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Influence of Lactobacillus and Bifidobacterium Combination on the Gut Microbiota, Clinical Course, and Local Gut Inflammation in Patients with Ulcerative Colitis: A Preliminary, Single-center, Open-label Study

Magdalena Pilarczyk-Żurek[1], Małgorzata Zwolińska-Wcisło[2], Tomasz Mach[2], Krzysztof Okoń[3], Paweł Adamski[4], Piotr B Heczko[1], Aleksandra Mikołajczyk-Cichońska[5], Grzegorz Stefański[5] and Magdalena Strus[5]*

[1]*Department of Microbiology, Jagiellonian University Medical College, Cracow, Poland*
[2]*Department of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College, Cracow, Poland*
[3]*Department of Pathomorphology, Jagiellonian University Medical College, Cracow, Poland*
[4]*Polish Academy of Sciences, Institute of Nature Conservation, Cracow, Poland*
[5]*IBSS BIOMED SA., Sosnowa 8 Ave., Cracow, Poland*

Abstract

Background: Ulcerative colitis (UC) is one of the chronic, relapsing, inflammatory disorders of the gut and is characterized by inflammation limited in most cases to the colon. Since gut microbiota play a critical role in the development and perpetuation of intestinal inflammation, the addition of probiotics to this complex system may exert a positive influence on gut inflammatory reactions.

Methods: A single center, open-label, intention-to-treat study involving patients with moderate-to-severe UC was performed to check whether a probiotic mixture containing *Lactobacillus plantarum*, *Lactobacillus rhamnosus,* and *Bifidobacterium longum* given together with a standard treatment could decrease clinical and histopathology indexes for UC evaluation.

Results: The mixture given once a day for at least 2 months together with mesalazine and ciprofloxacin to patients in the acute phase of UC significantly reduced their Mayo Clinic Index values. Moreover, numbers of *Lactobacilli* isolated from patients feces were significantly increased, while those of Gram-negative rods decreased. The mixture given together with mesalazine to patients with UC in remission also caused a decrease of their clinical scores, but a more prominent and significant decrease of the histopathological index values in biopsy samples was observed.

Conclusions: Supplementation of standard therapy with the probiotic mixture used in this study was efficacious in inducing and maintaining remission in UC, and this effect was related to modulation of dysbiosis in the gut microbiota.

Keywords: Ulcerative colitis; Probiotics

Abbreviations: UC: Ulcerative Colitis; IBD: Inflammatory Bowel Disease

Introduction

Ulcerative Colitis (UC) is one of the chronic, relapsing, inflammatory disorders of the gut, collectively termed Inflammatory Bowel Disease (IBD), and is characterized by inflammation limited in most cases to the colon. Histologically, UC shows superficial inflammatory changes limited to the colonic mucosa and submucosa with cryptitis and crypt abscesses. Recently, several genes and genetic loci that contribute to susceptibility to IBD have been identified [1]. Some, such as activation of G protein Ga_{12} (encoded by GNA12), which leads to destabilization of cell junctions, or mucus layer defects related to *Muc2* mutation, impair epithelial barrier function and enable direct interactions of the gut commensal microbiota with intestinal epithelia and dendritic cells [2]. There is increasing evidence showing that human gut microbiota play a role in the development and maintenance of UC [3]. Differences in the abundance of bacterial species and their functions can differentiate patients with UC from their healthy counterparts, which lead to hypothesis that UC, as well as other IBDs, is related to changes in gut microbial ecology [4].

Since gut microbiota play a critical role in the development and perpetuation of intestinal inflammation, the addition of probiotics to this complex system may exert a positive influence on gut inflammatory reactions by improving the mucosal barrier function to decrease immune reactions, displacing deleterious microbes from the luminal-mucosal interface, or altering the metabolic consequences of the microbiota [5].

• Several published studies report the use of probiotics for inducting remission in UC, and these have been systematically reviewed [6]. This review concluded that the addition of a probiotic to conventional therapy did not improve overall remission rates in patients with mild to moderate UC, but may reduce the severity of disease activity. Subsequently, probiotic mixture VSL#3 given to adult patients with mild-to-moderate UC caused a 50% decrease in the UC disease activity index at 6 weeks in a significantly higher number of the treated patients than in the placebo group [7]. A more recent review

***Corresponding author:** Magdalena Strus, PhD, Department of Microbiology, Jagiellonian University, Medical College, Czysta 18 street, 31-121 Cracow, Poland,Telephone number: +48 12 633 25 67; E-mail: mbstrus@cyf-kr.edu.pl

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analyzed randomized clinical trials and found that selected probiotics were efficacious in inducing and maintaining remission in UC [8]. Another review reached nearly the same conclusions on the efficacy of probiotics in UC [9].

