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ORIGINAL ARTICLE

Lactobacillus plantarum 299v Does Not Reduce Enteric Bacteria or Bacterial Translocation in Patients Undergoing Colon Resection

Peter Mangell · Henrik Thorlacius · Ingvar Syk · Siv Ahrné · Göran Molin · Crister Olsson · Bengt Jeppsson

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Abstract
Background Probiotics may exert beneficial effects in the gastrointestinal tract. This randomized trial investigated the effect of the probiotic *Lactobacillus plantarum* 299v on the intestinal load of potentially pathogenic bacteria, bacterial translocation, and cell proliferation in elective colon surgery.
Methods Seventy-five patients were randomized to pre- and postoperative oral intake of *Lactobacillus plantarum* 299v or placebo. Rectal swabs and mucosal biopsies were taken before the start of intake, after 1 week, at surgery, and after 6 days, weeks, and months. Viable counts were quantified for clostridia, *Enterobacteriaceae*, Gram-negative anaerobes, and lactobacilli. Bacterial translocation was determined by the analysis of bacterial DNA genes in mesenteric lymph nodes. Ki-67 was used as a marker of cell proliferation in normal mucosa and tumor.
Results *Lactobacillus plantarum* 299v was given without adverse effects. *Lactobacillus plantarum* 299v as well as *Enterobacteriaceae* and Gram-negative anaerobes increased in the colon 1 week after the administration of *Lactobacillus plantarum* 299v. There were no significant differences between patients receiving *Lactobacillus plantarum* 299v and placebo in the incidence of bacterial translocation (27 vs. 13 %) and postoperative complications (16 vs. 31 %).
Conclusions *Lactobacillus plantarum* 299v was established in the intestine, but no inhibitory effect on enteric bacteria, bacterial translocation, or postoperative complications was found. The mechanism behind the protective effects of probiotics found in animal and some human studies remain elusive and require further explorations. No adverse effects were recorded after the administration of high doses of *Lactobacillus plantarum* 299v.

Keywords Colon and rectal surgery · Probiotics · *Lactobacillus plantarum* · Bacterial translocation · Postoperative complications

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Introduction

The intestinal microflora plays an important role in maintaining health, e.g., by supplying the intestinal mucosa with short chain fatty acids such as butyrate. However, the microflora also hosts potentially pathogenic bacteria, which may induce or perpetuate disease. To reduce this risk, prophylactic antibiotics are routinely used in major abdominal surgery. Due to increasing bacterial multiresistance and the environmental effects of the excessive use of antibiotics, alternative means of modulating intestinal flora in order to prevent bacterial translocation (BT) and septic complications are needed. This is one reason why probiotic bacteria, i.e., living microorganisms with beneficial effects on the host [1], have gained increased interest in the last decade. Probiotics have been found to reduce BT in animal experiments [2–4]. However, clinical studies have been controversial. For example, probiotics were reported to reduce postoperative complications in liver transplant patients [5] and upper gastrointestinal surgery [6], but other investigators found no such effect in general surgery patients [7–10].

Studies on probiotics have used both single bacteria and mixtures of several bacteria. There is no evidence that multiple strains used together have favorable effects and it is often unclear why a mixture contains certain strains. *Lactobacillus plantarum* 299v (Lp 299v) has been extensively studied in animals and humans and has been shown to exert beneficial effects in severely compromised patients [11]. Lp 299v has also been shown to increase the diversity of intestinal bacterial flora [12].

In this double-blinded, placebo-controlled study on patients undergoing elective colon surgery, the effect of high doses of Lp 299v on the load of *Enterobacteriaceae* and lactobacilli in the rectum was examined, as well as BT at the time of surgery. Further, the effect of Lp 299v on cell proliferation and postoperative complications was studied.

Methods

Study Groups

Patients referred for colonic resection were eligible for entry into the study, which was double-blinded, randomized, placebo-controlled, and approved by the Ethics Committee, Lund University. Exclusion criteria were history of endocarditis, heart valve disease, or inability to follow the study protocol. Patients violating the protocol for any reason were excluded. Randomization was achieved by randomly linking the patient number to either the Lp 299v or placebo groups. Patients in the treatment group (299v group) were given sealed containers of an oatmeal-based drink containing 10^9 colony forming units (CFU) of Lp 299v per ml (Probi, Lund, Sweden). Patients in the control group (placebo group) were given an oatmeal-based drink without bacteria. The probiotic and placebo oatmeal drinks were identical in texture, smell, and taste. Patients, medical staff, and study personnel were blinded as to which group the patients were randomized.

