# Probiotics in Diverticular Disease of the Colon: an Open Label Study

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## Abstract

**Aim**: To investigate the effectiveness and safety of a symbiotic mixture in preventing recurrence of constipation-related abdominal pain in patients with uncomplicated diverticular disease of the colon. **Methods**: Forty-six consecutive patients (10 men, 36 women, mean age 62.5 years, range 49 to 77 years), previously affected by symptomatic uncomplicated diverticular disease of the colon, were enrolled in a 6-month follow-up study in a prospective, randomized, open-label study. The following symptoms were assessed at entry and through follow-up by using a quantitative scale: constipation, diarrhoea and abdominal pain. After recruitment, the patients were assigned to the following treatment: SCM-III symbiotic mixture, 10ml three times a day. The colonization of ingested Lactobacillus acidophilus 145 and *Bifidobacterium* spp. 420 was assessed by specie-specific PCR. Forty-five patients completed the study (97%). **Results**: Thirty-one patients (68%) were still symptom free after the 6th month of treatment. Treatment with SCM-III was regarded as "effective" or "very effective" in more than 78% of the patients altogether ($p<0.01$ vs baseline values). The microbiological study showed that, as compared to baseline values, SCM-III enabled a significant increase of the lactobacilli and bifidobacteria counting and a trend decrease of clostridia. Genomic analysis confirmed the survivability of the ingested strain as long as treatment was given. **Conclusions**: The present symbiotic mixture seems to be effective in preventing recurrence of symptomatic uncomplicated diverticular disease of the colon, especially in those patients with constipation-predominant features.

## Key words

Symbiotics – recurrence – constipation-predominant – diverticular disease – probiotics.

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## Introduction

Diverticular disease of the colon is very common in developed countries and its prevalence increases with age and up to an estimated 20% of patients may manifest clinically-relevant illness in their lives [1]. The treatment of uncomplicated diverticulitis (characterized by lower abdominal pain, fever, and bowel movement alterations) is generally non surgical whereas the latter option is usually required when serious complications supervene. In uncomplicated diverticular disease, treatment is aimed at relieving symptoms which may remarkably affect the quality of life of these patients requiring poorly absorbable antibiotics or anti-inflammatory drugs [2]. Nonetheless, despite the high prevalence of this disease, literature data on the treatment for maintaining remission in symptomatic diverticular disease is still a matter of debate. Stasis of luminal contents may occur within colonic diverticula, and it is probably associated with changes in the spectrum of intestinal microflora although such assumption has not been fully demonstrated yet. However, it is likely that faecal stasis and dysbiosis may trigger the formation and topical release of abnormal metabolites eventually causing those inflammatory/functional changes leading to abdominal symptoms. A recent study has showed that only less than 60% of patients using rifaximin for tentative maintenance treatment were symptom free at the 12th month of follow up [3]. On the other hand, concomitant constipation might represent a causative factor in the occurrence of symptomatic flare up. Dietary changes and disaccharides are among the most common prescriptions in such cases but their effective clinical benefit is not entirely established.

Given the complex interplay between the gut ecosystem and gastrointestinal function, the tentative manipulation of the gut flora through viable bacteria administration has in recent times being proposed as a further rational therapeutic option. Probiotics have been defined as viable micro-organisms which, when ingested, might exert beneficial effects in the prevention and treatment of a number of specific pathologic disorders [4-8]. A further possibility in microflora management procedures is the use of symbiotics in which the

live microbial species are combined with specific substrates (prebiotics) for growth and improved survival [9, 10] and such an approach might be an amenable tool in the weaponry of treating this disease. The rationale for using probiotics in the treatment of diverticular disease is likely to be due to their reported production of antimicrobials, competitive metabolic interactions with pro-inflammatory organisms, and inhibition of adherence and translocations of pathogens. They may also influence mucosal defence at the levels of immune and epithelial function, such as decreasing several pro-inflammatory cytokines. Moreover, Fric et al [11] have recently showed the effectiveness of probiotics in treating uncomplicated acute diverticulitis of the colon. About 60% of patients suffering from a previous attack of diverticulitis and not taking any medication in preventing recurrence of symptoms are symptomatic within 1 year. However, there is a large number of patients with diverticular disease, especially if with underlying constipation, who regularly undergo uncomplicated symptomatic phases requiring drug treatment. We have recently shown in experimental and clinical settings that a novel symbiotic could exert a significantly beneficial effect on gut translocation and local and systemic inflammatory and microbial metabolic parameters [12-14].