• The aim of this study was to investigate in a single center, open-label, intention-to-treat study involving patients with moderate-to-severe UC whether a probiotic mixture containing *Lactobacillus plantarum*, *Lactobacillus rhamnosus,* and *Bifidobacterium longum* given together with standard therapy could decrease clinical and histopathology indexes for UC.

Materials and Methods

Patients

Altogether, 51 patients (21 with UC in remission and 30 in the active phase of UC) of both sexes and between 18 and 72 years of age who were being treated in the Jagiellonian University Medical College Clinic of Gastroenterology in Cracow, Poland were enrolled. The study was conducted from 2008 to 2011 and was approved by the Jagiellonian University independent ethics committee (No KBET/5/B/2007). Written informed consent was obtained from all patients before enrollment.

Inclusion criteria included an existing diagnosis of UC based on disease history, colonoscopy results, and histopathologic evaluation of biopsies from the colonic mucosa. The patients were not treated with antibiotics for three months before enrollment. Exclusion criteria were: diabetes, autoimmune diseases, severe systemic diseases, alcohol abuse, cow milk allergy, and persistent treatment with non-steroidal anti-inflammatory drugs.

Probiotic preparation

The probiotic used was a commercially available food supplement (Lactoral®, IBSS BIOMED S.A., Krakow, Poland), kindly donated by the producer. One dose (one sachet) contained a mixture of three viable strains, *Lactobacillus plantarum* PL 02, *Lactobacillus rhamnosus* KL 53A, and *Bifidobacterium longum* PL 03, with a total of $\geq 1 \times 10^8$ cfu. The strains included in the probiotic have a documented human origin, having been isolated from the feces of healthy, breast-fed neonates, were deposited in an internationally recognized collection, and are covered by patents. Their identity and strain designation has been confirmed by phenotypic and molecular methods. They had been selected for commercial use on the basis of confirmed probiotic properties, i.e., high adherence ability to human Caco-2 and HT-29MTX cell lines, broad antagonistic activity towards pathogenic bacteria and fungi, and ability to survive in low gastric pH and bile. Moreover, the strains showed anti-inflammatory and tight junction-stimulating properties. The strains carry no extrachromosomal DNA elements able to transmit antibiotic resistance and do not show atypical resistance patterns to antibacterial agents.

Patients and treatment

The patients enrolled to the study were diagnosed on the basis of history, prior colonoscopy observations, and colon biopsies for histopathology and microbiology. A stool sample for microbial testing was collected at the initial visit by placing pea-size pellets in pre-weighed tubes with Schaedler Anaerobic Medium (SAB) (Difco, BD, Franklin Lakes, USA) with 10% glycerol. The samples were immediately snap frozen on dry ice and kept at -80°C or on dry ice until analysis. Disease activity was assessed according to the Mayo Clinic Disease Activity Index [10]. An acute phase of UC was diagnosed when the index was 4 or more, while a remission was diagnosed when the index was 3 or less. Following enrollment, the patients were divided into 2 groups: (A) patients with active disease and (B) those in remission. All patients were treated with mesalazine given orally in a dose of 3.0 g/d for patients with acute phase UC and 2 g/d for patients in remission. Patients in the acute phase of the disease were treated additionaly with ciprofloxacin i.v. 0.2 g/d for 10 days. Subsequently, approximately half of patients in both groups were randomly assigned to supplementary therapy with the probiotic preparation taken once a day for at least 2 months. Thus 4 subgroups were formed:

IA: patients in remission on standard therapy (n=13),

IB: patients in remission on standard therapy supplemented with the probiotic preparation (n=8),

IIA: patients in acute phase on standard therapy (n=18),

IIB: patients in acute phase on standard therapy supplemented with the probiotic preparation (n=12).

