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EFFICACY OF *KLUYVEROMYCES MARXIANUS* FRAGILIS B0399 ADDED TO A FERMENTED MILK CONTAINING *BIFIDOBACTERIUM LACTIS* BB12 IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

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ORIGINAL ARTICLE

Efficacy of *Kluyveromyces marxianus fragilis* B0399 added to a fermented milk containing *Bifidobacterium lactis* BB12 in patients with irritable bowel syndrome

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ABSTRACT

BACKGROUND: The aim of this study was to evaluate the effects on IBS symptoms induced by a new probiotic mixture compared to a standard formulation.

METHODS: Eighty-five prospectively enrolled Rome III irritable bowel syndrome (IBS) patients (23 M, 62 F; age 37.6±13.1) were randomized (42 *vs.* 43) to receive either a fermented milk ("active preparation") containing *Kluyveromyces marxianus fragilis* B0399, Bifidobacterium lactis BB12, *Lactobacillus bulgaricus* and *Streptococcus termophilus* or a "standard preparation" (*i.e.*, *Bifidobacterium lactis BB12*, *Lactobacillus bulgaricus* and *Streptococcus termophilus*). The study design was composed by a two-week run-in period, a four-week treatment period and a two-week wash-out period. During each period, IBS cardinal symptoms (abdominal pain, bloating and bowel movement disturbances) and a composite IBS score, calculated as the sum of the three symptoms indicated above, were recorded on a daily basis. Bowel movements and stool consistency were also recorded. The global self-assessment was collected at the end of treatment and wash-out period, using a four-point Likert Scale. All data were analyzed using SPSS package.

RESULTS: Both active and standard treatment were able to ameliorate abdominal pain/ discomfort in the two randomized groups: in the "active group" the improvement was reached at 4th week of treatment and persisted up to the first wash-out week; in contrast, in the "standard group" that improvement was obtained only at the first week of treatment and the first wash-out week. Abdominal bloating was significantly reduced starting from second week of treatment and lasting over the entire washout period in both study groups. Bowel movement abnormality was reduced only in patients receiving the "active treatment" starting from the fourth week and lasting for the entire wash-out period. Also, the composite score showed a reduction in treatment and wash-out period in the "active group" and a reduction during treatment period, but not during the washout, in the "standard group". Global self-assessment showed a significant better trend in patients treated with "active" vs. those receiving the "standard treatment".

CONCLUSIONS: The addition of the probiotic *Kluyveromyces marxianus B0399* yeast in the fermented milk containing *Bifidobacterium lactis* BB12, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* has improved bloating, bowel movement abnormality and showed a trend towards abdominal pain reduction.

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KEY WORDS: Irritable bowel syndrome; Probiotics; Cultured milk products.

Irritable bowel syndrome (IBS), one of the most common diseases in gastrointestinal clinical practice, a chronic disorder characterized by abdominal pain/discomfort and disturbed defecation not explained by structural and biochemical abnormalities.[1-3] The social impact of IBS on

