Original Article

Effect of DSS on Bacterial Growth in Gastrointestinal Tract

(IBD / DSS colitis / bacterial growth / *S. typhimurium* SL7207 / *E. coli* Nissle 1917)

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Abstract. Inflammatory bowel disease is an idiopathic autoimmune disorder that is mainly divided into ulcerative colitis and Crohn's disease. Probiotics are known for their beneficial effect and used as a treatment option in different gastrointestinal problems. The aim of our study was to find suitable bacterial vectors for gene therapy of inflammatory bowel disease. *Salmonella enterica* serovar Typhimurium SL7207 and *Escherichia coli* Nissle 1917 were investigated as potential vectors. Our results show that the growth of *Escherichia coli* Nissle 1917 was inhibited in the majority of samples collected from dextran sodium sulphate-treated animals compared with control growth in phosphate-buffered saline. The growth of *Salmonella enterica* serovar Typhimurium SL7207 in all investigated samples was enhanced or unaffected in comparison with phosphate-buffered saline; however, it did not reach the growth rates of *Escherichia coli* Nissle 1917. Dextran sodium sulphate treatment had a stimulating effect on the growth of both strains in homogenates of distant small intestine and proximal colon samples. The gastrointestinal tract contents and tissue homogenates did not inhibit growth of *Salmonella enterica* serovar Typhimurium SL7207 in comparison with the negative control, and provided more suitable environment for growth compared to *Escherichia coli* Nissle 1917. We therefore conclude that *Salmonella enterica* serovar Typhimurium SL7207 is a more suitable candidate for a potential bacterial vector, even though it has no known probiotic properties.

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Abbreviations: DSS – dextran sodium sulphate, *E. coli* – *Escherichia coli* Nissle 1917, GIT – gastrointestinal tract, IBD – inflammatory bowel disease, LB medium – Luria-Bertani medium, OD – optical density, PBS – phosphate-buffered saline, *S. typhimurium* – *Salmonella enterica* serovar Typhimurium SL7207, SD – standard deviation.

Introduction

Inflammatory bowel disease (IBD) represents a group of chronic gastrointestinal inflammatory diseases with an elusive aetiology caused by various genetic, immunological and environmental factors. Two main forms of IBD are known – ulcerative colitis and Crohn's disease, which share some similarities in pathogenesis. However, they can be clearly distinguished by several key clinical features, signs and symptoms (Xavier and Podolsky, 2007). Recently, the prevalence of IBD has been on the rise not only in developing countries, but also in northern Europe, the United Kingdom and North America. This trend is suggestive of environmental factors such as diet or lifestyle being as important in pathogenesis of IBD as commonly known immunologic and genetic predispositions (Baumgart and Carding, 2007). One of the key determinants is mucosal immunity, depending on the homeostasis of the gut microbiome and its stimulatory effect on properly established development of the intestinal immune system (McDermott and Huffnagle, 2014).

The murine dextran sodium sulphate (DSS) acute colitis model is well described and frequently used in research for its effectiveness (Wirtz et al., 2007). DSS directly attacks epithelial cells of the basal crypts in the colon and is responsible for the disruption of the mucosal barrier integrity (Wirtz et al., 2007). We therefore decided to use this model for our research, according to the method established by Wirtz et al. (2007).

Gene therapy has only been recently investigated as an alternative to corroborated treatment by commercial compounds for the treatment of various diseases by introducing transgenic nucleic acid into the target cell. The investigation continuously brings successful results. Although viruses are used most frequently for the transfer, bacterial vectors (bactofection) are an interesting option for gene transfer (Wirth et al., 2013). *Escherichia coli* has been previously used as a vector for plasmid delivery into mouse colitis-damaged mucosa with successful results of improved histology and decreased myeloperoxidase concentration in colonic tissue (Castagliuolo et al., 2005). *Escherichia coli* Nissle 1917 (*E. coli*) was successfully used as a vector for nematode immune modulator cystatin with positive results in the murine

acute colitis model and post-weaning pigs, a model with similar symptoms of colitis post weaning as in the tract of humans. Whelan et al. (2014) reported that cystatin was well received and the barrier function was improved, despite the lack of changes in cytokine expressions. *Salmonella enterica* serovar Typhimurium SL7207 (*S. typhimurium*) is an attenuated mutant with retained invasiveness that is considered as a favourable candidate for a vector (Basso et al., 2000). Our laboratory previously confirmed *S. typhimurium* SL7207 to be an efficient bacterial vector for plasmid delivery. A plasmid carrying genes encoding Cu-Zn superoxide dismutase and an N-terminal deletion mutant of monocyte chemoattractant protein-1 led to anti-oxidative and anti-inflammatory gene therapy in a mild colitis murine model (Palffy et al., 2011).