The disease status was checked at the control visit performed at least 2 months after patient enrollment. Only 31 patients finished the study: 6 in subgroup IA, 8 in subgroup IB, 8 in subgroup IIA, and 9 in subgroup IIB. The visit included an analysis of the patient's diary for the period of the study and a colonoscopy, including colon biopsies for histopathology and microbiology, and collection of a stool sample for microbiology as at the initial visit. The Mayo Clinic Index was then calculated.

Sampling of mucosa

All subjects underwent the same type of preparation prior to colonoscopy, with oral administration of sodium picophospate at dose of 0.001 g, magnesium oxide at a dose of 3.5 g, citric acid anhydrate at a dose of 10.97 g, and 5 mmol of potassium per sachet given twice daily. During colonoscopy, patients received intravenous sedation or general anesthesia, as required. Biopsy samples from patients with UC were obtained from inflamed and non-inflamed colonic mucosa sites, as revealed during colonoscopy. There were four biopsy specimens taken from each site: two for culture, one for FISH, and one for standard histopathological assessment. The biopsy samples for microbiology were transferred directly into pre-weighed tubes with SAB with 10% glycerol. The samples were immediately snap frozen on dry ice and kept at -80°C or on dry ice until analysis. All procedures were performed as quickly as possible, using sterile instruments and ensuring the integrity of the intestinal tissue. The codes of the biopsy samples were blinded before performing microbiological analysis.

Histopathology

The biopsy samples were fixed in buffered 10% formalin for 24 h, dehydrated with absolute ethanol, embedded in paraffin, cut, and stained with hematoxylin-eosin, Giemsa, and PAS using standard procedures. Pathological changes were evaluated according to the Geboes scale for 5 different parameters: (1) presence of a chronic inflammatory infiltrate, (2) neutrophilic and eosinophilic infiltrate in the lamina propria, (3) presence of neutrophils in the epithelium, (4) destruction of crypts, and (5) presence of erosions, ulcerations, or granuloma. The intensity of each parameter was graded from 0 to 5 [11].

Bacteriology

The frozen tissue or stool samples were thawed, weighed, homogenized in 1 ml of SAB, and quantitatively analyzed for their main bacterial constituents by cultures made on differential media

atient groups	Mean values of the Mayo Clinic Index		Mean decrease of the Mayo Clinic Index	P value
	Initial visit	Control visit		
IA	2.5	2.16	0.34	None
IB	2.25	1.5	0.75	None
IIA	7.0	4.0	3.0	<0.05
IIB	7.87	3.0	4.87	<0.05

Description of the group of patients: IA: patients in remission on standard therapy (n=6), IB: patients in remission on standard therapy supplemented with probiotic preparation (n=8), IIA; patients in acute phase on standard therapy (n=8), IIB; patients in acute phase on standard therapy supplemented with probiotic preparation (n=9)

Table 1: Mean Mayo Clinic Index values in the compared patient groups in acute versus remission UC phase in relation to probiotic dietary supplementation.

Sum values of the Geboes scale parameters	Group IA Patients in remission on standard therapy (n=6)	Group IB Patients in remission on standard therapy supplemented with probiotic preparation (n=8)	Statistical significant differences between groups	Group IIA Patients in acute phase on standard therapy (n=8)	Group IIB Patients in acute phase on standard therapy supplemented with probiotic preparation (n=9)	Statistical significant diffrences between groups
Initial visit	8,33	9,13	None	11,0	12,12	None
Control visit	7,50	6,00	None	7,28	8,87	Z=2,0058, P=0,0394
Difference between visits	-0,83	-3,12	Z=2,96057, P=0,0031	-3,75	-3,2	Z=1,03408, P=0,3011

Geboes scale parameters: (1) presence of the chronic inflammatory infiltrate, (2) neutrophilic and eosinophilic infiltrate in lamina propria, (3) presence of the neutrophils in epithelium, (4) destruction of crypts, (5) presence of erosions, ulcerations or granuloma. Intensity of each parameter was graded from 0 to 5. Figures represent sums of all 5 parameters calculated for all patents of each group.