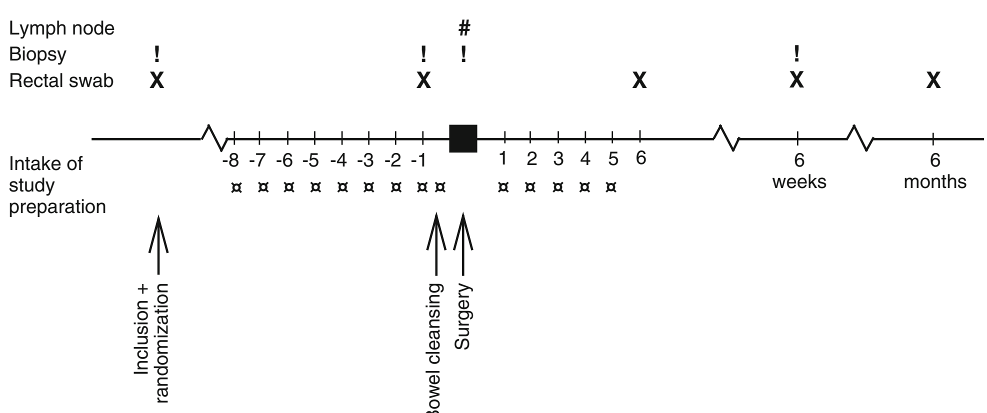
Study Protocol

Starting 8 days before surgery, each patient received 100 ml of the assigned preparation (Fig. 1). In the 299v group, this corresponds to a daily consumption of 10^{11} CFU of Lp 299v. Consumption was registered in a diary. Normal diet was continued during this period, except for a number of specified probiotic products. Preoperative bowel preparation was performed with 4 l of macrogol 4000 (Laxabon®), after which the patients were given 100 ml of the assigned preparation. Ingestion of the assigned preparation was resumed on the first postoperative day, continuing for another 5 days. Most patients ingested the preparation orally, while a few had their nasogastric tube up to 2 days after surgery. The postoperative parameters were registered by a nurse (Table 2). The operating time, perioperative bleeding, and days with epidural anesthesia were registered.

Bacterial Culturing

At intervals depicted in Fig. 1, rectal swabs and mucosal biopsies were taken at the 10-cm level in the dorsal rectum or from the resected specimen, using sterile instruments.

Fig. 1 Study design



All specimens were weighed, snap frozen in liquid nitrogen, and stored at −70°C. After thawing, samples were placed in an ultrasonic bath (Millipore, Sundbyberg, Sweden) for 5 min and vortexed using a Chiltern mixer (Thera-Glas, Gothenburg, Sweden) for 2 min. Bacterial viable counts were obtained by cultivation on violet red bile glucose agar (VRBG; Oxoid, Hampshire, England) incubated aerobically at 37°C for 24 h (*Enterobacteriaceae* counts) and on Rogosa agar (Oxoid) (lactobacilli counts), brain heart infusion agar with Gram-negative selective supplement (BHI-GN; Oxoid) (Gram-negative anaerobic counts), and Perfringens agar base with Perfringens selective supplement (Oxoid) (sulphite-reducing clostridia counts), all incubated anaerobically (Gas Pack, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) at 37°C for 72 h. Colonies were counted and expressed as CFU per gram of feces or tissue.

For the identification of ingested Lp 299v, randomly amplified polymorphic DNA (RAPD) was used. The colonies were grown anaerobically overnight at 37°C in *Lactobacillus*-carrying medium (LCM) [13] with 1 % w/v of glucose. The primer used in the polymerase chain reaction (PCR) amplification was a 9-mer with the sequences 3′-ACG CGC CCT-5′ (Scandinavian Gene Synthesis AB, Köping, Sweden). The preparation of samples and PCR amplification was performed as previously described [14].

Bacterial Translocation

At the induction of anesthesia, patients were given intravenous antibiotic prophylaxis (cefuroxime and metronidazole), according to clinical routines. Immediately after laparotomy, a mesenteric lymph node (MLN) at the ileocecal junction was harvested, cleared of fat, and placed in a sterile tube containing 3 ml of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The tube was snap frozen in liquid nitrogen and stored at −70°C for later analysis.