the general population is extremely high, as this syndrome affects 10-20% of people worldwide, accounts for 3% of visits to general practitioners and about 40% of all gastroenterology outpatient consultations.[1] These patients represent a considerable burden for society because of direct (*e.g.*, public healthcare use, drug consumption) and indirect (*e.g.*, absenteeism from work and lack of productivity) costs.[2] Patients with IBS are usually subdivided into three major subsets: diarrhea predominant, constipation-predominant, or alternating diarrhea and constipation. The pathogenesis is multifactorial; major mechanisms that are known to contribute to IBS symptoms include psychosocial factors, gut dysmotility, and enhanced perception of sensory stimuli conveyed from the gut wall to the central nervous system via sensory nerve pathways.[4] In addition, novel data concerning genetics, gut infection, food allergy or intolerance, modifications of gut microbiota, abnormal gas handling, impaired epithelial permeability, neuroplastic changes, stress and related hormone release, and gut wall immune activation have suggested that these factors may participate in symptom generation in subsets of IBS patients.[4, 5] Several lines of evidence indicate that an immune activation/inflammatory response at the mucosal level may play a role in generating and perpetuating symptoms in patients with IBS.[5-7] Clinically, it is well known that patients during the remission phase of an inflammatory bowel disease frequently develop symptoms mimicking those of IBS patients.[1] Following a bout of gastroenteritis (*i.e.*, *Campylobacter jejuni*, *Shigella*, or *Salmonella* infections), about one third of patients develop persistent IBS symptoms, thus being defined as postinfectious IBS. Postinfectious IBS has a clear onset and a better prognosis over time than conventional IBS.[8, 9] Finally, a minimal (or low-grade) inflammatory response, usually not identifiable by routine histology, has been shown in the intestinal mucosa of patients with either conventional or postinfectious IBS.[6, 7] Furthermore, enteric flora may also play a role in eliciting a minimal inflammatory response in the colonic mucosa of patient with IBS. In this respect, it has been suggested that patient with IBS may have bacterial overgrowth and that some IBS cardinal symptoms can be improved by nonabsorbable antibiotics.[10, 11] Nonetheless, the role exerted by the gut flora and its interaction with the enteric mucosa in IBS remains largely unknown. Probiotics, defined as live or attenuated bacteria (or related products) that confer a significant health benefit to the host, may be exploited to explore the lumen (*i.e.*, flora) to mucosa interactions occurring in the intestinal environment. Probiotics are endowed with a variety of effects including: 1) antibacterial and antiviral action (hence their possible usefulness in the special setting of postinfectious IBS); 2) anti-inflammatory effects by decreasing the mucosal inflammatory infiltrate (thereby inhibiting the immune-mediated activation of enteric neuronal reflexes and sensory nerve pathways conveying information from the enteric environment to the central nervous system); 3) modulatory properties on the enteric flora.[12-16] Further evidence indicates that probiotics may have an impact on gut functions via either a direct competition induced by the increased concentrations of commensal lactobacilli and bifidobacteria or indirectly through a reduction of pathogen-related inflammation and bacterial fermentation. Also, it is known that probiotics may affect gas production in the gut lumen and stool consistency.[17] Yeast probiotic preparation (*i.e.*, *Saccharomyces boulardii*), have increasingly been used throughout the world, providing empirical evidence of its efficacy as an adjuvant agent to treat gastrointestinal disorders. In the last decades increasing numbers of studies have been conducted each year to determine the mechanism of action of probiotic yeasts and possible beneficial properties for the host organism. Recently, RCTs have demonstrated the efficacy of *S. boulardii* as a probiotic medication and as a biotherapeutic agent in intestinal disorders, *e.g.* in *Clostridium difficile* associated disease, antibiotic-associated diarrheas, and acute infectious diarrheas.[18] *S. boulardii* is the only yeast tested on patients affected by IBS in a randomized controlled trial.[19] *Kluyveromyces marxianus* is a yeast closely related to the *Saccharomyces* spp.;[20] in fact, the genus *Kluyveromyces* was created by van derWalt[21] and later a number of species that had previously been assigned to Saccharomyces transferred to were *Kluyveromyces spp*.[22] *K. marxianus* can be

viewed as a possible probiotics due to some of its biological properties, such as a broad substrate spectrum, thermotolerance, high growth rates, low tendency to ferment when exposed to sugar, production of enzymes (beta-galactosidase, beta-glucosidase, inulinase and polygalacturonases and reduction of lactose content in food products. In a recent study, Kumura *et al*. Have investigated some yeast strains and their possible probiotic characteristics and *K. marxianus*, isolated from commercial blue cheese and kefir, showed the ability to adhere to Caco-2 enteric cells, proliferate under anaerobic conditions as well as acid and bile tolerance.[23] Based on these promising biological properties, the present study has been undertaken to test effects of *K. marxianus* in the clinical setting particularly focusing on patients with IBS. In this line we hypothesized that *K. marxianus fragilis* B0399 could be exploited to enhance immune modulation and therefore have a beneficial effect on symptoms related to IBS. The primary goal of this double-blind study was to evaluate the effects of a fermented milk formulation containing a new probiotic mix (*K. marxianus fragilis B0399*, *Bifidobacterium lactis BB12*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*) on IBS symptoms. Three main cardinal symptoms of IBS (*i.e.*, abdominal pain, bloating and bowel movement abnormality and a composite score calculated as the sum of these were investigated on a daily basis. Also, secondary aims of this trial were the evaluation of effect on bowel frequency, stool consistency and global self-satisfaction assessment.

