Original Article

# Clinical studies on alleviating the symptoms of irritable bowel syndrome with a probiotic combination

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Irritable bowel syndrome (IBS) is one of the most common diagnoses in gastroenterology, but current therapies are inefficient. Recent clinical trials suggest beneficial effects of certain probiotics in IBS. Because of the heterogeneity of IBS a probiotic combination may be more efficient than a single strain. We screened for optimal strains, and developed a multispecies probiotic combination consisting of *L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *shermanii* JS and *Bifidobacterium breve* Bb99. The clinical efficacy of the probiotic combination was evaluated in IBS patients in a randomised, double-blind, placebo-controlled six-month intervention. During six months the subjects received daily either probiotic supplementation or placebo. IBS symptoms were followed by symptom diaries. The probiotic supplementation demonstrated significant value in reducing IBS symptoms. At the end of the study period the total symptom score (abdominal pain + distension + flatulence + rumbling) had reduced with 42% in probiotic group versus 6% in the placebo group. The treatment difference in the baseline-adjusted symptom score between the groups was -7.7 points (95% CI -13.9 to -1.6) in the favour of the probiotic supplementation. The underlying mechanisms could involve for instance anti-inflammatory effects, balancing of the microbiota or motility-related effects induced by the probiotic. The probiotic activity may be enhanced by synergistic effects of the combination that each strain alone would not hold. In conclusion, we found a probiotic combination of LGG and three other strains to be effective in alleviating IBS symptoms.

**Key Words: gastrointestinal symptoms, irritable bowel syndrome, probiotics, probiotic combination**

## Introduction

Irritable bowel syndrome (IBS) is one of the most common diagnoses in gastroenterology, since it is estimated that approximately 10-20% of the adult population suffers from this syndrome worldwide.[1] IBS is a heterogeneous condition that presents as abdominal pain, distension, flatulence and irregular bowel movements. Certain probiotics have shown promising beneficial effects in IBS,[2-5] but the evidence can not yet be considered consistent. No single abnormality in the microbiota of IBS patients versus healthy controls has been found, but several studies have reported various and different alterations in the bacterial composition of subjects with IBS.[6-10] This finding combined with the diverse nature of IBS symptoms may indicate that a probiotic combination could be more efficient than a single strain in this particular disease. Timmerman and co-workers[11] have in a recent review defined a multispecies probiotic as "containing strains of different probiotic species that belong to one or preferentially more genera". They suggest that multispecies probiotics may in some conditions be more efficient than monostrain probiotics due to for instance enhanced adhesion and a greater variety of antimicrobial compounds. With similar thoughts, our aim was to develop a multispecies probiotic combination that could alleviate symptoms of IBS.

## Selection of efficient probiotic strains

*Lactobacillus rhamnosus* GG (ATCC 53103; LGG®) is one of the most studied probiotics worldwide. Its beneficial effects have been well-documented in the prevention and treatment of various gastrointestinal disorders, especially diarrhea.[12] In addition to this, LGG has been shown to possess immunomodulatory effects, e.g the lowering of proinflammatory cytokines, such as tumour necrosis factor (TNF)-α in allergic children[13] and interleukin (IL) -6 and TNF-α in healthy volunteers.[14] There is a growing body of evidence that IBS might be a state of low-grade mucosal inflammation,[15] and consequently a probiotic lowering pro-inflammatory responses may be hypothesised to be useful in IBS. LGG alone has, nonetheless, not been successful in alleviating IBS symptoms.[16,17] Therefore three other strains were, based on their promising *in vitro* properties, selected to be combined with LGG. Other strains selected

