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# Cell-free supernatants of *Escherichia coli* Nissle 1917 modulate human colonic motility: evidence from an *in vitro* organ bath study

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***Abstract*** *Clinical studies have shown that probiotics influence gastrointestinal motility, e.g. Escherichia coli Nissle 1917 (EcN) (Mutaflor®) proved to be at least as efficacious as lactulose and more potent than placebo in constipated patients. As the underlying mechanisms are not clarified, the effects of EcN culture supernatants on human colonic motility were assessed in vitro. Human colonic circular smooth muscle strips (n = 94, 17 patients) were isometrically examined in an organ bath and exposed to different concentrations of EcN supernatants. Contractility responses were recorded under (i) native conditions, (ii) electrical field stimulation (EFS), (iii) non-adrenergic non-cholinergic conditions, and (iv) enteric nerve blockade by tetrodotoxin (TTX). As concentrations of acetic acid were increased in EcN supernatants, contractility responses to acetic acid were additionally tested. EcN supernatants significantly increased the maximal tension forces both at low and high concentrations. Neither blockade of both adrenergic and cholinergic nerves nor application of TTX abolished these effects. EFS-induced contractility responses were not altered after exposure to EcN supernatants. Acetic acid elicited effects comparable to EcN supernatants only under TTX conditions. EcN supernatants modulate in vitro contractility of the human colon. As neither partial nor TTX blockade of enteric nerves abolished these effects, EcN supernatants appear to enhance colonic contractility by direct stimulation of smooth muscle cells. Active metabolites may include other substances than acetic acid, as acetic acid only partially resembled the effects elicited by EcN supernatants. The data provide a rationale for therapeutical application of probiotics in gastrointestinal motility disorders.*

***Keywords*** *enteric nervous system, Escherichia coli Nissle 1917, human colon, intestinal motility, organ bath, probiotics.*

**Abbreviations:** Ach, acetylcholine; AT, atropine sulphate; EcN, *Escherichia coli* Nissle 1917; EFS, electrical field stimulation; MMBT, maximal multiplication of basal tone; NANC, non-adrenergic non-cholinergic; Pap, papaverine hydrochloride; Phe, phentolamine; Pro, propranolol; SCFA, short-chain fatty acid; TTX, tetrodotoxin.

## INTRODUCTION

Probiotics have been defined as viable non-pathogenic microorganisms that, when administered to humans or animals in adequate amount, confer health benefits on the host. They are used either as dietary supplements or as pharmaceutical products for the treatment of gastrointestinal dysfunctions and diseases. Besides lactic acid bacteria and yeasts, the non-pathogenic *Escherichia coli* strain Nissle 1917 (EcN) (Mutaflor®, Ardeyphram GmbH, Herdecke, Germany) has been used widely in medical practice in Central Europe, e.g. for the

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treatment of collagenous colitis and Crohn's disease,[1,2] acute diarrhoea,[3] pouchitis,[4] pseudomembraneous colitis[5] and diverticular disease[6] as shown both in pilot and confirmatory studies. Recently, EcN has gained special attention due to its ability to be as effective as standard therapy (5-aminosalicylic acid, mesalazine) in maintaining remission in patients with ulcerative colitis as proven in large randomized clinical trials.[7–9] Anti-inflammatory activity of EcN has also been shown in murine models of acute and chronic colitis.[10,11]

In addition to their obvious immunomodulatory potential, probiotics are also capable of modulating gastrointestinal motility, and, thus, have been applied in functional gastrointestinal diseases, such as chronic constipation, diarrhoea and irritable bowel syndrome.[3,12–16] In two randomized clinical trials, EcN proved to be at least as efficacious as lactulose and more potent than placebo in the treatment of chronic constipation: in the first study,[12] 108 patients were treated for 14 weeks either with lactulose or with EcN. EcN increased the stool frequency significantly higher (6.3 per week) than lactulose (5.5 per week) ($P < 0.026$) and showed fewer side effects. In the second study ($n = 70$ patients), treatment with EcN for 8 weeks increased the stool frequency to 6.0 per week compared to 1.9 per week in the placebo group ($P < 0.001$).[13]

However, the mechanisms by which probiotics stimulate gastrointestinal motility remain largely unknown. Currently, it is discussed that stimulation of intestinal propulsion might be due to (i) a decrease in fecal pH caused by bacterial production of organic acids, (ii) osmotic effects of bacterial metabolites, and (iii) intestinal distension caused by increased intraluminal gas production (mainly $CO_2$, $H_2$ and $CH_4$) with subsequent reflectory smooth muscle contractions.[17–20] Up to now, efforts to elucidate the effects of probiotics on intestinal motility have been exclusively confined to animal studies. It has been shown that administration of probiotics significantly enhanced propulsive contractions of the terminal colon and increased defecation rate in pigs,[21] and abolished muscular hypercontractility in a mouse model of postinfectious gut dysfunction.[22] The underlying mechanisms by which probiotics influence human intestinal motility have not been systematically studied so far.