DNA Extraction

Lymph nodes were thawed, cut, and transferred to a tube with 190 µl of buffer G2 (DNA Tissue Kit, Qiagen, Hilden, Germany) and 10 µl of Proteinase K (Qiagen). Sterile glass beads were added and the cells were lysed at 56°C for 3–4 h in a shaking water bath. The tubes were shaken for 30 min in an Eppendorf Mixer 5432 (Eppendorf, Hamburg, Germany) at 4°C to disintegrate all bacteria. After centrifugation at 300×g for 1 min, the solution was transferred to a sample tube and total DNA was extracted by using BioRobot EZ1 (Qiagen), according to THE manufacturer's instructions. The DNA was eluted in 200 µl.

PCR

Primer sequences and PCR conditions are summarized in Table 1. In the PCR for the *Bacteroides fragilis* group, *irp*2 gene, and *Enterococcus* spp., each reaction contained 2.5 µl 10× PCR buffer, 200 µM of each deoxyribonucleotide (Roche Diagnostics, Mannheim, Germany), 1.25 U of Taq DNA Polymerase (Roche Diagnostics), and 0.5 µM of primer. For the amplification of *Clostridium perfringens* group, *Lactobacillus*, *Streptococcus*, *Escherichia coli* (*malB*), and type 1 fimbriae (*fim*A gene), HotStarTaq Master Mix (Qiagen) was used. For the amplification of *Escherichia coli*, 0.25 µM of primer and for *Lactobacillus* and *Streptococcus*, 0.5 µM of primer was used with 12.5 µl HotStarTaq Master Mix (Qiagen). For the amplification of *Clostridium perfringens* group and *fim*A gene, 1.0 µM of primer was used. The amount of template was 1–3 µl. An initial denaturation step at 95°C for 15 min was applied for activation of the Taq polymerase and PCR was ended with an elongation step at 72°C for 5 min. All amplifications were run for 40 cycles on a Tpersonal Combi (Biometra, Goettingen, Germany). The following positive controls were used: *Bacteroides fragilis* CCUG 4856^T, *Lactobacillus plantarum* DSM 9843, *Enterococcus gallinarum* CCUG 28696, *Streptococcus sobrinus* CCUG 25735^T, *Clostridium cadaveris* CCUG 24035^T, and *Escherichia coli* CCUG 29300^T. *Klebsiella planticola* CCUG 15718^T was used as the control for *irp*2, while *Escherichia coli* strain 233A and isolate 11F16 (kindly provided by Agnes Wold and Ia Adlerberth, Department of Immunology, Gothenburg University) were used as controls for *fim*A. For the negative control, ddH_2O was added instead of template. PCR products were run on agarose gel, stained with ethidium bromide, and photographed in UV light. Samples showing a band on the gel of the correct size were subjected to a second PCR for confirmation.

Cell Proliferation

Ki-67 was used as a marker of cell proliferation in normal and tumor tissue. Formaldehyde-fixed and paraffin-embed ded sections of tumor and normal tissue from 32 patients in the 299v group and the placebo group were used. Heat-induced epitope retrieval was done for 20 min at 98°C with Target Retrieval Solution (DakoCytomation, Glostrup, Denmark), followed by cooling for 20 min at room temperature. Incubation was performed with anti-human Ki-67 antigen (DakoCytomation) for 25 min, followed by visualization with the Envision Detection System (DakoCytomation) for 25 min. Hematoxylin was used for counter staining.

Table 1 Primer sequences, their targets, and polymerase chain reaction (PCR) conditions