In this study we aimed to determine which of the two bacterial strains used as vectors for IBD treatment had a potential to be better received by the host gastrointestinal tract (GIT). We compared bacterial survival in different locations and environments of GIT (tissues and contents) and investigated whether the DSS treatment and induction of colitis would display an effect on bacterial growth.

Material and Methods

Animals

C57BL/6 male mice (9 months old, Anlab, Prague, Czech Republic) had *ad libitum* access to food and water and were kept in controlled environment with 12-h light/dark cycle (Fig. 1). The tested animals were divided into two groups; DSS-treated (N = 7) and control (N = 6). The protocol described by Wirtz et al. (2007) was followed. The treated group was administered 2% DSS in drinking water for 7 days. A fresh solution of DSS was prepared every third day. After the treatment, mice were given water for 3 days for removal of residual DSS. Body weight and stool consistency (0 – normal; 1 – soft; 2 – watery; 3 – watery with blood) were monitored daily. The colitis stage was characterized by the presence of blood in the stool. All mice were sacrificed on day 10 and a range of samples was collected as described below.

Collecting samples

Stomach, proximal and distal small intestine, caecum, proximal and distal colon as well as their contents were collected and homogenized in phosphate-buffered saline (PBS) (1.5 ml) for 2 × 2 min at 25 Hz using a homogenizer (TissueLyzer, Qiagen, Germany). Samples were centrifuged for 5 min at 10,000 *g* at room temperature. Further experiments were performed using only collected supernatants.

Bacteria

E. coli Nissle 1917 (Darji et al., 1997) and *S. typhimurium* SL7207 (Cukrowska et al., 2002) were cultivated in Luria-Bertani (LB) medium (USBiological Life Sciences, San Antonio, TX) (18 h, 150 rpm, 37 °C). Cultures were diluted to OD_{600} = 0.4. One hundred microliters of independently dissolved cultures and controls, respectively, were mixed with 100 µl of samples in a 96-well plate in quadruplicates. LB broth and PBS were used as controls.

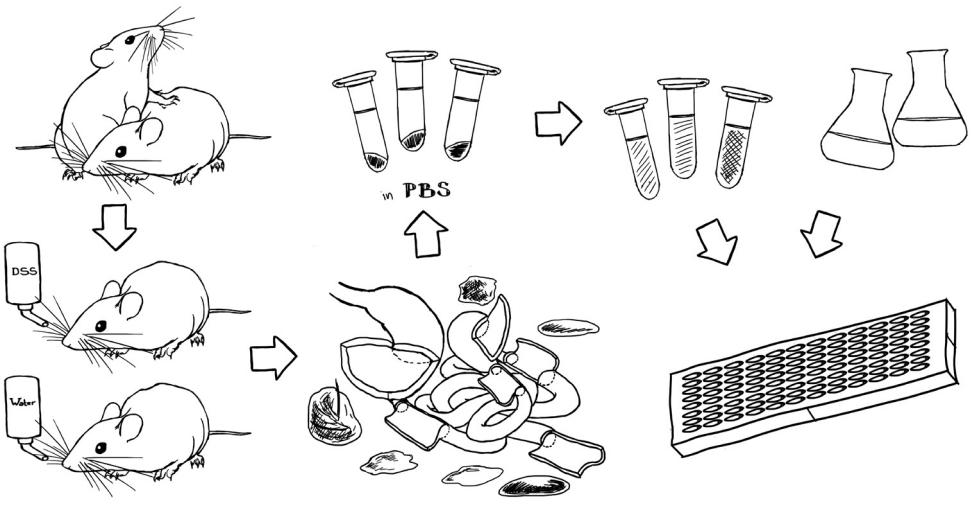

Fig. 1. Experimental protocol. Nine months old male C57BL/6 mice were divided into two groups – group treated with DSS in drinking water, and control group drinking tap water. After 7 days on DSS and 3 days on water, mice were sacrificed and samples of the contents and tissues from the stomach, proximal and distal small intestine, caecum, proximal and distal colon were collected. Samples were homogenized in saline and placed into 96-well plates. Bacteria *S. typhimurium* SL7207 and *E. coli* Nissle 1917 were diluted to OD_{600} 0.4 and added to the samples. PBS was used as a control. Optical density was measured at 595 nm every hour for 8 h since time point 0 (when bacteria were added). In between, the samples were cultivated at 37 °C and 500 rpm.