Materials and methods

Patients

Patients were enrolled from the Gastrointestinal Outpatient Clinic of the Department of Clinical Medicine, University of Bologna, Italy. Patients between 18-65 years who matched Rome III criteria for the diagnosis of IBS and in whom organic GI diseases (*i.e.*, inflammatory bowel disease or celiac disease) and systemic disorders have been ruled out, were considered for inclusion in this study. Pregnant women, patient with demonstrated lactose intolerance or food allergies as well as individuals who underwent any major abdominal surgery have been excluded. Patients taking concomitant medication such as antibiotics, corticosteroids or functional foods containing pre- or probiotic were also excluded from the study population. Each patient signed the informed consent prior to enter the study. The study protocol was approved by the Ethics Commitee of the St. Orsola Malpighi Hospital (156/2008/U/Sper) and it conforms to the ethical guidelines of the "World Medical Association Declaration of Helsinki".

Study design

This double-blind, controlled and randomized study was aimed at assessing the role of a new probiotic mix containing *K. marxianus fragilis B0399*, *Bifidobacterium lactis BB12*, *S. thermophilus*, *Lb. bulgaricus*, as compared to a standard mix composed by *Bifidobacterium lactis BB12*, *S. thermophilus* and *Lb. bulgaricus* on symptoms and a global self-assessment of patients with IBS (both constipated and diarrhea predominant). Each enrolled patient was assessed by review of clinical history and a thorough clinical examination as well as main hematologic and bio-humoral tests. Eligible subjects entered a two-week run-in period during which they recorded symptoms and stool frequency and form, each day on a daily card. During this period, and throughout the study patients were thought not to take any medications that could affect gut sensory motor function such as laxatives and antidiarrhea drugs as well as any preparation with potential effects on enteric flora. At the end of the run-in period subjects were randomized to receive either a fermented milk containing a probiotic mix with *Bifidobacterium lactis BB12, S. thermophilus, Lb. bulgaricus* (Standard preparation) or the same mix with *K. marxianus fragilis B0399* (active preparation).

Each formulation has been delivered as a fermented milk once a day for the entire duration of the four-week period of the study. Both standard and active preparation were identical in color, taste and consistency. Subjects were instructed to ingest the preparation and record symptoms and stool characteristics on a daily basis throughout the study. A randomization schedule was obtained before starting the patient enrollment by

choosing a subset from a published random number table (Scientific Tables, Documenta Geigy, 7th edition, Basle, Switzerland) and it was performed giving the subject an unlabeled pack of fermented milk (the code of the pack was known only to a study coordinator). All investigators as well as patients were blinded to the randomization process until completion of the study. On completion of the four-week treatment phase, patients continued to record symptoms on the daily card for a further two-week washout period, while off all therapy.

Probiotic preparations

The active test product was a fermented milk daily dosage containing *K. marxianus fragilis B0399* (10^7 CFU/dose) and the mix base: *Bifidobatterium lactis BB12* (10^9 CFU/dose), *S. thermophilus* (10^9 CFU/dose) and *Lb. bulgaricus* (10^8 CFU/dose). The standard product was a fermented milk containing same mix base: Bifidobacterium lactis BB12 (10^9 CFU/ dose), *S. thermophilus* (10^9 CFU/dose) and *Lb. bulgaricus* (10^8 CFU/dose). Both active and standard preparation were similar in flavor, appearance, texture, and taste. Each daily dose was a pot contained 125 g of product and was provided by Coop Italia (Casalecchio di Reno, Bologna, Italy).

Assessments

During the trial, subjects were seen and diary cards were collected every two weeks. Three main IBS symptoms were assessed: 1) abdominal pain/discomfort; 2) bloating/distension; 3) bowel movement abnormality (including urgency in diarrhea-predominant IBS patient and straining or a sense of incomplete evacuation in patient with constipated-predominant IBS). Each symptom was evaluated using a ten-centimeter visual analogue scale (VAS, 0-10). A composite score was also calculated as the sum of the three IBS symptoms (VAS, 0-30). Bowel movement frequency was recorded as number per day, and consistency was evaluated using the Bristol Stool Scale. At the end of the treatment period and wash-out period global self-assessment was assessed using a 4-point Likert scale (1=markedly worsened; 2=mildly worsened; 3=mildly improved; 4=markedly improved).

Statistical analysis

Sample size

The sample size calculation was made according to Dupont and Plummer[24, 25] by using the software PS Power and Sample Size Calculations (Version 2.1.30, February 2003) developed at the Department of Statistics, Vanderbilt University, Nashville, TN, USA. The mean±SD cumulative score of the standard group at the end of the treatment was estimated by the data previously reported by O'Mahony *et al.* after four weeks of treatment with Bifidobacterium infantis 35624 (4.7±3.4).[26] We had hypothesized a two-unit reduction in the active group and a sample size of 46 subjects per group was calculated by choosing a 0.05 value of the alpha probability and a power of 0.80.