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Accepted 30th August 2006

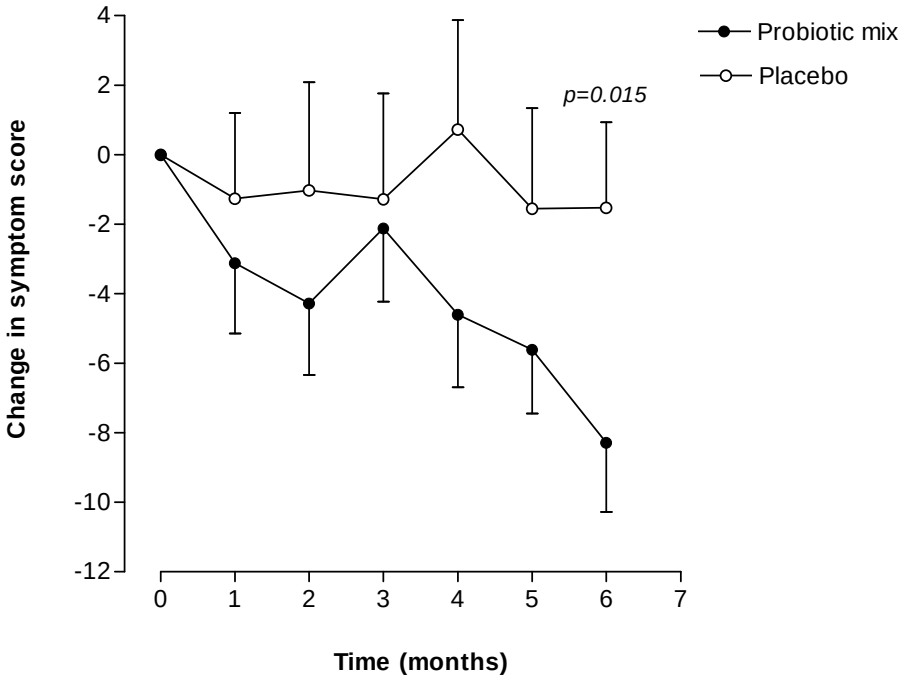


**Figure 1.** Change (mean ± SEM) in total symptom score (abdominal pain + distension + flatulence + rumbling) during the six month intervention (p=0.015 at six months; n=81). Figure reprinted from reference 4 with the permission of Blackwell Publishing.

to the combination were *Lactobacillus rhamnosus* Lc705 (DSM 7061; Lc705), *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7067; PJS) and *Bifidobacterium breve* Bb99 (DSM 13692; Bb99).

Lc705 has been shown to produce an antimicrobial agent, 2-pyrrolidone-5-carboxylic acid.[18] Together with PJS it inhibits yeasts and moulds in food and feed[18] and seems slightly to alleviate constipation.[20] Other strains of *P. freudenreichii* have in earlier experiments showed successful *in vitro* and *in vivo* adhesion to intestinal epithelial cells[21] as well as beneficial activity in the GI tract via producing apoptosis-inducing SCFA.[22] *B. breve* was isolated from a normal gut microbiota of a healthy infant, and there is an increasing amount of data indicating that bifidobacteria are advantageous probiotic agents.[23,24] Regarding synergistic effects especially adhesion can be remarkably increased in probiotic combinations: the presence of LGG more than doubled the adhesion of *Bifidobacterium* Bb-12 and tripled the adhesion of a *P. freudenreichii* strain. [25,26]

**Clinical trial with a multispecies probiotic combination**

A randomized, double-blind, placebo-controlled six-month trial was conducted in order to investigate the therapeutic value of the probiotic combination (LGG, Lc705, PJS and Bb99) in IBS.[4] Altogether 103 IBS patients with a well-established IBS diagnosis took part in the trial, and 86 subjects completed the study. All the patients fulfilled the Rome criteria I[27] and the majority (68%) also fulfilled the Rome criteria II.[28] During the six-month intervention period all subjects took daily either one multispecies probiotic capsule (Valio Ltd, Helsinki, Finland; total amount of bacteria 8-9 x $10^9$ cfu/day; equal amount of each strain) or one placebo capsule. Abdominal symptoms and bowel habits were followed by a symptom diary that the subjects were instructed to fill in regularly. The RAND-36 questionnaire[29] was used to monitor health related quality of life.