Spectrophotometry

Growth rates of the mixtures – samples with bacteria or controls, respectively, were measured spectrophotometrically (Minilyser Spectra II, Tecan, Salzburg, Austria) (OD) in time points 0 (right after bacterial inoculation) and every consequent hour for the period of 8 h at 595 nm. Plates with samples were incubated at 37 °C and 500 rpm in a thermoshaker (Biosan, Riga, Latvia) between the measurements. The growth rates of bacteria in samples were compared with inoculated bacteria in LB broth or PBS.

Statistical analyses

Data were analysed using repeated measures ANOVA with Bonferroni correction (GraphPad5.0). The P value lower than 0.05 was significant. Data are presented as mean with standard deviations (SD).

Results

DSS-induced colitis was highly effective; blood in the stool was observed on day 4–5. Weight loss started to appear after DSS replacement – a rapid decrease occurred on day 7, resulting in around 10% weight loss on day 10 (Fig. 2).

While presenting our results, we compared the growth rates of samples with PBS as a negative control, showing bacterial growth in a non-manipulated environment. The focus was on presenting these results among the groups of healthy control animals and DSS-treated animals and supernatant collected from the contents or tissue homogenates of their GIT. In general, *E. coli* displayed around 1/4 higher OD levels than *S. typhimurium*; however, unlike *S. typhimurium, E. coli* was inhibited in all samples, both of contents and homogenates, compared with PBS.

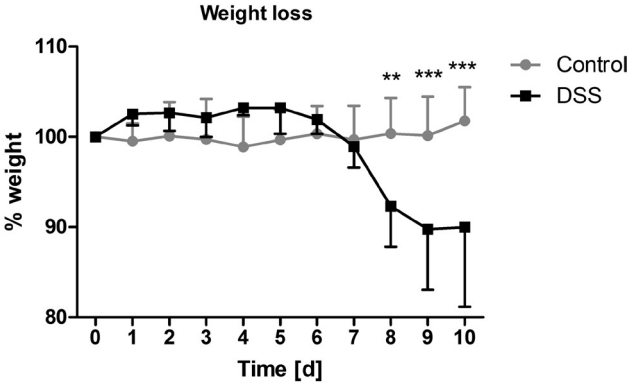

Fig. 2. Weight loss of mice. Nine months old male C57BL/6 mice were divided into two groups – group treated with DSS in drinking water (black), and control group drinking tap water (grey). After 7 days on DSS and 3 days on water, mice were sacrificed and samples collected. Approximately 10% weight loss can be seen in DSS-treated animals on day 10. ** $P < 0.01$, *** $P < 0.001$ between groups. All data are presented as mean ± SD.

The growth of *E. coli* in samples compared with PBS was higher in healthy control animals. In their contents, the growth rates were similar or higher than in PBS with the exception of the stomach, where the growth was inhibited in time point over 7 h with significant differences. In the contents of DSS-treated animals, *E. coli* was inhibited in all samples, with the exception of proximal small intestine, without significant differences. Inhibition in the contents of the DSS-treated group was more permanent in the stomach (*7 h, **8 h), caecum (*6, 7 h, **8 h) and proximal colon (*7 h, **8 h) (Fig. 3). In tissue homogenates of the healthy controls the growth of *E. coli* was slightly elevated in the proximal part of GIT compared with PBS and the distal part until 5-h time point; afterwards, the growth rates decreased under PBS levels. The highest inhibition of the growth of *E. coli* was observed in the stomach at *7 and 8 h compared to PBS. All samples from DSS-treated animals followed the same curve, where the samples were inhibited after 5 hours of incubation compared to PBS levels. Significant differences were observed in the case of distal small intestine (*8 h) and distal colon from the 2nd hour (*6 h, **7 h, ***8 h).

On the other hand, results from growth rates of *S. typhimurium* were either the same or higher than its growth in PBS; no inhibition such as in the case of *E. coli* was observed. *S. typhimurium* prospered in all locations of the proximal part of GIT, mainly in proximal small intestine (samples in PBS: healthy content *2 h, **3–6 h, *7, DSS content **2–6 h, healthy tissue: *5–7 h, DSS homogenate: *2 h, **3–6 h, *7 h) (Fig. 3). Higher growth rates with significant differences were observed in the stomach as well (*2 h, **3–6 h, *7, 8 h). Most of the samples shared a similar to identical growth curve in the remaining conditions. *S. typhimurium* prospered in tissue homogenates of the stomach and caecum from healthy mice, but the differences compared to PBS were not significant. What is most important, the growth rate of *S. typhimurium* in proximal colon tissue homogenates of DSS-treated animals was, although non-significantly, elevated as well.