Therefore, this study aimed at characterizing the effects of EcN on the contractility pattern of human intestinal specimens by using standardized *in vitro* organ bath techniques. In particular, it should be clarified whether putative effects induced by EcN are mediated via interaction with enteric nerves or by direct stimulation of the smooth musculature. Furthermore, the effects of the short-chain fatty acid (SCFA) acetic acid were tested in similar manner, as acetic acid is produced by EcN in high amounts, and as SCFAs are known to stimulate intestinal motility in both animals[23–30] and humans.[31–33]

## MATERIALS AND METHODS

### Patients

Segments of distal colon were obtained from patients (eight males, nine females, mean age 65.1 years, range 41–86 years) who underwent partial colectomy for non-obstructing colorectal carcinoma. All patients reported normal bowel habits with bowel movements at regular intervals. Radiographic studies showed a normal anatomy of the colon and rectum with no evidence for anorectal out-let obstruction. Specimens were obtained immediately after surgical removal and harvested in an area not involved by the neoplasm (>5 cm from the tumour). The study of human tissue received an approval from the Local Ethic Committee of the Faculty of Medicine, University of Luebeck (002/97).

### Preparation of muscle strips

After cleansing in a Krebs-bicarbonate solution ($Na^+$ 137; $K^+$ 5,9; $Ca^{2+}$ 2.5; $Mg^{2+}$ 1,2; $Cl^-$ 124; $HCO_3^-$ 25; $H_2PO_4^-$ 1.2; glucose 11.5, concentrations in mmol $L^{-1}$, pH 7.4) the serosal covering, mucosa and entire submucosa were removed to expose the muscular layers. Muscle strips of the muscularis propria (length: 20 mm, cross-section: 1 $mm^2$) were excised parallel to the circular muscle layer by razor blades and transferred into a 30-mL organ bath containing continuously oxygenated (5% $CO_2$, 95% $O_2$) Krebs-bicarbonate solution at 37 °C to allow recording of circular smooth muscle contractility.

### Organ bath, electrical field stimulation

Muscle strips were suspended with 10 mN $mm^{-2}$ tension and were allowed to equilibrate for 60 min. Isometric tension forces were recorded using a force transducer (Type F30/K30; Hugo Sachs Elektronik, March-Hugstetten, Germany) coupled to an amplifier (Type 301; Hugo Sachs Elektronik). The amplified tension forces were converted into digital signals by an analogue-digital converter (ADC, Type DT21-EZ; Data Translation GmbH, Bietigheim-Bissingen, Germany) and continuously recorded on a computer. Analysis of the recorded data was performed by using Visual Engineering Environment software (Hewlett Packard,

Version 5.0.; Boblingen, Germany). Electrical field stimulation (EFS) was carried out by electrodes positioned adjacent to each muscle strip at a distance of 1 mm. For selective EFS of enteric nerves, a series of square-impulses (50 V, 50 Hz, 0.5-ms duration) was applied for 30 s. Using these parameters, EFS consistently evoked a relaxation (on-relaxation during application of the electrical field) followed by a contraction (off-contraction after turning-off the electrical field) of human colonic circular muscle strips.

## Drugs

Acetylcholine chloride (Ach, $10^{-7}$–$10^{-4}$ mol $L^{-1}$; Sigma) was used for control of viability and for exogenous cholinergic stimulation to evoke maximal contraction forces. Propanolol (Pro, $10^{-5}$ mol $L^{-1}$; Sigma), phentolamine (Phe, $10^{-5}$ mol $L^{-1}$; Sigma) and atropine sulphate (AT, $5 \times 10^{-5}$ mol $L^{-1}$; Sigma) were used for blockade of adrenergic and cholinergic nerves to establish non-adrenergic non-cholinergic (NANC) conditions. Tetrodotoxin (TTX, $10^{-5}$ mol $L^{-1}$; Alomone) was used for blockade of enteric nerves. Remaining contractile activity after TTX administration has been considered negligible. At the end of each experiment, viability of the muscle strips was checked by confirmation of spontaneous mechanical activity and proper response to EFS. Finally, muscle strips were exposed to papaverin hydrochloride (Pap, $2 \times 10^{-3}$ mol $L^{-1}$; Karls-Pharma) for complete relaxation.