In light of these data, we conducted a prospective study evaluating the effectiveness and tolerability of a symbiotic mixture preventing recurrence of symptomatic diverticular disease of the colon and, in particular, in those patients presenting a constipation-predominant pattern. We also checked the survivability of ingested bacteria by genomic tools which have been highlighted as possible gold-standard in the field, recently pointed out in a detailed review by Yadav et al [15].

## Material and methods

From July 2006 to September 2007, a prospective, open-label study was conducted on 46 consecutive patients (10 men, 36 women, mean age 67.5 yrs, range 39 to 77 yrs) presenting with a history of recurring constipation-predominant symptomatic uncomplicated diverticular disease of the colon (average 1.2 episodes/month). Diagnosis of symptomatic uncomplicated diverticular disease of the colon was defined as nonspecific abdominal complaints (intensity over 3 on a VAS evaluation) in patients with diverticula of the colon, without any systemic sign or symptom of inflammation, according to well-defined criteria and requiring medical treatment (either by self-administration or by health professionals) such as antispasmodic drugs and/or painkillers and/or oral or enema laxatives. In particular, spasmolytics were used in 38 (82.6%), non absorbable antibiotics in 29 (63%), fibers in 6 (13%). Twenty-one out of 46 (45.6%) patients referred had a history of diverticulitis. Diagnosis of diverticular disease was established by colonoscopy or bowel enema in all patients. Diverticula were localized in overall colon in 3 patients (6.5%), overall leftsided colon in 17 patients (36.9%) and only in the sigma in 25 (54.3%).

Criteria for exclusion from the study were an active or recent peptic ulcer, chronic renal insufficiency and presence of ongoing or past major diverticulitis complications. Moreover, all systemic and non-absorbable antibiotics and antifungal medications, suppositories, herbal supplements and food containing live active cultures were considered as exclusion criteria. Patients were also given a dietary questionnaire in order to detect any major changes during the study period.

### Study design

The study period began with the baseline evaluation (day 0) and ended with the final visit, on the 6th month. Stool samples were collected at 0, 1, 3 and 6 months. We assessed the following symptoms in all enrolled patients: (1) constipation, (2) diarrhoea and (3) abdominal pain. The intensity of the symptoms was scored on a quantitative scale of 0 to 3 according to worsening of symptoms: 0: absence; 1 - slight; 2- mild/moderate; 3 - severe. All patients were asymptomatic at the time of enrolment and showed a score of 0 in all parameters evaluated. Patients were also instructed to rate the below-mentioned parameters on diary cards. The overall clinical improvement (OCI) as compared to their status in the past three months was assessed at each visit using a four-point scale (1: very effective, 2: effective; 3: unchanged, 4: worsened). All patients were assigned to the following treatment schedule: SCM-III 10 ml t.i.d. (Microflorana-F, Named, Lesmo, Italy. Composition for 100 ml: *L. acidophilus* strain 145 1.25 x 106, L. helveticus ATC 15009 1.3 x 109, *Bifidobacterium* spp. 420 4.95 x 109 in a phytoextracts-enriched medium. This consists of: urtica dioica, ribes nigrum, vaccinium myrtillus, taraxacum officinalis leaves and roots, daucus carota, equinacea purpurea leaves and roots in the amount of 82g/100ml).

Specific genomic primers for *L. acidophilus* 145 and *Bifidobacterium* spp 420 contained in the mixture were developed to identify the specific strain survival along the gastrointestinal tract. Patients were enrolled in a 6-month follow-up study taking into account the symptoms by using a quantitative scale. All patients were asymptomatic at the time of enrolment and showed a score of 0 in all parameters evaluated.

Clinical check ups were planned at the end of the 1st, 3rd and 6th month of treatment. Measurements of acceptability and tolerability, the presence of possible side effects and patients' compliance were recorded also and a detailed form was provided to patients and physicians. Clinical examination and biochemical tests were made available to all patients in each possible case of querying side effects or a supervening complication related to the underlying disease. The intention-to-treat analysis included all patients and 95% confidence intervals (CI) were provided.