The probiotic supplementation demonstrated significant value in reducing IBS symptoms. At the end of the intervention the total symptom score (abdominal pain + distension + flatulence + rumbling; possible range 0-112) had reduced with 42% in probiotic group versus 6% in the placebo group. At six month the treatment difference in the baseline-adjusted total symptom score was -7.7 points (95% CI -13.9 to -1.6) when the probiotic group was compared to placebo ($P$=0.015; Fig. 1). There were no significant differences between the groups regarding changes in bowel habits or quality of life. Consistently, a pilot-trial involving subjects under *H. pylori* eradication has shown the beneficial GI effects of the probiotic combination, since the probiotic supplementation improved tolerance to eradication.[30]

**Possible mechanisms of probiotic therapy**

Several putative mechanisms have been suggested to play a role in the IBS symptom-relieving effects of probiotics. Probiotics could influence IBS directly through balancing the microbiota, and hence normalising an aberrant gas-production or production of short chain fatty acids observed in some cases of IBS.[31,32] An inflammatory component seems also to be one possible deviance in IBS, especially in so-called post-infectious IBS, a form of the disease that affects 10–15% of patients after acute infectious enteritis.[33] LGG alone is able to modulate the immune response.[13,14] Also the probiotic combination has in other trials involving atopic children been shown to be immunomodulatory, but in a different way from LGG.[34-36] Animal models clearly imply that inflammation could contribute to symptoms of irritable bowel, since there is

an indication of a causal relationship between the presence of mucosal inflammation and altered sensory-motor function.[37]

In addition to balancing the microbiota and having immunomodulatory effects probiotics may influence intestinal motility. *In vitro* studies on isolated intestines of guinea pigs have shown that probiotics, especially bifidobacteria, have a relaxing effect on the colon.[38] *L. paracasei* seems also to attenuate post-infective dysmotility and visceral hypersensitivity in murine models of IBS.[39,40] A recent cell line study also introduces new ideas about possible probiotic mechanisms in the GI tract.[41] LGG was shown to modulate the activity of certain signalling pathways in intestinal epithelial cells by activation of MAP kinases. LGG treatment of gut epithelial cells seemed to protect them from oxidant stress, possibly by preserving cytoskeletal integrity.

## Conclusions

A multispecies probiotic consisting of LGG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *shermanii* JS and *B. breve* Bb99 seems to alleviate IBS symptoms significantly in a six-month placebo-controlled trial. This is the first long-term clinical intervention to demnostrate efficacy of a certain probiotic in irritable bowel syndrome. Studies investigating the mechanisms of action are under way.

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# 使用益生菌結合體減少大腸急躁症的症狀之臨床研究

大腸急躁症(IBS)是腸胃疾病中最常見的診斷，但是目前的治療效率不夠。最近的臨床試驗建議益生菌對於IBS有好的影響。因為IBS的異質性，益生菌的結合體可能較單一菌株較具有功效。我們篩選理想的菌株，並且發展多菌種益生菌結合體，包括*L. rhamnosus* GG, *L. rhamnosus* Lc705, *P. freudenreichii* ssp. *shermanii* JS 及 *Bifidobacterium breve* Bb99。 益生菌結合 體的臨 床效 力以 IBS 病人的隨機雙盲，安慰劑控制之六個月介入臨床試驗來評估。研究對象在六個月期間，每日接受益生菌補充品或是安慰劑。IBS症狀記載在症狀日記中。益生菌補充品表現出顯著降低IBS症狀的能力。研究終了，益生菌組的總症狀分數(腹痛+腹脹+脹氣+腹鳴)降低42%比上安慰劑組的6%。在校正啟始的症狀分數後，兩組的差異為-7.7分(95% CI -13.9到-1.6), 益生菌補充 品較佳。 基本 的機制包含如抗發炎、平衡菌群或是被益生菌誘導蠕動相關影響。益生菌的活性可能因為益生菌結合體中各單一菌株間產生的協同作用而增強。總而言之，我們發現包含LGG及其他三種菌株的益生菌結合體可以有效的減少IBS徵狀。

關鍵字：腸胃道症狀、大腸急躁症、益生菌、益生菌結合體。