Table 2: A comparison of the cumulated Geboes scale values for histological evaluation of the inflammation intensity in colon samples taken from UC patients in acute versus remission phase and taking or not taking probiotic preparation.

in aerobic and anaerobic conditions. All these manipulations were done aseptically in an anaerobic chamber (MACS-MG 500 Work Station, DW Scientific, Shipley, UK) in N(85%)+H_2(10%)+CO_2(5%) atmosphere. Homogenized samples were serially diluted with SAB, and 100 µl aliquots plated on the following media: McConkey agar (Oxoid, Basingstoke, UK) for *Enterobacteriaceae,* Columbia blood agar (Difco) with 5% sheep blood for streptococci, enterococcosel agar (BBL, BD, Franklin Lakes, USA) for enterococci, MRS agar (Oxoid) for lactobacilli and other lactic acid bacteria (LAB), BL agar for bifidobacteria, and Wilkins-Chalgren agar base (Oxoid) with supplements for *Bacteroides*.

The dilutions were then spread over the plate surface using a glass rod, and the plates were incubated aerobically at 37°C for 24 h, except for the cultures for anaerobic bacteria, which were kept in the anaerobic chamber for up to 4 days, depending on the type of media. The morphology of the colonies was analyzed using a magnifying glass, and several colonies were picked of each morphological type, subcultured on appropriate aerobic and anaerobic media, and Gram-stained. After further incubation and culture purity checks, phenotypic identification was performed using commercial identification systems (API 20E, API20A, APIStaph, APIStrept, bioMerieux, Marcy l'Etoile, France; BBL Crystal ID System, BD, Franklin Lakes, USA). All isolates of the *Lactobacillus* and *Bifidobacterium* genera were checked for their identity with the probiotic strains given to patients by testing their resistance to ciprofloxacin and co-trimoxazole using a disk diffusion test (Oxoid) on MRS or BL agar (Oxoid), since the *L. plantarum* PL02 strain is known to be resistant to ciprofloxacin and sensitive to co-trimoxazole, while *L. rhamnosus* KL53A is sensitive to ciprofloxacin and resistant to co-trimoxazole and *B. longum* PL03 is resistant to both antibiotics.

Fluorescent in situ hybridization

Fluorescent *in situ* hybridization (FISH) was performed on smears made of the tissue samples on microscope slides (Super Frost Plus, Menzel-Glaser, Braunschweig, Germany). The slides were incubated at 37°C for 30 min., fixed in 4% paraformaldehyde for 20 min. at 4°C and in 96% methanol at -20°C for one hour. The following FISH probes (Eurogentec, Seraing, Belgium) were used: EUB338 specific for all bacteria (5'-GCT GCC TCC CGT AGG AGT-3') labeled with fluorescein at the 5' and 3' ends (green fluorescence), STREP for *Streptococcus (*5'-GGT ATT AGC AYC TGT TTC CA-3'), Lab158 for *Lactobacillus* and *Enterococcus* (5'- GGT ATT AGC AYC TGT TTC CA-3'), Bif164 for *Bifidobacterium (*5'-CAT CCG GCA TTA CCA CCC-3'), BAC303 for *Bacteroides* (5'-CCA ATG TGG GGG ACC TT-3'), ECOLI for *E. coli* (5'-GCA AAG GTA TTA ACT TTA CTC CC-3'), and Erec for *Clostridium coccoides* species (5'-GCT TCT TAG TCA RGT ACC G-3'). All probes except for EUB338 were labeled with CY3 at the 5'end (red fluorescence). Hybridization was performed according to the conditions described for each probe.

Statistical analysis

Comparisons were made using Student's t-test for variables with a normal distribution and the χ^2 test. For comparison of bacterial populations and ratios of particular bacterial groups, the likelihood ratio was used. These statistical methods were chosen because the data distribution was significantly different from the normal distribution. All analyses were conducted using SAS 9.1 package and SAS Enterprise Quide 3.0 (SAS Institute, USA).

Results

The study included 51 patients: 21 with UC in remission and 30 in the active phase of UC. There were 31 who completed the study: 6 in remission on standard therapy (subgroup IA), 8 in remission on standard therapy supplemented with the probiotic preparation (subgroup IB), 8 in acute phase on standard therapy (subgroup IIA), and 9 in acute phase on standard therapy supplemented with the probiotic preparation (subgroup IIB). Their disease status was evaluated using clinical, histological, and microbiological parameters. Analysis of the changes in values of the Mayo Clinic Index among these patients revealed that a decrease of the mean index values between the initial and control visit was found in all groups, but it was more prominent in both groups of patients in the acute phase of the disease (Table 1). Moreover, the decrease of the mean index values was higher in the patients in the acute phase taking the probiotic preparation (p=0.076).