### Stool microflora analysis

Stool specimens were collected in pre-weighed collection tubes containing liquid phosphate buffered saline + 1% gelatin medium. Collection tubes were frozen until used. A stool sample of approximately 2-5 g was placed in the

collection tube and then dispersed in the transport media. The tube was sealed, immediately agitated until the stool and liquid were mixed thoroughly, and then refrigerated. The stool sample was weighed, and cultured within 72 hours of collection. Determination of bacterial flora was carried out as reported by Mitsuoka et al [16]. Briefly, 9ml of a diluent was added to 1g of the faecal sample, the mixture was vigorously shaken and tenfold serial dilutions of the suspension were prepared. Each dilution was set in aliquots of 0.05ml onto agar plates of media which was appropriate for the target organisms as follows. The following organisms were counted on selective media to the genus level: *Bifidobacterium* – Reinforced Clostridial agar plus nalidixic acid (20 mg/L), polymixin B (8.5 mg/mL), kanamycin (12.5 mg/mL), iodoacetic acid (12 mg/L), and 2,3,5 triphenyl tetrazolium chloride (25 mg/mL); *Enterococcus* – KF Streptococcal agar; *Clostridium* - Clostrisel agar. The quantitative culture was performed by plating 100 µl of diluted stool specimen onto each agar medium. The plate surfaces were allowed to dry for 15 minutes and then placed in an appropriate environment. Lactobacilli, bifidobacteria, and clostridia plates were incubated in an anaerobic chamber at 37º C for a minimum of 4 days. Enterococci plates were incubated for 48 hours in a 5% $CO_2$ atmosphere at 37º C. Bacterial identification was assessed upon the morphology of the colonies, microscopic examination of Gram-stained slides, tests for growth under aerobic conditions and appropriate biochemical tests. For all organisms, total colony counts were reported as less than detectable ($<10^2$) or detectable ( $\geq 10^2$). Using these criteria, this methodology detected at least $10^2$ colony forming units (CFU)/g stool.

**Extraction of DNA from faecal samples**

Each collected faecal sample was stored at -80°C until DNA was extracted. Faecal samples (20 mg) were washed 3 times in 1.0 mL of phosphate buffered solution and centrifuged at 14 000g. Faecal pellets were resuspended in 450 µL of extraction buffer (100 mM Tris-HCl, 40 mM EDTA, pH 9.0) and 50 µL of 10% SDS. Glass beads (300 mg; diameter, 0.1 mm) and 500 µL of buffer-saturated phenol were added to the suspension and the mixture was vigorously vortexed for 30 seconds. Then, 50 µl of cell lysing solution (60 mg/ml of lysozyme, 1 mg/ml of N-acetylmuramidase) was added to the bead solution to assure efficient degradation of bacterial cell walls. After centrifugation at 14 000g for 5 minutes, 400 µL of supernatant was extracted with phenol-chloroform and 250 µL of the supernatant was precipitated with isopropanol. DNA was purified using a High Pure Polymerase Chain Reaction (PCR) Template Preparation kit (Roche, Basel, Switzerland) and suspended in 200 µL of TE buffer and DNA was finally extracted. The DNA yields from faecal samples was 211 ± 55 ng.

**Species-specific quantitative real-time PCR detection of *L. acidophilus* and *Bifidobacterium* colonization**

For the selection of primer and probe sequences, the 16S-23S intergenic spacer regions of the different Lactobacillus species was retrieved from the GenBank, EMBL, and DDBJ databases. Specific sequences were identified to design primers and probes for *L. acidophilus* and the primer and probe were tested for specificity using the basic local alignment search tool which proved to fulfill the related criteria (F GAA AGA GCC CAA ACC AAG TGA TT R_acid_IS CTT CCC AGA TAA TTC AAC TAT CGC TTA P_acid_IS TAC CAC TTT GCA GTC CTA CA). The 5' reporter dye VIC and the 3' quencher NFQ-MGB served as probes for the detection of the genus *L. acidophilus* 145. Moreover, TaqMan minor groove binding probes were also used to further increase specificity and sensitivity.

*Bifidobacterium* spp. colony morphologies from each fecal sample were randomly selected from each bifidobacteria culture plate for PCR analysis. Part of the colony was suspended in 20 µL of sterile distilled water and 4µL of this suspension served as a template for PCR. Amplification of the template DNA was carried out in 45 µL of PCR Supermix and 0.5 µL (100 pmol/µlLl) of each of the two primers. *Bifidobacterium* spp. 420-spp. primers were used for PCR amplification. Primer sequences are as follows: Bflact2 (5' - GTGGAGACACGGTTTCCC-3') and Bflact5 (5' -CACACCACACAATCCAATAC -3'). Amplification of DNA was performed in a thermal cycler programmed as such: 10 min at 95°C and 30 cycles of 30 sec at 95°C, 1 min at 58°C, 4 min at 72°C, followed by 7 min at 72°C. Amplicons were identified by ethidium bromide staining following electrophoresis in 1.2% TAE agarose gels. *Bifidobacterium* spp. 420 specific colonies were expected to yield a single band of 680 bp, in contrast to non- *Bifidobacterium* spp. 420 samples which did not yield any amplicons. When no amplicons were present after the *Bifidobacterium* spp. 420-specific PCR, the presence of template bacterial DNA was confirmed to rule out a false negative. For this PCR, amplification of template DNA was performed as described above and directed by 16S rRNA primers under the following cycling conditions: 5 min at 94°C and 35 cycles of 15 sec at 94°C, 30 sec at 57°C, 90 sec at 72°C, followed by 10 min at 72°C. Colonization was defined as the PCR-based detection of *Bifidobacterium* spp. 420 in any of one or more of the five selected unique colony morphotypes. The relative amounts of the different bacterial species in faecal samples were calculated after correction for differences in the amplification efficiencies of the PCR.