Bacterial group or gene	Primer	Sequence 5′-3′	Size (bp)	Denaturation[a]	Annealing[b]	Elongation[c]	Reference
Bacteroides fragilis group (16S rRNA)	Brfa-F	ata gcc ttt cga aag raa gat	495	94°C/20 s	50°C/20 s	72°C/45 s	[31]
	Brfa-R	cca gta tca act gca att tta					
Lactobacillus (16S rRNA)	Lac-F	agc agt agg gaa tct tcc a	341	95°C/15 s	58°C/20 s	72°C/45 s	[32]
	Lac-R	cac cgc tac aca tgg ag					
Enterococcus spp. (16S rRNA)	Ent-F	ccc tta ttg tta gtt gcc atc att	144	95°C/15 s	61°C/20 s	72°C/30 s	[32]
	Ent-R	act cgt tgt act tcc cat tgt					
Streptococcus (*tuf* gene)	Str1	gta cag ttg ctt cag gac gta tc	197	95°C/15 s	55°C/20 s	72°C/45 s	[33]
	Str2	acg ttc gat ttc atc acg ttg					
Clostridium perfringens group (16S rRNA)	Clo-F	atg caa gtc gag cga(g/t)g	120	95°C/15 s	55°C/20 s	72°C/45 s	[32]
	Clo-R	tat gcg gta tta atc t(c/t)c ctt t					
High pathogenicity Island (*irp*2)	*irp*2-F	aag gat tcg ctg tta ccg gac	280	94°C/30 s	63°C/30 s	72°C/60 s	[34]
	*irp*2-R	tcg tcg ggc agc gtt tct tct					
Type 1 fimbriae (*fim*A)	*fim*A-F	cga cgc atc ttc ctc att ctt ct	721	94°C/15 s	59°C/20 s	72°C/45 s	[35]
	*fim*A-R	att ggt tcc gtt att cag ggt tgt t					
Escherichia coli (*malB*)	*malB*-F	gac ctc ggt tta gtt cac aga	585	94°C/15 s	50°C/20 s	72°C/45 s	[36]
	malB-R	cac acg ctg acg ctg acc a					

[a] Denaturation, [b] annealing, and [c] elongation temperature and time

Statistics

Statistical analysis was performed using SigmaStat software for Windows (Systat Software Inc., CA, USA). The Chi-square test was used to compare qualitative data. Quantitative data are expressed as medians (interquartile range [IQR]). For non-parametric data, the Wilcoxon signed-rank test was used within groups, while the Mann–Whitney rank sum test was used between groups. A *P*-value < 0.050 was considered to be significant. A previous pilot study on 12 patients, of whom two were excluded due to protocol violation, indicated that 22 patients in each group were needed in order to show a reduction in postoperative *Enterobacteriaceae* counts with Lp 299v treatment compared to placebo at a 5 % significance level with a power of 80 %.

Results

Study Population

Seventy-two patients were included and randomized, with 36 patients in each study group. The demographic details are found in Table 2. Four patients in the 299v group were excluded: two patients due to protocol violation (inability to ingest the study preparation or undergo postoperative sampling) and two patients who were operated at another hospital or received emergency surgery. Four patients in the placebo group were also excluded: three due to protocol violation (two patients were unwilling to undergo postoperative sampling and one patient decided not to proceed with surgery) and one patient had surgery cancelled due to heart failure. There were no differences in the measured parameters between the two groups. Indications for surgery and performed procedures are summarized in Table 2. All patients presented protocols of adequate pre- and postoperative intake of the study preparation.

Postoperative Complications

Five patients in the 299v group and 10 patients in the placebo group experienced postoperative complications (Table 3). Two patients in each group had severe complications necessitating re-laparotomy and intensive care. There was one case of mortality in the 299v group and two cases in the placebo group during the 30-day postoperative period. Most of the complications were infectious, confirmed by culture and treated with antibiotics. There was no statistically significant difference in the complication rate between the two groups, neither in regard to infectious or nor non-infectious complications.

Bacterial Culture

At Inclusion

Rectal swabs and mucosal biopsies taken at study inclusion showed no differences in the bacterial concentrations between the two study groups (Tables 4 and 5).

Table 2 Demographic details

	299v group	Placebo group
Randomized	36	36
Excluded	4	4
Male/female	16/16	20/12
Age, years	74 (70–80)	70 (64–79)
Operating time, min	187 (57)	198 (67)
Bleeding, ml	300 (287)	300 (475)
Days with postoperative epidural anesthesia	4 (1)	4 (3)
Passage of gas (postoperative day)	2 (2)	3 (3)
Passage of stool (postoperative day)	4 (2)	4 (2)
Free fluid intake (postoperative day)	3 (2)	3 (3)
Free food intake (postoperative day)	5 (2)	5 (2)
Diagnosis		
Adenocarcinoma	26	24
Benign adenoma	4	2
Diverticulitis	1	3
Polyposis		1
Carcinoid		1
Mb Crohn		1
Other	1	
Type of resection		
Ileocecal	2	1
Right hemicolectomy	17	12
Transverse colon	1	2
Left hemicolectomy	2	2
Sigmoid colon	8	14
Total colectomy	1	1
No resection	1	