## EcN supernatants

Cell-free EcN culture supernatants (in short 'EcN supernatants') corresponded to spent culture supernatants obtained after culturing of EcN bacteria in Standard-I-Bouillon (Merck, Darmstadt, Germany). Bacteria were grown in 750-mL flasks up to the stationary phase under aerobic conditions for 16 h at 37 °C yielding suspensions with ca. $10^9$ bacteria $mL^{-1}$. EcN supernatants were obtained after separation of the bacteria from the culture medium by centrifugation for 15 min at 10 ***g*** at +4 °C (Suprafuge 22, Heraeus/Thermo Fisher, Langenselbold) followed by sterile filtration of the supernatants using 0.45-$\mu$m filters. EcN supernatants were kept frozen at −80 °C until use.

## Acetic acid

Gas chromatographic analyses showed that Standard-I-Bouillon after growth of EcN contained clearly higher concentrations of acetic acid (19 mmol $L^{-1}$) than fresh Standard-I-Bouillon alone (10 mmol $L^{-1}$). To test the effect of acetic acid on *in vitro* contractility, Standard-I-Bouillon (without EcN supernatants) was supplemented with increasing concentrations of acetic acid (Merck) (see study protocol for acetic acid).

## Study protocol for EcN supernatants

In a first series of experiments, the effects of EcN supernatants were studied on 94 specimens obtained from 17 patients (five to six muscle strips per patient). Ach was applied in cumulative doses ($10^{-7}$–$10^{-4}$ mol $L^{-1}$) to test viability of muscle strips and to define the maximal contraction amplitudes. After several changes of organ bath solution, muscle strips were exposed to low (dilution 1 : 50, 0.6 mL into 30 mL-organ bath) and high (dilution 1 : 5, 6 mL into 30 mL-organ bath) concentrations of EcN supernatants. Fresh Standard-I-Bouillon (without EcN supernatants) administered at same concentrations (dilution 1 : 50 and 1 : 5) served as control. Contractility responses were recorded (i) under native conditions, (ii) after EFS, (iii) under NANC conditions (addition of At, Phe, Pro), and (iv) after blockade of enteric nerves by TTX. Time duration of the experiments was ca. 5 h for each muscle strip.

## Study protocol for acetic acid

In a second series of experiments, the effects of acetic acid were studied on 24 specimens obtained from four patients (three male, one female, mean age 51 years, range 21–63 years). Muscle strips were exposed to Standard-I-Bouillon containing a basal concentration of 10 mmol $L^{-1}$ acetic acid and to Standard-I-Bouillon supplemented with increasing amounts of acetic acid (20, 40 and 80 mmol $L^{-1}$). Contractility responses were recorded for low (1 : 50) and high (1 : 5) dilutions under native conditions and after blockade of enteric nerves by TTX. The final pH within the organ bath solution was 7.46 after administration of Standard-I-Bouillon and 6.66 after administration of the highest concentration of acetic acid (80 mmol $L^{-1}$, dilution 1 : 5). Time duration of the experiments was ca. 5 h for each muscle strip.

## Analysis of data

For statistical comparison, the following parameters were calculated from the recorded data:

1 *Maximal multiplication of basal tension* (*MMBT*): The effect of an administered substance on muscular contractility was measured by comparing the basal tension before administration with the maximal amplitudes elicited after administration. The ratio

between the maximal amplitude and the basal tension (absolute values) corresponded to the maximal multiplication of the basal tension expressed as relative value. A ratio has been used to account for the variability of basal tension in different muscle strips and during different time periods of the experiments, thus allowing adequate comparison of the data.

2 *Alteration of median tension*: The median tension was measured before and after administration of a given substance. The difference corresponded to the alteration of median tension induced by a given substance.

3 *Relative relaxation after EFS*: The proportion of EFS-induced relaxation was expressed as relative value calculated by the ratio between the relaxation after EFS and the maximal (complete) relaxation recorded after administration of papaverine.

Prior to statistical analysis, the data recorded from muscle strips (five to six per patient, total number $n = 94$) were meant for each patient ($n = 17$). The parameters were compared by using non-parametric Wilcoxon-test for two related variables (Statistical Package for Social Sciences, SPSS). A difference of median values with a $P$-value <0.05 was considered significant. The data are graphically presented by box-whisker plots showing the median (horizontal black line), 50% of values (grey box) and 99% of values (whiskers). Statistical consulting was provided by the Institute of Medical Biometry and Statistics (University of Luebeck).