Means, standard deviations (SD), and frequencies were used as descriptive statistics The Mann-Whitney, Wilcoxon, Fisher's Exact and Pearson χ^2 tests were applied to analyze the data. Data were managed by means of the SPSS (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA). Two-tailed P values less than 0.05 were considered statistically significant.

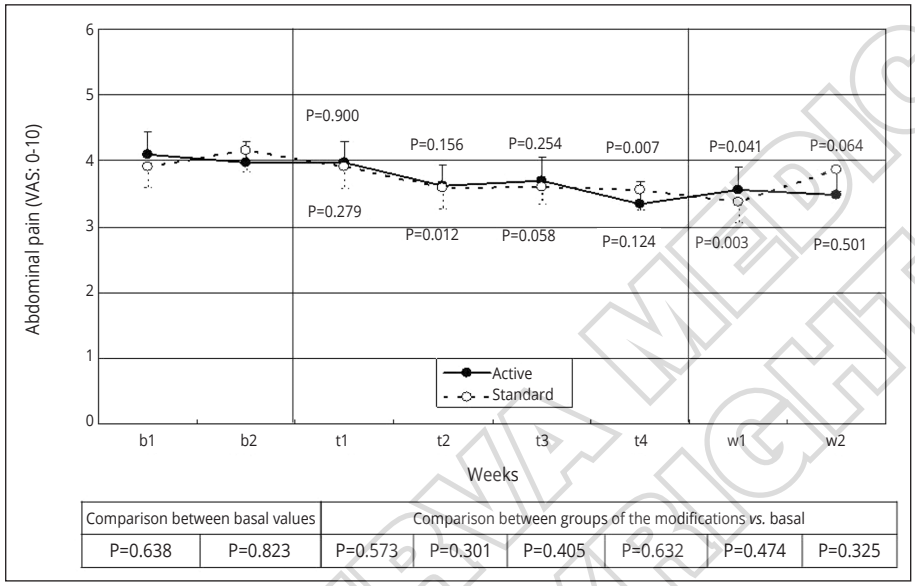
Results

Of the 92 randomized patients, seven declined to commence the trial. Of the 85 studied patients (92.4%), 42 patients received the active treatment, while 43 were on the standard one. All subjects were Caucasian, the mean age was 37.6±13.1 years, 62 were women and 23 were men. Patients characteristics at baseline are described in Table I. Sex, age and IBS subtypes were balanced in both treatment groups. Figure 1, 2, 3, 4 show the symptom score profiles of IBS patients in the active and standard groups. During the baseline observation period, the two groups of patients had similar scores as far as all the four symptoms were concerned. Concerning the three main symptoms examined in the present study, the active treatment was effective in reducing abdominal pain/discomfort but for a shorter period of time than the reduction of abdominal bloating (fourth week of treatment and first week of washout [Figure 1]). In contrast, the standard treatment was effective, on abdominal pain, at the

TABLE I.— *Patient baseline characteristics.*

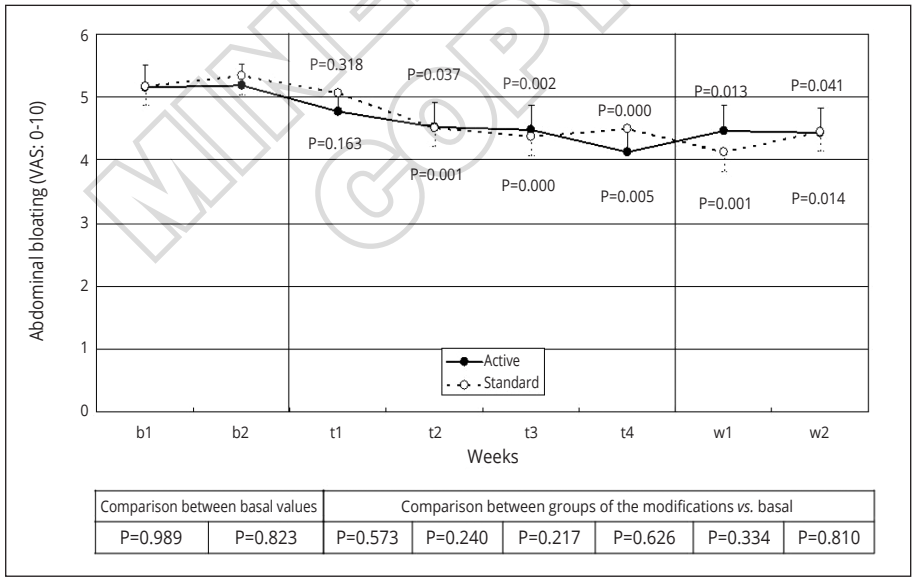
	Active group (N. 42)	Standard group (N. 43)	P value
Gender:			
Male	12 (28.6%)	11 (25.6%)	P=0.810 [a]
Female	30 (71.4%)	32 (74.4%)	
Age (years): mean±SD	35.3±12.4	39.8±13.50	P=0.106 [b]
IBS subtypes:			
Diarrhea-IBS	19 (45.2%)	19 (44.2%)	
Constipation-IBS	11 (26.2%)	11 (25.6%)	P=0.986 [c]
Mixed-IBS	12 (28.6%)	13 (30.2%)	

