration of Probiotics Compared With Placebo

	Group A		Group B		Group C		A Versus B	A Versus C	B Versus C
Parameter	**Mean ± SD**	***P****	**Mean ± SD**	***P****	**Mean ± SD**	***P****	$P^{\dagger}_{\ddagger}$	$P^{\dagger}_{\ddagger}$	$P^{\dagger}_{\ddagger}$
No. weekly evacuations									
T_0	5.61 ± 2.24	†	5.30 ± 2.24	†	5.79 ± 2.19	†	0.343	0.584	0.102
T_{15}	5.86 ± 2.11	0.148	7.29 ± 1.95	< 0.001	7.11 ± 1.94	< 0.001	< 0.001	< 0.001	0.517
T_{30}	5.81 ± 1.98	0.507	7.26 ± 1.88	< 0.001	6.93 ± 1.75	< 0.001	< 0.001	< 0.001	0.200
Consistency of feces									
T_0	1.79 ± 0.71	†	1.63 ± 0.65	†	1.80 ± 0.78	†	0.107	0.909	0.074
T_{15}	1.84 ± 0.69	0.339	2.14 ± 0.64	< 0.001	2.17 ± 0.64	< 0.001	0.003	0.001	0.773
T_{30}	1.89 ± 0.63	0.151	2.22 ± 0.66	< 0.001	2.30 ± 0.61	< 0.001	0.001	< 0.001	0.364
Ease of expulsion									
T_0	1.94 ± 0.75	†	1.80 ± 0.60	†	1.96 ± 0.79	†	0.164	0.818	0.085
T_{15}	1.99 ± 0.66	0.508	2.27 ± 0.54	< 0.001	2.19 ± 0.66	0.001	0.002	0.044	0.338
T_{30}	2.04 ± 0.61	0.300	2.44 ± 0.59	< 0.001	2.48 ± 0.57	< 0.001	< 0.001	< 0.001	0.663
Sensation of complete emptying									
T_0	2.01 ± 0.65	†	1.88 ± 0.54	†	1.99 ± 0.68	†	0.130	0.826	0.190
T_{15}	2.05 ± 0.63	0.593	2.23 ± 0.59	0.259	2.31 ± 0.61	< 0.001	0.054	0.006	0.333
T_{30}	2.00 ± 0.68	0.859	2.35 ± 0.59	0.0013	2.44 ± 0.59	< 0.001	< 0.001	< 0.001	0.296
Anal itching, burning, and pain									
T_0	0.33 ± 0.47	†	0.30 ± 0.46	†	0.30 ± 0.46	†	0.715	0.715	1.000
T_{15}	0.40 ± 0.49	0.278	0.22 ± 0.42	< 0.001	0.20 ± 0.40	0.027	0.007	0.002	0.684
T_{30}	0.41 ± 0.49	0.321	0.10 ± 0.30	< 0.001	0.11 ± 0.31	< 0.001	< 0.001	< 0.001	0.874
Abdominal bloating									
T_0	1.88 ± 0.68	†	1.70 ± 0.60	†	1.78 ± 0.61	†	0.062	0.325	0.317
T_{15}	1.94 ± 0.68	0.508	2.48 ± 0.56	< 0.001	2.43 ± 0.57	< 0.001	< 0.001	< 0.001	0.550
T_{30}	1.90 ± 0.62	0.770	2.59 ± 0.55	< 0.001	2.63 ± 0.56	< 0.001	< 0.001	< 0.001	0.599

*Comparison between time zero (T_0) and the following analysis (T_{15} and T_{30}) within each group.
†Comparison reference time.
‡Comparison between the 3 groups at T_0, T_{15}, and T_{30}.

Sensation of Complete Emptying

A 2-weeks treatment with *B. lactis* BS01 was able to significantly improve the sensation of complete emptying ($P = 0.006$ vs. placebo). *L. plantarum* LP01 + *B. breve* BR03 induced a moderate improvement of this parameter, even if not statistically significant ($P = 0.054$). After 30 days of treatment, the improvement is significant in both active groups compared to placebo ($P < 0.001$ in group B, $P < 0.001$ in group C).