**Statistical analysis**

Significance was established by analysis of variance and the level of significance was determined by employing a Duncan's multiple-range test. Data were expressed in the text as means (SD) and a probability value of $<0.05$ was set as indicating that a statistically significant difference existed between experimental groups. The scores of efficacy and the intensity and frequency of the symptoms were evaluated as compared to baseline values by Mann-Whitney-Wilcoxon rank test.

## Results

No evident clinical (diarrhoea, abdominal pain, nausea) or biochemical adverse side effects were recorded nor any

diverticulitis sign was recorded throughout the study period. Compliance was satisfactory as all patients took more than 98% of the symbiotic prescribed. About 9% of patients (4/46) reported a poor palatability of the symbiotic. Forty-five patients completed the study (97.8%): 1 patient was withdrawn from the study for protocol violation (the use of a systemic antibiotic for extra intestinal disease). Thirty-one patients (31/45 patients: 68.8 %) were still symptom free after the 6th month of treatment ($p<0.05$). Treatment with SCM-III was regarded as "effective" or "very effective" in 77.2% of the patients altogether ($p<0.01$ vs baseline values, data not shown). No worsening was reported as a final assessment at the end of the study period and about 6% (3/45) reported a "not effective" result. The total number of constipation-related abdominal pain expected (intensity ≥2) showed a significant decrease with an overall reduction of two-third of expected episodes which was maintained throughout the 6-month observation period ($p<0.01$, Fig. 1). At a further analysis, it appeared that two-thirds of the patients reporting episodes of mild discomfort at entry (intensity <2, 10/13 patients) had improved or normalized their bowel habits (data not shown).

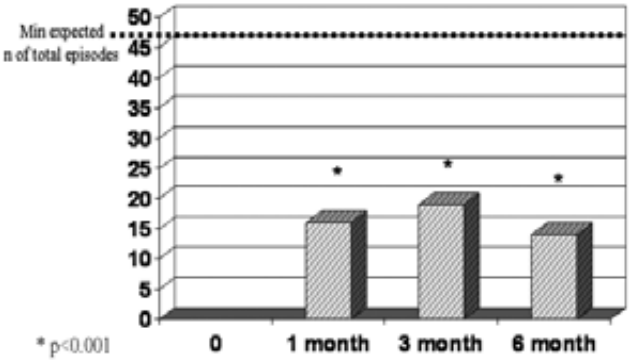


**Fig 1.** Total number of episodes of constipation-related abdominal pain (intensity ≥2). Effect of SCM-III.

The microbiological study showed that, as compared to baseline values, SCM-III enabled a significant increase of total anaerobes and in particular of lactobacilli and bifidobacteria together with a trend decrease of clostridia counting ($p<0.05$, Table I). Streptococci counting remained unchanged. Such effect occurred in all patients and was not related to the baseline counting of each strain nor to the clinical variables examined (duration of the disease, past antibiotics treatment, age, gender, main dietary style). Genomic analysis confirmed the significant survivability of ingested *L. acidophilus* and *Bifidobacterium* contained in the symbiotic mixture. In particular, at baseline no stool samples contained *L. acidophilus* 145 nor *Bifidobacterium* spp. 420. However, both bacteria were recovered from the stools of patients along the symbiotic supplementation period and peaking at the 3rd month observation (Fig. 2, $p<0.001$ vs baseline).

Finally, 12 patients positive in both strains at 6-month observation discontinued the treatment and were subjected to follow up. Genomic analysis revealed that already after two weeks of the discontinuation of the administration of SCM-III, both the species strains were no longer detected.