[a]Fisher's Exact test; [b]Mann-Whitney Test; [c]Pearson χ^2.



Comparison between basal values		Comparison between groups of the modifications *vs.* basal					
P=0.638	P=0.823	P=0.573	P=0.301	P=0.405	P=0.632	P=0.474	P=0.325

Figure 1.—The abdominal pain score (VAS 0-10; Mean values±SE) during the 8-week study period. Comparison between basal value and between groups (*vs.* basal).



Comparison between basal values		Comparison between groups of the modifications *vs.* basal					
P=0.989	P=0.823	P=0.573	P=0.240	P=0.217	P=0.626	P=0.334	P=0.810

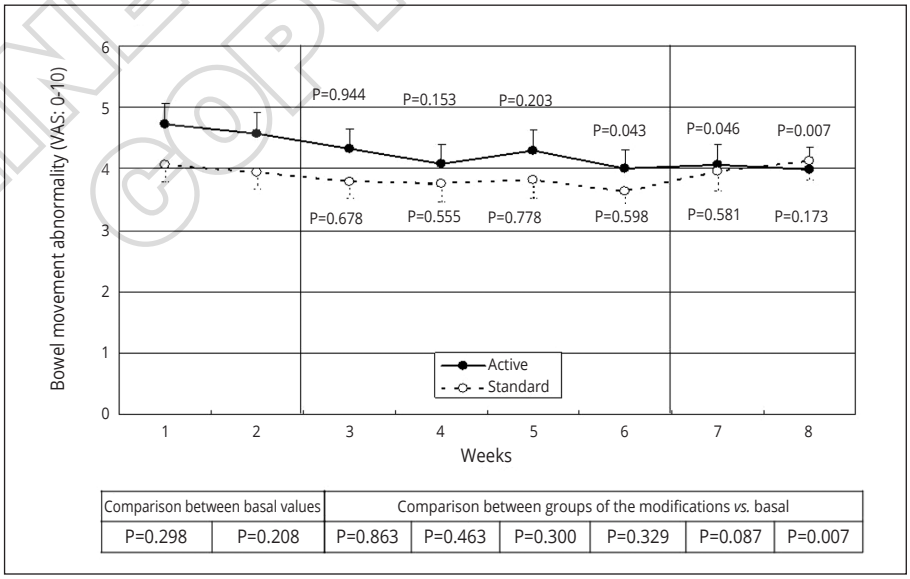
Figure 2.—The abdominal bloating score (VAS 0-10; Mean values±SE) during the 8-week study period. Comparison between basal value and between groups (*vs.* basal).

second week of treatment and at the first week of the wash-out period. No significant differences between active and standard treatment were obtained regarding abdominal pain/discomfort. The most striking effect of both active and standard treatment was a significant decrease in abdominal bloating starting from the second week of treatment ment and lasting over the entire wash-out period (Figure 2). The standard preparation was also effective in reducing significantly bloating at the same time-period with no differences between the two study groups. Compared to basal, the active treatment significantly reduced the bowel movement abnormality score starting from the fourth week of treatment and lasting for the whole wash-out period (Figure 3) while no significant modifications were observed during the standard treatment. The reduction induced by the active treatment at the second week of the wash-out period was significantly different than the modification observed in the standard group (P=0.007). The cumulative score, calculated as the sum of pain, bloating and bowel movement abnormality score (VAS 0-30) was significantly reduced in patients receiving the active treatment starting from the second week of treatment and lasting over the whole treatment and wash-out period (Figure 4). The reduction observed in the standard group was significant in the last three weeks of treatment only, while it was non-significant during the wash-out period, although no significant differences were reached in comparison with the active treatment group.