Median (interquartile range)

Table 3 Postoperative complications

	299v Group $n = 32$	Placebo group $n = 32$	*P*-value
Complications	5 (16 %)	10 (31 %)	0.238
Septic	4 (13 %)	6 (18 %)	0.731
Non-septic	1 (3 %)	4 (13 %)	0.352
Mild	Pneumonia	Pneumonia	
	Superficial wound infection	Superficial wound infection	
	Urinary infection	Urinary infection (3)	
		Urinary retention	
		Pleural exudate (sterile)	
		Delayed postoperative paralysis	
Severe	Multi-organ failure	Total wound dehiscence	
	Intestinal obstruction	Intra-abdominal abscess	

Preoperative Colonization of the Study Product

The concentration of lactobacilli on rectal swabs and biopsies increased in the 299v group (Tables 4 and 5). This increase remained at 6 weeks postoperatively on rectal swabs but not on the mucosa. By using RAPD, it could be established that the study preparation with Lp 299v were recovered from the rectum after 1 week of ingestion and was also found on the mucosa of the intestinal segment resected (Fig. 2). With continuing intake of the preparation in the 299v group, the concentration of lactobacilli remained elevated on postoperative day 6 (Table 4) and the ingested lactobacilli were detected with RAPD. However, 6 weeks after surgery, the lactobacilli found on the rectal swabs and mucosal biopsies belonged to the indigenous flora in all but a few patients, and did not have the same band pattern on PCR as the ingested Lp 299v (Fig. 2).

After intake of the study preparation for 1 week, viable counts of *Enterobacteriaceae* and Gram-negative anaerobes increased significantly on rectal swabs in both the 299v and placebo groups (Table 4), but between the two study groups, there was only a significant difference in *Enterobacteriaceae*. Viable counts of clostridia on rectal swabs were unchanged in both groups and on the mucosa in the placebo group. On biopsies, preoperative intake in the 299v group increased the load of all the studied bacteria, whereas bacterial viable counts in the placebo group were unchanged (Table 5). However, the differences in the mucosal bacterial levels were not significant between the two groups.

Postoperative Colonization

On postoperative day 6, rectal swabs in the 299v group showed increased viable counts of clostridia, *Enterobacteriaceae*, and Gram-negative anaerobes compared with the load at inclusion (Table 4). In the placebo group, only *Enterobacteriaceae* and Gram-negative anaerobes were increased. Comparing preoperative with postoperative viable counts, the placebo group showed a significantly increased load of *Enterobacteriaceae*. A postoperative increase in *Enterobacteriaceae* was also seen in the 299v group, although this was not significant compared with preoperatively.

Table 4 Rectal swab

	Inclusion		Preoperative			Postoperative day 6			Postoperative 6 weeks			Postoperative 6 months		
	Median	IQR	Median	IQR	*P*-value[a]	Median	IQR	*P*[a]	Median	IQR	*P*-value[a]	Median	IQR	*P*-value[a]
299v group														
Clostridia	5.1	1.0	5.9	1.5	0.080	6.2	1.6	0.005	5.5	1.3	0.196	5.7	1.0	0.268
Enterobacteriaceae	5.4	1.2	6.5	1.2	<0.001 0.012[b]	6.9	1.1	<0.001	6.2	2.0	0.004	6.1	2.4	0.093
Gram-negative anaerobes	6.3	1.1	7.2	1.1	<0.001	7.4	1.2	<0.001	7.1	1.4	0.002	7.0	1.1	0.002
Lactobacilli	6.0	1.3	6.6	1.3	0.002	6.3	1.5	0.011	6.7	1.6	0.006	6.1	1.6	0.098
Placebo group														
Clostridia	5.4	1.6	5.5	1.6	0.104	6.2	1.3	0.087	5.8	1.6	0.045	5.6	1.5	0.487
Enterobacteriaceae	5.3	1.5	5.6	1.1	0.016	6.8	1.7	<0.001 <0.001[c]	6.1	1.2	0.004	6.1	2.1	0.030
Gram-negative anaerobes	6.1	1.3	7.0	1.7	<0.001	7.1	1.7	<0.001	6.9	1.7	0.006	6.8	1.2	0.003
Lactobacilli	6.1	1.4	6.1	2.1	0.430	6.1	2.7	0.580	6.6	1.8	0.306	5.9	2	0.583