**Table I.** Faecal flora assessment in symptomatic diverticular disease: effects of SCM-III

| Bacterial species (log no./g wet feces) (healthy control) | Baseline | SCM-III |
|---|---|---|
| Total aerobes | 8.2 ± 0.22 | 8.18 ± 0.34 |
| Total anaerobes | 10.1 ± 0.12 | 10.8 ± 0.10* |
| Lactobacillus | 7. 1 ± 0.11 | 8. 4 ± 0.09* |
| Bifidobacterium | 9.3 ± 0.34 | 10.1 ± 0.22* |
| Enterococcus | 6. 2 ± 0.21 | 6. 3 ± 0.33 |
| Clostridium | 8. 5 ± 0.16 | 8. 1 ± 0.27 |

*p < 0.05

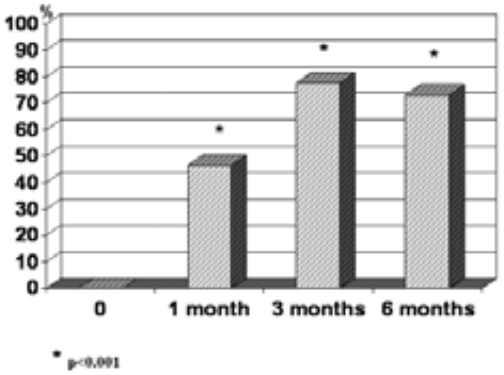


**Fig 2.** PCR detection of concomitant *L. acidophilus* 145 and *Bifidobacterium* 420 colonization.

## Discussion

The gastrointestinal tract is a complex microbial ecosystem, comprised of several hundred species of bacteria at a concentration of more than $10^{11}$ bacteria per gram of contents. These microbes have a potent influence on host health and a delicate equilibrium exists between organisms believed to benefit the host and those that may be detrimental [17]. Accordingly, tentative gut flora manipulation through probiotics offers an ideal theoretical, albeit still cumbersome to evaluate, approach to gastrointestinal disease and gut-related systemic illnesses. The symbiotic mixture we used in this study seems to be effective in preventing recurrence of symptomatic diverticular disease of the colon, expecially in those patients with constipation-predominant features. This specific subset of patients was chosen when considering the prokinetic-like properties exerted by this symbiotic in an experimental setting [18]. As a matter of fact, the majority of patients who were symptom-free at the entry into the study remained asymptomatic under SCM-III treatment while those who had minor discomfort did not experience any worsening while reporting an improvement/normalization of their bowel habits.

Moreover, we have recently shown that this symbiotic is able to beneficially modify the gut flora and its metabolic

activity in patients with liver cirrhosis [13] as well as to lower ammonia, benzodiazepine-like substances and endotoxin in this complex clinical setting [19], the latter being of some potential interest also in the triggering of severe inflammatory changes of complicated diverticulitis [20].

Moreover the PCR-based genomic approach employed in the present study confirmed the effective colonization of the symbiotic mixture through a significant survival of its two main probiotic strains in most but not all patients. In the present study the possible interference of small intestinal bacterial overgrowth which has been shown by Tursi et al [21] to be a very frequent associated phenomenon in this clinical set up was not assessed.

Taken altogether and with the limitation of a study addressing a rather heterogeneous population, it would appear that SCM-III exerts a significant beneficial effect in this condition and, namely, in those patients presenting with diverticular disease and constipation-related abdominal pain. These data finally suggest that treatment with balanced and viable symbiotic compounds may be a valuable and a likely preferable therapeutic option than repeat antibiotic treatment alone. Such a schedule should ideally be administered in a cyclic/subcontinuous regimen when considering the temporary efficacy on the modification of the faecal flora [22, 23] as also shown by genomic tools in our study. Indeed, cyclic probiotic administration for 10-15 days is able to maintain intestinal colonization for a further 15-20 days without any decreasing in bacterial concentration [19, 24]. On the other hand, long-term or repeat antibiotic treatment in the asymptomatic phase, and not specifically required for complicated diverticulitis, may increase also bacterial resistance, when used alone, having no prokinetic or sound microecological rationale in this setting. Moreover, it is known that bacterial re-colonization may take up to two weeks [25].

The optimal therapeutic regimen remains to be established using symbiotics and intertwining with anti-inflammatory drugs as suggested by Tursi et al [26-28] which may exert further specific intracellular benefit at the mucosal level [29].

## Conclusion

The present symbiotic mixture seems to be effective in preventing recurrence of symptomatic diverticular disease of the colon, especially in those patients with constipation-predominant features while positively altering the gut flora.

## Conflicts of interest

None to declare.

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