Mean numbers of main groups of bacteria (cfu/g)	Group IA Patients in remission on standard therapy (n=6)		Group IB Patients in remission on standard therapy supplemented with probiotic preparation (n=8)		Group IIA Patients in acute phase on standard therapy (n=8)		Group IIB Patients in acute phase on standard therapy supplemented with probiotic preparation (n=9)	
	Initial visit	Control visit	Initial visit	Control visit	Initial visit	Control visit	Initial visit	Control visit
Enterobacteriaceaae	1,09E+07^{+}	2,83E+05^{+}	5,03E+05^{+++}	1,33E+03^{+++}	2,60E+06^{+++++}	2,89E+04^{+++++}	2,10E+06	5,34E+06*
Enterococcus	5,18E+06^{++}	9,47E+04)$^{++}$	5,89E+07^{++++}	2,41E+02^{++++}	2,52E+07	1,14E+07	3,30E+07	1,84E+06
Streptococcus	4,05E+06	1,66E+07	1,81E+07	1,44E+ 07	9,45E+06	4,76E+05**	8,61E+07	3,97E+06**
Lactobacillus	1,28E+05	1,84E+06	2,58E+05	7,83E+04	2,60E+07^{++++++}	5,41E+04**$^{++++++}$	4,88E+06	2,03E+07***
Bifidobacterium	1,60E+07	4,39E+05	2,69E+05	4,53E+05	3,24E+06$^{+++++++}$	6,20E+04**** $^{+++++++}$	5,70E+07	2,57E+07****

Statistical differences between groups: (*)p=0,046, (**)p=0,05, (***)p=0,05, (****)p=0,05
Statistical differences between visits: ($^{+}$)p=0,023, ($^{++}$)p=0,024, ($^{+++}$)p=0,031,($^{++++}$) p=0,031,($^{+++++}$)p=0,0017, ($^{++++++}$)p=0,0093, ($^{+++++++}$) p=0,064

Table 3: Mean numbers of bacteria representing main bacterial groups present in patients faecal samples.

Groups of bacteria	Group IA Patients in remission on standard therapy (n=6)		Group IB Patients in remission on standard therapy supplemented with probiotic preparation (n=8)		Group IIA Patients in acute phase on standard therapy (n=8)		Group IIB Patients in acute phase on standard therapy supplemented with probiotic preparation (n=9)	
	Initial visit	Control visit	Initial visit	Control visit	Initial visit	Control visit	Initial visit	Control visit
Enterobacteriaceaae	3,62E+06^{+}	2,09E+05^{+}	4,26E+06^{++}	1,04E+03^{++}	2,61E+07^{+++}	3,36E+04^{++++}	2,19E+07	4,37E+06*
Enterococcus	1,63E+07	2,53E+07	3,01E+07	4,17E+07	1,19E+07	3,35E+06	2,11E+07	9,17E+05
Streptococcus	3,33E+07	2,00E+06	8,41E+06	5,10E+05	5,78E+06	2,39E+06	1,79E+05	1,40E+05
Lactobacillus	5,44E+06	1,49E+07	5,74E+05	6,25E+05	5,83E+06^{++++}	3,40E+05**$^{+++++}$	1,82E+07	1,98E+06**
Bifidobacterium	2,88E+06	1,27E+06	2,69E+06	2,31E+06	1,81E+06^{+++++}	2,44E+04***$^{++++++}$	4,29E+06	2,01E+06***

Statistical differences between groups: (*)p=0,046, (**)p=0,016, (***)p=0,021
Statistical differences between visits: ($^{+}$)p=0,0645, ($^{++}$) p=0,0645, ($^{+++}$)p=0,0017, ($^{++++}$)p=0,0785, ($^{+++++}$) p=0,0195

Table 4: Mean numbers of bacteria (log) representing main bacterial groups present in biopsy samples.

Histopathology

Cumulative Geboes scale values for histological evaluation of the inflammation intensity in colon samples taken from patients with UC in acute versus remission phase and taking or not taking probiotic preparation were compared (Table 2). The intensity of inflammation decreased from the initial to control visits in all 4 groups, though to different degrees. The most significant differences were observed between groups of patients in remission supplemented or not with the probiotic preparation (p=0.003). It is of interest that the intensity of inflammation in patients with acute colitis decreased significantly in both groups from the initial to control visit (p=0.039) but no significant difference was noted between the groups.