## RESULTS

### Characterization of muscular contractility pattern

After adequate preload and equilibration muscle strips showed regular spontaneous contractions with a basal tension ranging from 7 to 25 mN mm$^{-2}$. Maximal stimulation with $10^{-4}$ mol L$^{-1}$ Ach evoked a mean response of 58.9 mN mm$^{-2}$ (24.1–170.6 mN mm$^{-2}$) in viable muscle strips. Those muscle strips which did not respond adequately to Ach were excluded from the experiments.

### Contractile responses under native conditions

Under native conditions EcN supernatants evoked a significantly ($P \leq 0.05$) higher MMBT compared to Standard-I-Bouillon alone. The increase in maximal tension forces was not transient confined to a singularly increased amplitude but lasted for about 10 min including a series of increased maximal amplitudes. The responses to EcN supernatants were reproducible and did not significantly decline after repetition ruling out desensitation. Alterations in frequency ranges have not been observed after addition of EcN supernatants or Standard-I-Bouillon. MMBT was 1.76 after administration of EcN supernatants *vs* 1.41 after administration of Standard-I-Bouillon at low concentrations (dilution 1 : 50) and 1.95 *vs* 1.70 at high concentrations (dilution 1 : 5) (Fig. 1). The percental increase of MMBT caused by EcN supernatants compared to Standard-I-Bouillon alone was 25% (1 : 50) and 15% (1 : 5) respectively. No differences between EcN supernatants and Standard-I-Bouillon were found concerning alteration of median tension.

### Contractile responses under NANC conditions

Under NANC conditions EcN supernatants evoked a significantly ($P < 0.01$) higher MMBT compared to Standard-I-Bouillon alone showing an enhancement of maximal tension forces similar as observed in native conditions. The MMBT was 1.56 after administration of EcN supernatants *vs* 1.28 after administration of Standard-I-Bouillon at low concentrations (dilution 1 : 50) and 1.70 *vs* 1.33 at high concentrations (dilution

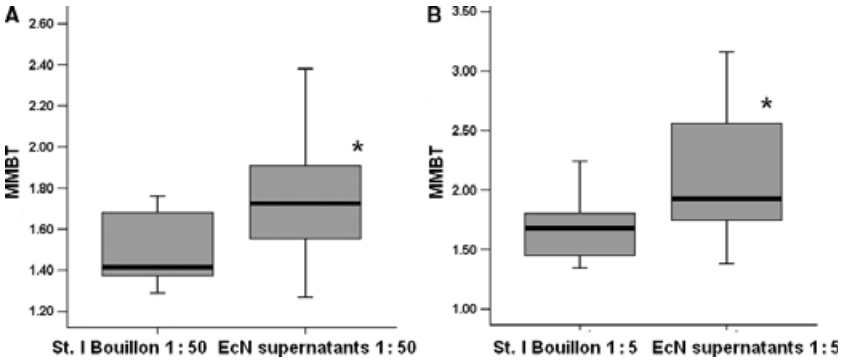


**Figure 1** Maximal multiplication of basal tension (MMBT) under native conditions. EcN supernatants evoked a significantly (*$P \leq 0.05$) higher MMBT compared to Standard-I-Bouillon. Data are shown for dilution 1 : 50 (A) and 1 : 5 (B), $n = 17$ patients.

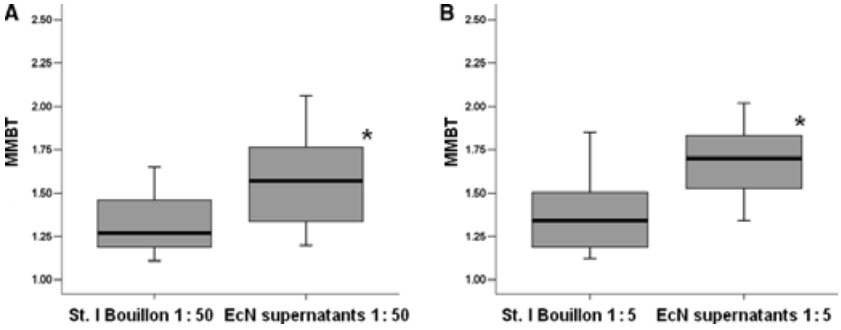


**Figure 2** Maximal multiplication of basal tension (MMBT) under NANC conditions. EcN supernatants evoked a significantly (*$P < 0.01$) higher MMBT compared to Standard-I-Bouillon. Data are shown for dilution 1 : 50 (A) and 1 : 5 (B), $n = 17$ patients.