Log10 CFU/g of feces, $n = 18–32$

[a] Versus inclusion

[b] Versus placebo

[c] Versus preoperative

Table 5 Mucosal biopsy

	Inclusion		Preoperative			Preoperative			Postoperative 6 weeks		
	Median	IQR	Median	IQR	*P*-value[a]	Median	IQR	*P*-value[b]	Median	IQR	*P*-value[a]
299v Group											
Clostridia	5.0	0.7	5.6	0.9	0.019	4.2	1.8	0.004	5.1	0.6	0.301
Enterobacteriaceae	5.5	1.4	5.8	0.9	<0.013	4.9	1.2	<0.001	5.6	2.2	0.426
Gram-negative anaerobes	6.0	1.0	6.7	1.0	0.002	5.5	1.5	<0.001	6.6	0.7	0.348
Lactobacilli	5.7	1.2	6.3	1.4	0.005	5.8	1.8	0.004	6.5	1.5	0.547
Placebo group											
Clostridia	4.8	0.9	5.3	1.0	0.426	4.0	0.9	0.012	5.6	0.7	0.105
Enterobacteriaceae	5.8	1.1	5.4	1.0	0.266	4.6	1.5	<0.025	5.7	1.5	0.742
Gram-negative anaerobes	6.0	1.2	6.5	0.9	0.054	4.9	1.4	<0.001	6.8	1.0	0.009
Lactobacilli	5.6	2.0	5.9	1.6	0.583	4.7	1.7	0.006	6.0	1.1	0.813

Log10 CFU/g of mucosa, $n = 11–32$

[a] Versus inclusion

[b] Versus preoperative

Effect of Bowel Preparation and Antibiotic Prophylaxis

Viable counts of all the studied bacterial groups were significantly lower in both the 299v group and the placebo group when comparing preoperative mucosal biopsies with those obtained from the surgical specimen (Table 5).

Bacterial Translocation

Lymph nodes from 46 patients, 22 from the 299v group and 24 from the placebo group, were examined for the presence of specific bacterial DNA. Translocation was found and reproduced in nine lymph nodes (20 % of all the studied lymph nodes), of which six were from the 299v group and

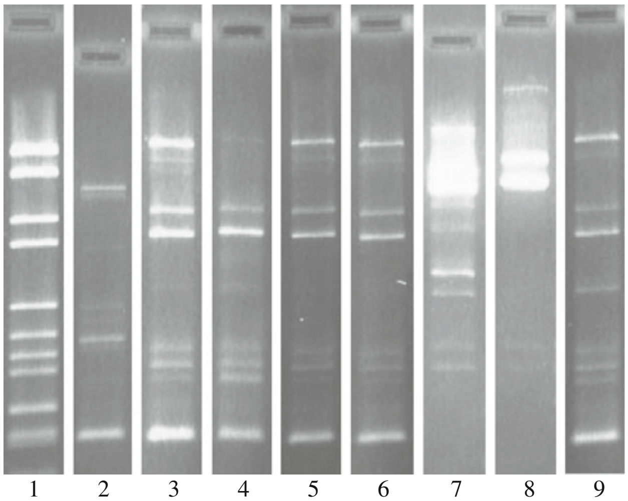


Fig. 2 Typical band patterns of randomly amplified polymorphic DNA (RAPD) gels for bacterial colonies picked from the lactobacilli plate count (Rogosa) from a patient randomized to the 299v group. *1*: DNA size markers and *9*: the *Lactobacillus plantarum* 299v strain. *2*: rectal swab at inclusion (before intake of the study preparation); *3*: preoperative rectal swab and *4*: mucosal biopsy (1 week after intake of the study preparation); *5*: mucosal biopsy from resected colon; *6*: rectal swab on postoperative day 6; *7*: rectal swab 6 weeks postoperatively; and *8*: mucosal biopsy 6 months postoperatively

three were from the placebo group (Table 6). There was no significant difference in translocation between the study groups. None of the patients in the 229v group with postoperative complications had positive lymph nodes. Out of the ten patients with postoperative complications in the placebo group, one patient with a urinary infection had a lymph node positive for *Streptococcus*. Of all the patients, translocated lactobacilli genes were only found in lymph nodes from the 299v group.

Cell Proliferation

Ki-67 analysis showed a significantly higher proliferation index in tumor cells compared to normal mucosa (Fig. 3). Lp 299v did not affect cell proliferation in tumor or normal tissue.