In each group a trend in reduction of the number of daily bowel movement is reported, but there were no differences between two study groups (Table II). Also stool form, measured using Bristol Stool Scale, did not significantly differ in subjects of both groups (Table III). The global self assessment, assessed with a four-point Likert Scale, showed a better response in subjects randomized to active treatment vs. those receiving the standard mix both at the end of the four-week treatment and the wash-out period (P=0.002, and P=0.001, respectively; Figure 5). No adverse events were reported during the study period.

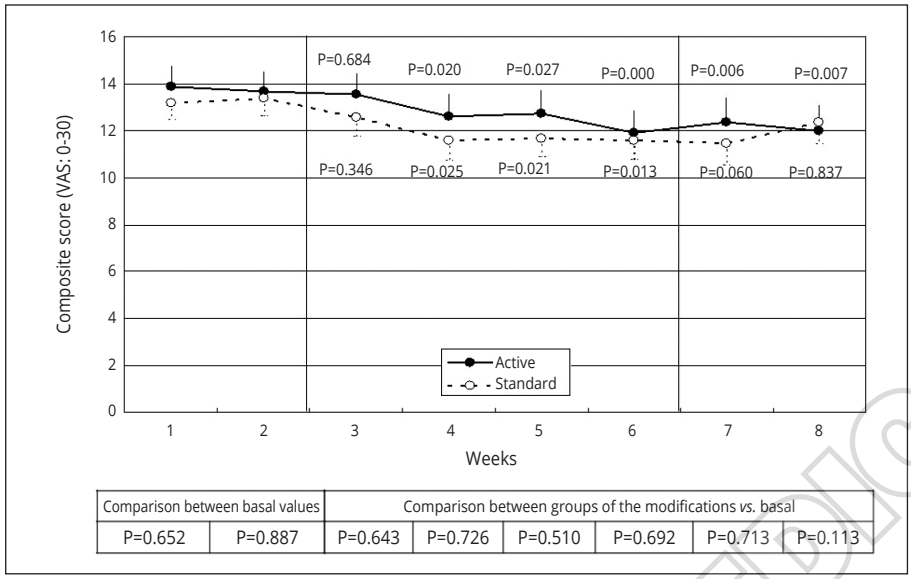
Discussion

In our study we evaluated the effects of a fermented milk containing a probiotic mix, composed by *K. marxianus fragilis B0399*, *Bifidobacterium lactis BB12*, *S. thermophilus* and *Lb. bulgaricus* ("active formulation") on IBS symptoms. This probiotic formulation has been compared to a "standard formulation" composed by Bifidobacterium lactis BB12, *S. thermophilus* and *Lb. bulgaricus*. The fermented milk containing a multi-



Comparison between basal values		Comparison between groups of the modifications *vs.* basal					
P=0.298	P=0.208	P=0.863	P=0.463	P=0.300	P=0.329	P=0.087	P=0.007

Figure 3.—The bowel movement abnormality score (VAS 0-10; Mean values±SE) during the 8-week study period. Comparison between basal value and between groups (*vs.* basal).



Comparison between basal values		Comparison between groups of the modifications *vs.* basal					
P=0.652	P=0.887	P=0.643	P=0.726	P=0.510	P=0.692	P=0.713	P=0.113

Figure 4.—The composit IBS symptoms core (VAS 0-30; Mean values ± SE) during the 8-week study period. Comparison between basal value and between groups (*vs.* basal).

TABLE II.—*Daily bowel movement.*

Week	Active group (N. 42)	Standard group (N. 43)	P value
b1	1.56±0.83	1.25±0.66	0.113 [a]
b2	1.47±0.76	1.40±0.74	0.638 [a]
t1	1.34±0.65	1.34±0.63	0.812 [b]
t2	1.34±0.52	1.25±0.65	0.494 [b]
t3	1.38±0.66	1.28±0.66	0.670 [b]
t4	1.30±0.67	1.24±0.82	0.781 [b]
w1	1.26±0.69	1.21±0.68	0.960 [b]
w2	1.32±0.68	1.19±0.65	0.602 [b]

Data were reported as Mean±DS; [a]comparison between basal values; [b]comparison between groups of the modifications *vs.* basal: Mann-Whitney Test.