1 : 5) (Fig. 2). The percental increase of MMBT caused by EcN supernatants compared to Standard-I-Bouillon alone was 22% (1 : 50) and 28% (1 : 5) respectively. No differences between EcN supernatants and Standard-I-Bouillon were found concerning alteration of median tension.

## Contractile responses under TTX conditions

Under TTX conditions EcN supernatants evoked a higher MMBT compared to Standard-I-Bouillon alone. At low concentrations (dilution 1 : 50), MMBT was 2.10 after administration of EcN supernatants *vs* 1.80 after administration of Standard-I-Bouillon ($P \leq 0.05$) (Fig. 3A). At high concentrations (dilution 1 : 5), MMBT was 2.75 after administration of EcN supernatants *vs* 2.06 after administration of Standard-I-Bouillon ($P < 0.01$) (Fig. 3B). The percental increase of MMBT caused by EcN supernatants compared to Standard-I-Bouillon alone was 17% (1 : 50) and 33.5% (1 : 5) respectively. No differences between EcN supernatants and Standard-I-Bouillon were found concerning alteration of median tension. Original traces illustrating the effects of EcN supernatants and Standard-I-Bouillon under TTX conditions for both concentrations are shown in Fig. 4.

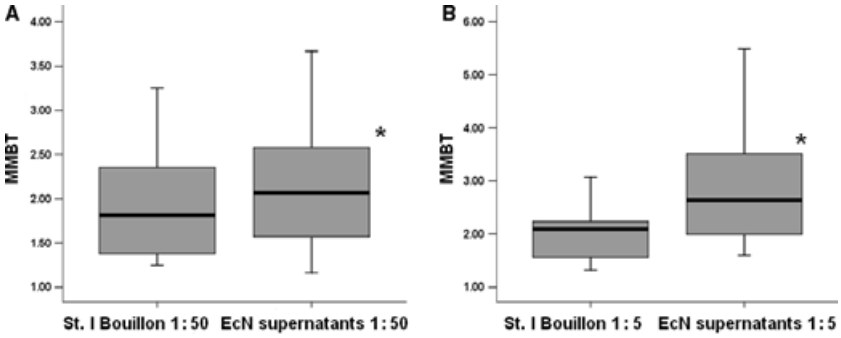


**Figure 3** Maximal multiplication of basal tension (MMBT) under TTX conditions. EcN supernatants evoked a significantly higher MMBT compared to Standard-I-Bouillon both at low (1 : 50) (*$P < 0.05$) and high (1 : 5) (*$P < 0.01$) dilution, $n = 17$ patients.

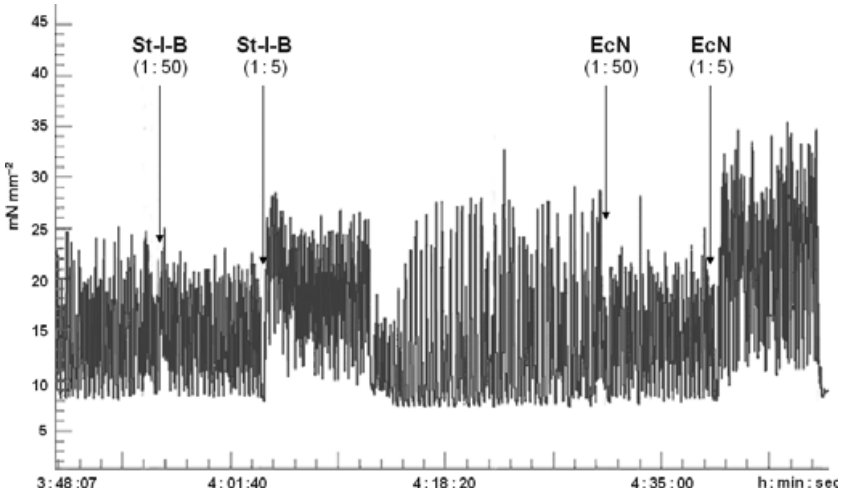


**Figure 4** Contractile responses of a smooth muscle strip to Standard-I-Bouillon (St-I-B) and EcN supernatants (EcN) under TTX condition at low (1 : 50) and high (1 : 5) concentrations. While EcN supernatants evoked higher maximal contraction amplitudes (tension forces, mN $mm^{-2}$) compared to Standard-I-Bouillon at high concentrations (1 : 5), both media did not differ significantly at low concentrations (1 : 50). The median tension did not differ significantly between Standard-I-Bouillon and EcN supernatants, when compared for both low and high concentrations.