Table 6 16S rRNA genes found in mesenteric lymph nodes (MLNs)

	299v group $n = 22$	Placebo group $n = 24$	*P*-value
Positive lymph nodes	6 (27 %)	3 (13 %)	0.374
Translocated bacteria			
Lactobacillus	2	–	
Clostridium perfringens group	2	–	
Streptococcus	1	2	
Bacteroides fragilis group	1	1	

Discussion

This study shows that Lp 299v given orally to patients undergoing colon surgery survives passage through the gastrointestinal tract and can be recovered from the feces and mucosa of the rectum. Pretreatment with Lp 299v did not reduce viable counts of *Enterobacteriaceae*, clostridia, or Gram-negative anaerobes, or prevent BT to MLNs. In addition, we found no statistically significant reduction in postoperative complications in patients treated with Lp 299v. Importantly, the intake of a large amount of Lp 299v in this setting did not elicit any adverse events.

Gastrointestinal surgery, especially colorectal, is associated with a high rate of infectious complications caused by enteric bacteria that have crossed the intestinal wall into sterile compartments of the body. With trauma, infection, irradiation, or cytotoxic treatment, potentially pathogenic bacteria, such as *Enterobacteriaceae*, may increase in number and translocate across the intestinal mucosa. Prophylactic antibiotics are routinely used in colorectal surgery, but have not eliminated infectious complications. Further, the frequent use of antibiotics induces bacterial multiresistance and is an environmental burden. Probiotics have been considered to be an alternative to antibiotics in an attempt to modulate and stabilize the intestinal microflora.

In experimental studies, probiotics have been shown to attenuate BT [2–4]. BT occurs in humans [15] and its relation to sepsis is presumed but not proven. Probiotics given to healthy volunteers decreased the number of *Enterobacteriaceae* [16] and clostridia [17] in the colon, and some studies showed that probiotics might reduce postoperative infections [5, 18] and sepsis [19]. However, other studies did not show a preventive effect of probiotics

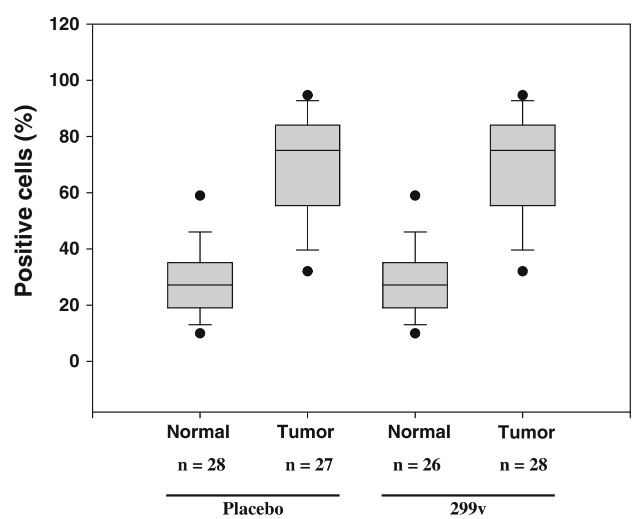


Fig. 3 Ki-67 expression in the colon. Samples are from normal and tumor mucosa

[7, 8, 10] on septic complications or BT in patients undergoing colon surgery. Treatment with probiotics has been reported to even increase BT and mortality in patients with acute pancreatitis and organ failure [20].

In the present study, fecal and mucosal loads of *Enterobacteriaceae*, clostridia, and Gram-negative anaerobes were determined before intake of the probiotics or placebo and then followed over time. Levels of *Enterobacteriaceae* and Gram-negative anaerobes increased after 1 week of lactobacilli intake, but only to a significant degree for *Enterobacteriaceae*. One explanation might be that the duration of pretreatment was too short to achieve a reduction of potential pathogens. However, in a colonoscopy study, lactobacilli with the same concentration but twice as long as herein was administered without any effect on the bacterial composition in the colon [21]. The finding that some bacteria increased after both Lp 299v and placebo intake indicates that oatmeal conceivably acts as a substrate for intestinal bacteria. Another reason for the lack of effect could be that the lactobacilli given did not survive the passage through the gastrointestinal tract. However, by using RAPD, it was found that the ingested lactobacilli were, indeed, recovered from the rectum, proving that they were able to pass through the intestine and colonize the mucosa.