Microbiology (general)

Between the initial and control visit, the numbers of Gram-negative rods belonging to the Enterobacteriaceae family significantly decreased from initial to control visit in groups of patients in remission, with and without probiotics (IA, IB), and in patients in the acute phase not supplemented with probiotics (IIA). The numbers of *Lactobacilli* and *Bifidobacteria* also decreased in the latter group. On the other hand, probiotic supplementation in patients in the acute phase of UC (group IIB) was associated with a significant increase of *Lactobacilli* and *Bifidobacteria* numbers in feces (Table 3).

Populations of main groups of cultivable bacteria found in tissue samples taken from inflamed sites of the patients with acute-phase disease or in remission of UC, taking or not taking the probiotic preparation (groups IB versus IA and groups IIB versus IIA), are shown in Table 4. Numbers of Enterobacteriaceae rods decreased in all groups from the initial to control visit, although the most prominent decreases were in group IB getting probiotics and in group IIA without probiotics (p=0.001). A decrease was observed for *Lactobacilli* and *Bifidobacteria* in group IIA and less prominently in patients of group IIB getting probiotics. It appeared, when numbers of bacteria present in fecal samples were compared between patients groups on control visit, that significant changes were observed only in patients with acute-phase UC. Application of the probiotic preparation was related to an increase of Enterobacteriaceae, *Lactobacilli*, *Bifidobacteria*, and *Streptococci* numbers (Table 3). Practically the same changes were observed when biopsy samples were compared, with the exception of the *Streptococci* numbers (Table 4).

Microbiology (colonization)

All isolates belonging to *Lactobacillus* and *Bifidobacterium* genera cultured and isolated from tissue samples taken from inflamed samples of the patients gut mucosa were analyzed for their phenotypic identity with the applied probiotic strains, based on their characteristic differential resistance to ciprofloxacin and co-trimoxazole. Presence of such isolates was confirmed in 4 samples taken from patients of group IB and 6 patients of group IIB. Moreover, using specific probes for *Lactobacillus plantarum* and FISH technology, we demonstrated higher populations of these bacteria attached to the mucosa of patients taking the probiotic preparation compared with those on standard therapy (Figure 1).

Discussion

A systematic review concluded that the addition of a probiotic to conventional therapy did not improve overall remission rates in patients with mild to moderate ulcerative colitis, but the addition of probiotics may reduce disease activity [6]. Our studies support this view, since Mayo Clinic Index values were lower in patients with acute UC getting probiotics compared with control patients. It is of interest that the same was not observed in patients in remission, because their index values were low at the beginning of the study, and the difference between the study groups, although noted, was too small to be statistically significant. However, when looking at histological evaluation of the inflammation

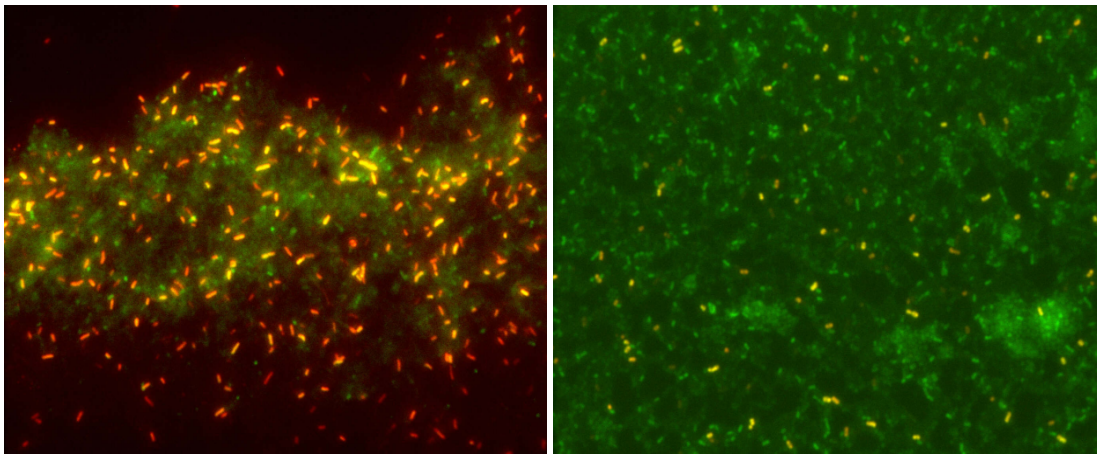

Figure 1: *Lactobacillus plantarum* cells (glowing red) present in stool samples taken from a patient (JW) with acute UC receiving probiotic bacteria (left photo) and patient (WA) not taking probiotic preparation (right photo). FISH technique, fluorescent microscope magnification 400 x.