### Effects of EcN supernatants at low and high concentrations

To verify whether the enhancement of maximal contraction forces evoked by EcN supernatants depended on the concentrations administered, MMBT recorded for both low (dilution 1 : 50) and high (dilution 1 : 5) concentrations of EcN supernatants was compared. Under each of the conditions tested (native, NANC, TTX), MMBT was significantly higher ($P < 0.01$) with increasing concentrations of EcN supernatants. In contrast, comparison of low and high concentrations of Standard-I-Bouillon alone (dilutions 1 : 50 *vs* 1 : 5) showed no statistically significant changes of MMBT neither under native ($P = 0.08$), NANC ($P = 0.14$) nor TTX ($P = 0.22$) conditions.

### Relaxation after electrical field stimulation

To test whether EcN supernatants influence the EFS-induced relaxation of muscle strips, the extent of relative relaxation evoked by EFS was compared before and after administration of either EcN supernatants or Standard-I-Bouillon (dilutions 1 : 50 and 1 : 5). Both EcN supernatants and Standard-I-Bouillon did not significantly alter the EFS-induced relaxation under native and NANC conditions (data not shown).

### Contractile responses after exposure to increasing concentrations of acetic acid

Standard-I-Bouillon supplemented with increasing concentrations of acetic acid evoked an enhancement of maximal tension forces only under TTX conditions. The MMBT elicited by acetic acid (20 mmol $L^{-1}$) was significantly ($P \leq 0.01$) higher in comparison to unsupplemented Standard-I-Bouillon (10 mmol $L^{-1}$ acetic acid). Although the mean values of MMBT for higher concentration of acetic acid (40, 80 mmol $L^{-1}$) were also elevated compared to unsupplemented Standard-I-Bouillon, they were not statistical significant due to the high variability of the data (Fig. 5). The enhancement of maximal tension forces induced by increasing concentrations of acetic acid was less pronounced than after addition of EcN supernatants (compare Figs 3 and 5) and was not observed under native conditions.

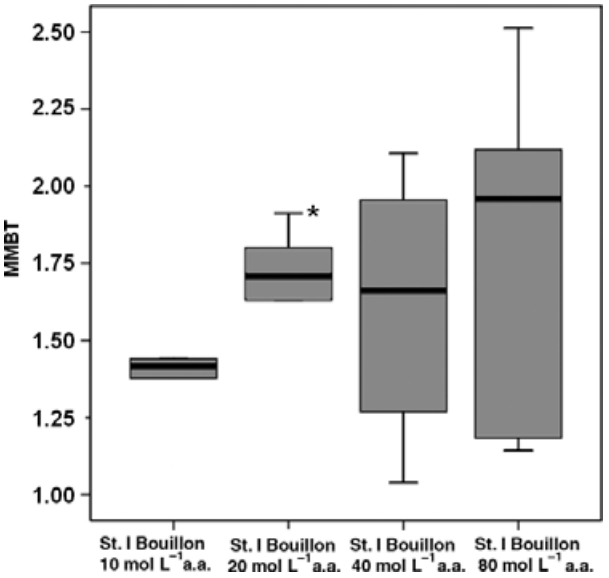


**Figure 5** Maximal multiplication of basal tension (MMBT) after exposure to increasing concentrations of acetic acid (a.a.) under TTX conditions. MMBT elicited by 20 mmol $L^{-1}$ acetic acid was significantly ($P \leq 0.01$) higher compared to unsupplemented Standard-I-Bouillon (10 mmol $L^{-1}$ acetic acid), whereas 40 and 80 mmol $L^{-1}$ acetic acid did not differ significantly. Data are shown for dilution 1 : 5, $n = 24$ muscle strips.