TABLE III.—*Bristol Stool Scale.*

Period	Active group (N. 42)	Standard group (N. 43)	P value
Basal (b1-b2)			0.574 [a]
t1-t2	P=0.711 [b]	P=0.058 [b]	0.170 [c]
t3-t4	P=0.319 [b]	P=0.080 [b]	0.583 [c]
Wash-out (w1-w2)	P=0.230 [b]	P=0.180 [b]	0.808 [c]

[a]Comparison between basal values; [b]comparison between values *vs.* basal; [c]comparison between groups of the modifications *vs.* basal: Mann-Whitney Test.

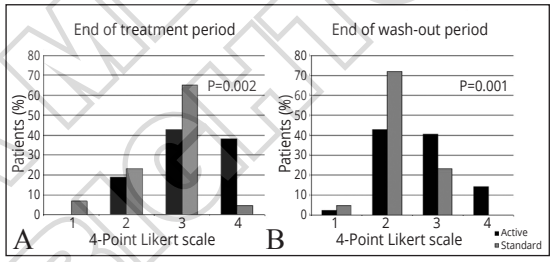


Figure 5.—A, B) The global self-assessment (four-point Likert Scale) at the end of treatment period (A) and wash-out period (B).
1=markedly worsened; 2=mildly worsened; 3=mildly improved; 4=markedly improved.

strain mix of bacterial and yeast probiotics were effective in reducing each cardinal symptom of IBS investigated in this study, such as abdominal pain/discomfort, bloating and bowel movement abnormality (*i.e.*, straining or urgency). Moreover, a composite score of these three symptoms showed a significant improvement in patients treated with the new formulation starting from the treatment period and lasting over the entire wash-out period. Compared to the standard formulation-treated group, the "active" group showed a significant reduction in bowel movement abnormality during the last week of treatment and the entire wash-out period (P=0.01). These effects were observed without any change in frequency and consistency of bowel movement, thus they cannot be attributed to an intrinsic "laxative" or, even, "antidiarrheal" effect. The reduction in bowel movement abnormality showed in patients receiving the "active" treatment could also justify the difference observed in self-assessment improvement reported by patients. Compared to

those receiving the standard formula, patients on active treatment reported a significant improvement of global self-assessment at the end of treatment (P=0.002) and wash-out period (P=0.001). Finally, both probiotic preparations were well tolerated and devoid of untoward effects in IBS patients. The basis to understand the improvement of the symptoms indicated above could be the modulation of the minimal mucosal inflammation which has been thought to play a role in symptom generation in IBS.[5, 6] Indeed, growing evidence demonstrates that an immune activation/inflammatory response detectable at the mucosal level may play a pathogenetic role in eliciting gut dysfunction and symptom generation in patients with IBS.[3, 27] The basis for this concept is centered on several clinical data.

First, it is well known that patients with quiescent inflammatory bowel disease frequently develop symptoms overlapping those of IBS patients.[28] Second, approximately a third of patients develop persistent IBS symptoms after an acute episode of gastroenteritis (*i.e.*, *Campylobacter jejuni*, *Shigella*, or *Salmonella* infections), thus leading to the term postinfectious IBS.[29] Finally, a low-grade inflammatory response, which is usually undetectable endoscopically and at routine histology, has been demonstrated in the intestinal mucosa of patients with either conventional or postinfectious IBS.[3, 29] Several factors might contribute to such minimal inflammatory changes, including abnormalities to the resident gut microbiota occurring in IBS patients. For example, small bowel bacterial overgrowth, qualitative alteration of gut microbiota and abnormal gas fermentation and handling have all been reported in patients with IBS.[10, 17, 30, 31] Hence the implication that probiotics might be beneficial in the treatment of IBS symptoms owing to their potential effects on a variety of factors, which, in addition to the above indicated modulation of minimal inflammation, include motility, colonic fermentation and formation of gas. Several clinical trials evaluated the efficacy of probiotics on IBS; however, the available data are controversial and there are a lot of difficulties in comparing studies using probiotics that varied in terms of species, strains, preparations, and doses.[32] A recent meta-analysis showed that overall probiotic therapies have a statistically significant effect in improving a number of IBS related symptoms, in particular, most published trials indicated a positive outcome for global IBS symptoms. In contrast, trials based on *Lactobacilli* and on *Bifidobacteria* did not show statistical significance, whereas multistrain probiotic formulations did show a significant effect in improving IBS symptoms.[32] This study is innovative since it tests a fermented milk containing the yeast *K. marxianus fragilis* B0399 along with a multistrain mix in IBS patients. *Bifidobacterium lactis* BB12, contained in a bacterial multistrain mix, has been previously evaluated because of its probiotic properties (*e.g.*, survival through the gastrointestinal tract) and the results showed no significant effects on IBS symptom relief as compared to a control (fermented milk only).[33] Concerning *K. marxianus*, this yeast has a documented ability to survive throughout the gut, can transiently colonize both the colonic lumen and mucosa (Caco-2 enteric cells adherence), proliferate under anaerobic conditions and high chloride and bile acid tolerance.[23] Furthermore, other relevant biological properties showed by *K. marxianus* include a broad substrate spectrum (*i.e.*, lactose and other disaccharides), thermo-tolerance, high growth rates, low tendency to ferment when exposed to sugar, production of enzymes (*e.g.*, alpha-galactosidase, alphaglucosidase, inulinase, and polygalacturonases) and reduction of lactose content in foods.[34] Based on this ample array of microbiological and biochemical features, it is not surprising that *K. marxianus*, as other probiotics, had a role in symptom reduction in the IBS patients investigated in the present study. Taken together, these properties may account for the positive effect observed on the primary end-point of this study, *i.e.*, the efficacy of the probiotic mix containing *K. marxianus* on a composite score calculated as the sum of abdominal pain, bloating and bowel movement abnormality. The results showed a significant effect in both groups (starting from the second week of treatment), without significant difference between two groups. The lack of an adequate control preparation did not allow the identification of a specific effect evoked by *K. marxianus*. Indeed, the effect of the standard preparation containing *Bifidobacterium lactis* B12 cannot be underestimated and recent