intensity in biopsy samples, it is more evident that the applied probiotics ameliorated inflammatory reactions in patients in remission but not in those with acute UC. This discrepancy may be explained by the fact that a decrease of inflammatory changes observed under the microscope usually precedes clinical [12]. Recently, Rowland and his colleagues have stated in their overview on probiotics in UC, that the evidence for the role of some probiotic strains in prolonging remission in patients with UC is promising and deserves further investigation. Indeed, some trials have shown additional efficacy when probiotics were administered with conventional therapy [13,14]. Application of the studied probiotic preparation caused favorable changes in major constituents of the patients' gut microbiota, as observed in fecal and biopsy samples, conventionally representing planktonic and adhered parts of the biota. Again, these changes, based on the increase of *Lactobacilli* and *Bifidobacteria*, were more pronounced in patients with acute UC. Dysbiosis in the intestinal microbiota of persons with IBD has been described, but there are still varied reports on changes in the abundance of *Bifidobacterium* and *Lactobacillus* organisms in patients with IBD. We have shown that, among others, the numbers of *Bifidobacteria* are low in children with early, acute UC [15]. Wang [16] concluded recently that *Bifidobacteria* and *Lactobacilli* are increased in patients with active IBD after application of probiotics.

A recent meta-analyses of Sand et al. [17], Chibbar and Dieleman [18] and Derikx et al. [19] confirmed the opinion on positive effects of administration of probiotics and stressing importance of *Bifidobacterium* component of the administered probiotic preparations. It is of interest that probiotics application was related to an increase of Enterobacteriaceae in our patients with acute UC. Although *E. coli,* as a representative of this bacterial group, are regarded as a harmful intestinal bacteria, their role in controlling gut inflammation may be also beneficial. *E. coli* can inhibit hydroxyl radical formation and can affect the initiation and/or prolongation of remission of UC, as we have shown in our previous study [20]. Increased understanding of the normal intestinal microflora and better characterization of probiotic strains at the phenotypic and genomic levels is postulated as well as clarification of the mechanisms of action of these microflora in different clinical settings. A reduced diet containing evaluated probiotics may improve symptoms in IBD [21]. It should be stressed that the standard treatment of the patients with acute UC, ciprofloxacin plus mesalazine, administered to our patients invariably caused a decrease of all bacteria group numbers contained in feces and biopsies of our patients. This effect was related to clinical improvement, which was more prominent in the patient group receiving probiotics, and to the increase of *Lactobacilli* numbers in the patients' feces. It seems that the probiotic preparation given to them was able to overcome the suppressive effect of ciprofloxacin for Lactobacilli, due to the natural resistance of *L. plantarum* to this drug as well as to constant supplementation [22].

Strategies modulating dysbiosis in the gut microbiota might be a therapeutic option in IBD. Antibacterial treatment has been used, but with limited effect. Probiotics may improve intestinal microbial balance, enhancing gut barrier function, and improving local immune response. Their effects are strain-specific, so that comparisons and meta-analyses of studies using different probiotics are problematic [23].

This intention-to-treat study was performed in order to check if the combination of three well-characterized probiotic strains could be effective in supplementary treatment of UC in both active and remission states. The probiotic strains used in this study have been fully characterized by us in functional studies performed *in vitro* in human intestinal cell line systems. Moreover, safety, probiotic properties, and colonizing ability of the tested strains were checked on gnotobiotic (germ-free) mice and on rat neonates.

Further double-blind, controlled clinical trials will be needed to evaluate the clinical and microbiological effects of the probiotic preparation on a larger population of patients with UC. The risk of bacterial translocation and subsequent bacteremia also has to be considered for safety evaluation.

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