## DISCUSSION

From controlled clinical trials, it has been concluded that EcN is able to modulate gastrointestinal motility.[3,12,13] However, up to now, all experimental studies designed to elucidate the underlying mechanisms by which probiotic agents influence gastrointestinal motility have been carried out in animal models: Thus far, two *in vivo* studies could show that administration of probiotics induces increased colonic propulsive contractions and defecation rate in pigs[21] and abolishes muscular hypercontractility in a mouse model of post-infectious gut dysfunction.[22] Ohashi *et al.*[21] monitored colonic contraction patterns in conscious pigs using strain gauge force transducers and observed enhanced propulsive contractions of the terminal colon and an elevated defecation frequency following administration of *Lactobacillus casei* strain Shirota. In the study of Verdu *et al.*,[22] mice were gavaged with different probiotic bacteria after recovery from intestinal infection with *Trichinella spiralis*. *Lactobacillus paracasei*, but not *Lactobacillus johnsonii*, *Bifidobacterium lactis* and *Bifidobacterium longum*, attenuated postinfectious muscle hypercontractility. Muscle hypercontractility was attenuated both by *Lactobacillus paracasei* and *Lactobacillus paracasei*-free supernatants. The effects were abolished after heat treatment of *Lactobacillus paracasei*-free supernatants suggesting that a heat-labile metabolite may be involved – an observation which does not speak in favour for SCFAs as active agents.

An *in vitro* study[34] has investigated the effects of entire bacteria, bacterial cell debris and crude extracts

of the probiotic mixture VSL#3 on motility patterns in guinea-pig ileum and proximal colon. While neither entire bacteria nor bacterial cell debris had any effects, crude extracts evoked a dose-dependent contraction of ileal segments and relaxation of colonic muscle strips. The underlying mechanisms did not involve muscarinic receptors, as the administration of atropine did not reverse the described effects. The results suggest that crude extracts from probiotic strains contained in VSL#3 (e.g. *Bifidobacterium*, *Lactobacillus, Streptococcus*) are able to modulate intestinal motility by direct interaction with the smooth muscle cells.

The only *in vitro* study[35] available for EcN has evaluated the effects of EcN supernatants on intestinal motility patterns using a study design similar to the present investigation. Colonic muscle strips from rats were exposed to either EcN culture supernatants or pure culture medium. Isometric tension forces were recorded under native conditions and after cumulative carbachol stimulation to evaluate maximal contraction amplitudes. While the culture medium itself dose-dependently reduced maximal contraction amplitudes, EcN supernatants abolished this inhibition in 50% of the samples. It has been concluded that EcN may influence colonic motility by its metabolic activity due to either a breakdown of inhibitory compounds or production of stimulating agents.

This study has assessed the impact of probiotic compounds on intestinal motility for the first time in the human gut. Standard-I-Bouillon supplemented with EcN supernatants evoked a significantly higher MMBT in colonic circular muscle strips compared to Standard-I-Bouillon alone. The increase of maximal tension forces was more pronounced when the concentration of EcN supernatants was elevated. These effects were observed under native conditions as well as after blockade of either extrinsic nervous inputs (NANC conditions) or the entire enteric nervous system abolishing also most of the intrinsic nervous input (TTX conditions).

Both partial and complete blockade of enteric nerves led to a continuous increase of maximal contraction forces induced by EcN supernatants which were 15% (native conditions), 28% (NANC conditions) and 34% (TTX conditions) higher than those evoked by Standard-I-Bouillon alone (dilution 1 : 5). Thus, the overall input of enteric nerves and in particular of NANC innervation seemed to attenuate the contractile effects of EcN supernatants – which is in line with the mainly inhibitory role of NANC transmitters in regards to smooth muscle contraction. However, modulation of contractility pattern by EcN supernatants was confined to the phasic activity by elevating maximal tension forces, as the mean tension (tonic activity) was not affected.

*Escherichia coli* Nissle 1917 supernatants did not alter EFS-induced relaxation of smooth muscle strips. Regardless of the concentrations of EcN supernatants (dilutions 1 : 50 and 1 : 5) and the different conditions tested (native, NANC), the relative relaxation evoked by EFS remained unchanged suggesting an action pathway of EcN supernatants independent from the enteric nervous system. This assumption is supported in particular by the fact that after blockade of enteric nerves by TTX EcN supernatants still exerted their contractile effects. Thus, if nerve-mediated mechanisms are unlikely, direct action onto smooth muscle cells should be considered.