Discussion

E. coli Nissle 1917 is a well-defined probiotic known for a century and commercially available as Mutaflor (Ardeypharm, Germany), used for the treatment of a variety of intestinal diseases. Its positive effect on intestinal microbiota and mucosa was demonstrated in numerous studies (Orel and Kamhi Trop, 2014; Sha et al., 2014). This strain was previously used as a bacterial vector in therapies and had a potential in the treatment of human IBD. Westendorf et al. (2005) presented a study encouraging the usage of *E. coli* Nissle 1917 as a bacterial carrier for possible gene therapy in mice. However, Petersen et al. (2014) did not recommend adding *E. coli* Nissle 1917 as a treatment for ulcerative colitis without pre-treatment with antibiotics. On the other hand, previous studies in mice showed *S. typhimu-*

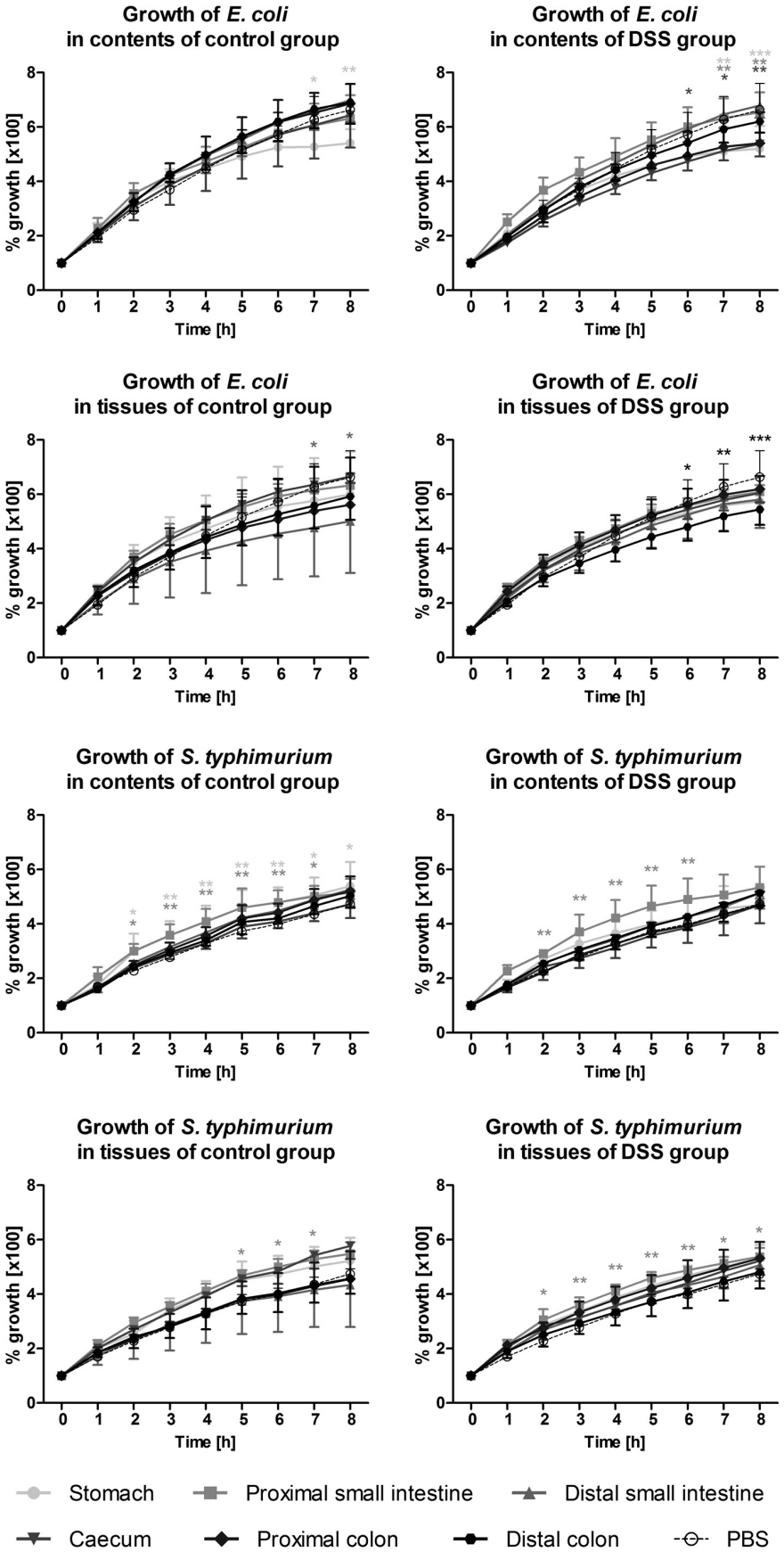

Fig. 3. Bacterial growth in the contents and tissue homogenates. Bacteria were added to the contents and tissue homogenates of the stomach, proximal and distal small intestine, caecum, and proximal and distal colon at time 0, and optical density was measured every hour for 8 h. PBS was used as a negative control (dotted). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to PBS. All data are presented as mean ± SD.

rium SL7207 to be a favourable strain for its attenuated, non-pathogenic nature, longer survival in the colon (compared to the upper parts of GIT) and better adaptation for colonization of the inflamed gut (Pálffy et al., 2010, 2011; Gardlik et al., 2011, 2014).

Our results showed similar growth rates of bacteria in different environments in the murine DSS-induced colitis model and control mice. *S. typhimurium* showed better growth than *E. coli*. The growth of *S. typhimurium* was more gradual; on the other hand, it increased more stably in different environments in comparison with the growth in PBS and also in comparison with the growth of *E. coli*. The latter was, on the other hand, inhibited or stagnant in comparison with PBS control growth.

Even though the growth of *E. coli* was consistently similar in all samples (supernatants of the contents and tissues), the growth rate was inhibited in both animal groups after 5-h time point in most investigated samples. On the other hand, *S. typhimurium* showed the capability to grow in both tested environments (contents and tissue homogenates), and in both animal groups (controls and mice with DSS-induced colitis) and with much less variation in all investigated locations than *E. coli*.

The results of this study can be analysed from three different viewpoints: a) what was the difference in bacterial growth between the contents and tissues; b) what was the difference among the specific locations of GIT; and c) what was the effect of DSS on bacterial growth?