Stress, such as surgical trauma, induces an increase of *Enterobacteriaceae* in the gut [22], which was found also in the present study. In a study using a combination of several probiotics and a prebiotic, together with oral antibiotic and mechanical bowel preparation, a reduction in perioperative *Enterobacteriaceae* was found [23]. The *Enterobacteriaceae* count in the postoperative period was, however, not determined. A pilot study by our group showed that pretreatment with Lp 299v decreases the increase in *Enterobacteriaceae* after surgery (data not shown). However, we were not able to confirm that finding in this larger study. A better effect in reducing postoperative *Enterobacteriaceae* levels might have been achieved with the intake of Lp 299v for a longer time period. An alternative explanation is that the viable count of *Enterobacteriaceae* is representative of several different genera, which may respond differently to Lp 299v. A tentative reduction in any one of the genera by the probiotic may result in an expansion of another, thus, leaving the total load of *Enterobacteriaceae* unchanged.

The incidence of postoperative complications in the present study was not statistically different between the two groups, despite a higher number of complications in the placebo group. This contrasts to the abovementioned study by Rayes et al. [5]. The reason for this might be due to the liver transplant patients having a more advanced disease than patients undergoing elective colorectal surgery. This is further emphasized by a study on patients undergoing major general surgery, where probiotics mainly prevented postoperative infections in the most severely ill patients [24]. In addition, it cannot be excluded that our present study might have included too few patients to detect differences in surgical complications.

Biopsies revealed increased concentrations of all studied bacteria in the 299v group, while they remained unchanged in the placebo group. This might be due to a thicker mucus layer in the 299v group, as this is found with the administration of probiotics [25, 26]. The mucus is important in preventing luminal bacteria to adhere to enterocytes. Mucus traps bacteria on its way to the mucosa, and is then discharged by peristalsis. In this study, mucosal specimens were not rinsed, thus, enteric bacteria trapped in the mucus are included in the cultures. Therefore, a high concentration of bacteria on biopsies does not necessarily mean that more enteric bacteria are in contact with enterocytes.

To our knowledge, this is the first double-blinded, placebo-controlled study of patients undergoing colonic resections in which the impact of a single strain of lactobacilli on the intestinal flora and BT has been studied. We found that Lp 299v did not reduce BT in this setting. We chose to use PCR instead of conventional culturing of MLNs to detect BT as culturing only detects live bacteria and might, thus, miss bacteria that are killed while translocating. Furthermore, many anaerobes might be difficult to culture. The PCR technique has been used to study BT to blood [27] and lymph nodes [28], where bacterial 16S rRNA genes were detected in 14–40 %. The incidence of positive lymph nodes in this study was higher than when using conventional culture methods [7, 8], but, still, bacterial DNA was found in only 27 % of the patients, which might be because primers for only selected bacterial species or groups were used. It is noteworthy that the MLNs were harvested before handling of the gut, which increased the risk of BT [9]. Further, only one lymph node was harvested from each patient, which may underestimate the real incidence of translocation. Interestingly, lactobacilli were found in two out of six positive MLNs in the 299v group, but none in the placebo group, indicating that the administered bacteria do indeed colonize the intestine and have the ability to translocate. Despite the use of three different primers, we did not find any *Escherichia coli* in the lymph nodes. The reason for this discrepancy between PCR and conventional culturing is unclear at present.

Lp 299v had no effect on cell proliferation in normal mucosa or tumors, which is consistent with a previous study using prebiotics [29]. Thus, probiotics given in the present setting seem to be safe in patients with colon tumors. There are indications that probiotics might even reduce cell proliferation, as shown in the normal mucosa of rats [30]. Whether probiotics might have such an effect on humans remains to be elucidated.

The patients included in this study had undergone a number of different surgical procedures that might affect the outcome of the parameters studied, as may have other factors, such as the duration of procedures, size and location of the tumor, and patient-associated factors. These influences are minimized as much as possible in our randomized and controlled study, but cannot be fully eliminated.

A possible limitation in the present study might be the duration of treatment with lactobacilli, both in the pre- and postoperative periods. This might explain the transient effect on the intestinal microflora and further studies should address the issue of the duration of treatment.

In conclusion, administration of the probiotic Lp 299v to patients undergoing colonic resection does not reduce viable counts of some potentially pathogenic bacteria in the rectum before or after surgery. Further, the administration of this probiotic bacteria does not prevent BT or reduce the incidence of postoperative complications.

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