Standard-I-Bouillon itself was also able to elevate maximal tension forces, however, less intensively than Standard-I-Bouillon supplemented with EcN supernatants suggesting that both media contain either different agents able to elevate maximal tension forces or a common contractility-enhancing agent in different concentrations. Gaschromatographic analysis revealed that pure Standard-I-Bouillon and Standard-I-Bouillon after cultivation with EcN differed in their content of acetic acid (10 *vs* 19 mmol $L^{-1}$). Acetic acid is one of the major metabolic products of EcN (G. Sollorz and U. Sonnenborn, unpublished data) and an important member of the colonic SCFA family. SCFAs are putative candidates to act on enteric smooth muscle: e.g. intraluminal administration of SCFAs induced an increased motility of both ileum and colon in dogs.[23] It has been suggested that a mechanism involving $Ca^{2+}$-channel proteins may be responsible for the accelerated intestinal transit, because the effects vanished, when $Ca^{2+}$-free solution was used.[24,25] In rats, SCFAs accelerated stomach-to-caecum transit time[28] and evoked contractile effects in the terminal ileum[29] and isolated colon.[26] In humans, SCFAs were able to stimulate ileal motility.[31] However, when Standard-I-Bouillon was supplemented with increasing concentrations of acetic acid instead of EcN supernatants, comparable effects could only be observed under TTX conditions. The enhancement of maximal tension forces induced by acetic acid was less pronounced than after exposure to EcN supernatants and was not observed under native conditions. Thus, the effects elicited by EcN supernatants cannot be fully attributed to acetic acid. Although previous data on acetic acid have shown its prokinetic properties, the present findings suggest that EcN supernatants contain contractility-enhancing agents other than acetic acid.

One of the major methodical limitations inherent to *in vitro* studies of intestinal motility is the artificial

separation of the enteric musculature from the rest of the intestinal wall (submucosal and mucosal tissue) – in particular, if the impact of intraluminal agents, such as probiotic metabolites, on muscular contractility is evaluated. However, most of the metabolites of lower molecular weight released from probiotic bacteria including acetic acid are able to cross the epithelial barrier and are transported via blood vessels to the intestinal muscle layers. There is evidence from the literature that SCFAs are readily absorbed and reach peripheral tissues.[36,37] Acetic acid represents 90% of all absorbed SCFAs which are detectable in peripheral blood.[38]

In the human colon, the daily production of total SCFAs has been estimated to be 100–200 mmol $L^{-1}$[39] with a ratio of 60 : 20 : 20 between acetic, propionic and butyric acid[39,40] implying a production of 60–120 mmol $L^{-1}$ acetic acid per day. In germfree mice, it has been found that monoassociation with *E. coli* resulted in an increase of total SCFAs in the caecum from 1.0 to 6.9 mmol $kg^{-1}$ content, with acetic acid (6.7 mmol $kg^{-1}$) being the main metabolite.[41] This is nearly 10% of the total acetic acid produced by the entire microbiota of conventional animals (74.2 mmol $kg^{-1}$). Assuming similar relationships in humans the contribution of *E. coli* to the daily acetic acid production will amount to 6–12 mmol $L^{-1}$. This is well within the range of acetic acid produced in 24 h by EcN under aerobic conditions (5.0–20.3 mmol $L^{-1}$) *in vitro* (G. Sollorz and U. Sonnenborn, unpublished data). In the organ bath experiments, EcN supernatants have been diluted 1 : 5 and 1 : 50, respectively, and therefore should have contained 1–4 and 0.1–0.4 mmol $L^{-1}$ acetic acid. These values might slightly underestimate the total amount of acetic acid, as the culture medium itself already contained 10 mmol $L^{-1}$ of acetic acid. In the experiments with addition of acetic acid (20, 40, 80 mmol $L^{-1}$), the actual acetic acid concentration in the organ bath would have amounted to 4, 8 and 16 mmol $L^{-1}$ (1 : 5 dilution) and 0.4, 0.8 and 0.16 mmol $L^{-1}$ (1 : 50 dilution) plus the amount already present in the medium. In isolated segments of rat colon, SCFAs evoked an increased contraction with maximal responses at 0.1 mmol $L^{-1}$ acetic acid which were not further enhanced at 10 mmol $L^{-1}$.[42] Thus, the acetic acid concentrations used in our organ bath experiments lie well within these dimensions.

## CONCLUSION

*Escherichia coli* Nissle 1917 supernatants increased the maximal tension forces of smooth muscle strips from human colon both at low and high concentrations. As neither partial nor TTX induced blockade of enteric nerves abolished these effects, EcN supernatants appear to enhance maximal contraction amplitudes by direct stimulation of smooth muscle cells. Active metabolites produced by EcN most likely include other substances than acetic acid, as this SCFA only partially resembled the effects elicited by EcN supernatants. The present data illustrate the potential of probiotics to trigger *in vitro* motility patterns of the human colon and may provide a rationale for their clinical application in the treatment of gastrointestinal motor dysfunctions, such as severe constipation or constipation-predominant irritable bowel syndrome. Further studies on human tissue are required to characterize in greater detail the physiological mechanisms by which probiotics and their luminally released metabolites influence intestinal motility in health and disease.

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