evidence demonstrated an active effect of *Bifidobacteria* supplementation on IBS symptoms.[32] However, it is noteworthy that only the multistrain formulation containing both yeast and bacteria (the "active preparation") had positive effects on the composite score lasting over the entire wash-out period. The mechanisms underlying this very interesting effects should be defined and further analysis is necessary (*e.g.*, ad hoc studies investigating the ability of the active probiotic mix with *K. marxianus* on the supposed minimal inflammatory infiltrate detectable in a subsets of IBS patients). The secondary endpoints of the study, *i.e.* the analysis of each cardinal IBS symptoms, showed that both active and standard preparations had an effect on pain reduction and improvement of bloating and bowel movement abnormality. Our results confirm the trend showed by Moayyedi *et al.*,[32] who found a significant heterogeneity of probiotic effects among ten trials with 834 IBS participants reported on abdominal pain. Bloating, the most common abdominal symptom reported by patients with IBS which has been demonstrated by several studies to be improved by different types of probiotics, including the VSL#3 mix[35, 36] and *Bifidobacterium animalis*.[37] In our experience both active and standard formulations were able to induce a significant reduction in abdominal bloating starting from the second week of treatment and lasting over the entire wash-out period. In term of quantitative effect, bloating reduction was the most striking effect observed in our study. In contrast with previous data, which cannot find a significant effect on reduction in urgency,[35, 36, 38] our study did demonstrate a significant improvement in the bowel movement abnormality (*i.e.*, urgency in diarrhea-predominant-IBS and straining or sense of incomplete evacuation in constipated predominant-IBS) only in patients who received the "active" formulation. This difference between the two study groups maintained its statistical significance up to the end of the entire wash-out period. Probiotic formulation containing multiple strains of *Bifidobacteria* and *Lactobacilli* previously showed a significant effect on overall symptoms reduction in IBS.[38] Some authors have speculated that *Bifidobacteria* represent the active component of probiotic combinations based on the finding that Lactobacilli per se have no effects on IBS symptoms and that *Bifidobacteria* (especially *Bifidobacterium infantis 35624*) may relief IBS symptoms because of their well-known anti-inflammatory action and neuromodulatory properties.[15] Also, a synergistic effect of different species of probiotics on IBS symptom improvement has been hypothesized.[32] Another important finding emerged by this study, has been a superior effect on symptom reduction induced by the "active" *vs.* the "standard formulation" as confirmed by patient's global self-assessment based on a four-point Likert scale. This suggest that the active probiotic mix used in this study had a favorable effect of wellbeing perception which is another important aspect emerging from our active probiotic formula.

Conclusions

In conclusion, this study shows the potential benefit of adding the probiotic *K. marxianus fragilis B0399* yeast in the fermented milk containing *Bifidobacterium lactis BB12*, *S. thermophilus* and *Lb. bulgaricus* in the management of some cardinal IBS symptoms. Whether this formula might have an actual efficacy deserves further studies based on a broader number of patients and *ad hoc* experimental design.

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