No differences have been registered at T_0 (group B vs. placebo: $P = 0.130$; group C vs. placebo: $P = 0.826$) and between the 2 active formulations at T_{15} and T_{30} ($P = 0.333$ and $P = 0.296$, respectively).

Anal Itching, Burning, and Pain

The administration of both probiotic formulations compared with placebo was able to significantly reduce discomfort associated with evacuations ($P = 0.007$ at T_{15} and $P < 0.001$ at T_{30} in group B, $P = 0.002$ at T_{15} and $P < 0.001$ at T_{30} in group C).

No differences have been registered at T_0 (group B vs. placebo: $P = 0.715$; group C vs. placebo: $P = 0.715$).

Abdominal Bloating

A significant reduction of bloating was recorded after 2 weeks of treatment with probiotics ($P < 0.001$ in both groups compared with placebo). After 30 days, a slight further improvement compared with T_{15} was reported by the volunteers, confirming the efficacy of both active formulations.

No differences have been registered at T_0 neither between the 2 active groups nor between each active group and placebo (group B vs. placebo: $P = 0.062$; group C vs. placebo: $P = 0.325$; and group B vs. group C: $P = 0.317$). The 2 active formulations showed a similar efficacy in the relieve of bloating both at T_{15} and at T_{30}.

DISCUSSION

As known earlier, bacteria can be used to improve human health. A "probiotic" can be defined as a bacterium ensuring specific favorable effects if present in food or added to food. Consensus on the definition of the term probiotic was reached only a few years ago, when it was established that oral probiotics were "living microorganisms which, when ingested in specific quantities, are beneficial to human health."[24]

Consistent with this definition, it is not necessary for probiotics to colonize the human intestine. The crucial point is to show a distinct beneficial effect on health with the use of a specific strain. In fact, the efficacy of a probiotic is strain-specific. Controlled, randomized, and double-blind studies would be required[24] to show the efficacy of a probiotic.

The objective of our randomized double-blind controlled study in volunteers versus placebo was to evaluate the performance of 2 probiotic strains in the management of evacuation disorders, a very frequent problem in healthy population at any age with important economic and social implications.

In the groups of volunteers taken into consideration, approximately 30% of the subjects could be identified as suffering from evacuation disorders (< 3 to 4 defecations/wk, hard feces, and incomplete defecation) and abdominal bloating. Many individuals (about 20%) with a normal number of defecations considered themselves affected by defecation problems (incomplete evacuation, hard feces, and difficult defecation).

It is interesting to note that the strains tested, particularly *B. animalis* subsp. *lactis* PROBIAL BS01 and the association of *L. plantarum* PROBIAL LP01 and *B. breve* PROBIAL BR03 proved to be useful in the management of evacuation disorders and/or related troubles. Improvements in the evacuation disorders can be explained by the activity of some strains of probiotic (eg, *Bifidobacteria*) on nondigestible carbohydrates present in the colon (bran, cellulose, hemicellulose, pectins, fructo-oligosaccharides, and galacto-oligosaccharides).[25,26] The final metabolism of all these substrates is their fermentation with production of short-chain fatty acids (SCFA; particularly acetate, propionate, and butyrate) and carbon dioxide and lower quantities of other gases. Small quantities of SCFA are also produced by proteins catabolism.[27–29] SCFA are able to promote intestinal peristalsis and progression of the feces mainly for their slight and physiological "irritant activity" on the intestinal mucosa.

In the groups treated with probiotic strains not only showed an improvement of evacuation disorders and abdominal bloating, but also the absence of side effects throughout the study.

Some of the positive effects on evacuation disorders and hard stools continued for 10 to 15 days after the suspension of the active formulations, indicating either an active reproduction of the strains in the intestinal lumen or a substantial change in the habitat with prevalence of fermentative over putrefactive processes.

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