Bacteria grew better in the contents

When comparing bacterial growth in the contents and tissues, we needed to take into account the bacterial strain as well. Generally, there were higher (less inhibited compared with PBS) growth rates of *E. coli* in the contents than in tissues, but they seemed less essential that those of *S. typhimurium*. The growth of *S. typhimurium* in the homogenates was more pronounced in comparison with PBS controls. On the other hand, *E. coli* showed better growth in PBS compared to the inoculated homogenates or contents.

E. coli had higher variation in different GIT locations; S. typhimurium was stimulated in all locations

When focusing on bacterial growth with regard to intestinal locations, there was much higher variation in growth rates of *E. coli* than in those of *S. typhimurium*. The only exception was the growth of *E. coli* in the contents of stomach of healthy animals that showed a decrease starting at 5-h time point, while slight stimulation of growth was observed in the healthy colon. The variation rose in the contents of DSS-treated animals, when growth rates of the colon dropped under the PBS-treated control. In tissue homogenates the variance was higher in healthy controls than in the DSS-treated group. While in healthy tissues of proximal small intestine and caecum *E. coli* was stimulated until 7-h time point more prominently, the growth in the distal part of small intestine showed prominent variation compared to the PBS-treated control. This difference was eliminated in the DSS-treated group, where the growth was mostly the same, with the exception of distal colon, where the growth rate was decreased even more, compared to the healthy control and PBS. Usage of *E. coli* as a bacterial vector for the treatment of tissue of distal colon might not be strongly recommended. Our results confirm the findings by Petersen et al. (2014), who did not recommend adding *E. coli* Nissle 1917 as a treatment for ulcerative colitis without pre-treatment with antibiotics.

The first fact worth noticing when looking at the growth rates of *S. typhimurium* in different GIT locations was that no specific location inhibited its growth. *S. typhimurium* grew in all samples at least as well as in the PBS-treated control. No such variance as in the case of *E. coli* was observed – the only prominent exception was the growth of *S. typhimurium* in the proximal small intestine, which seemed to be more welcoming to the bacteria in all conditions – not depending on the health status or difference in the environment (contents/tissue). To conclude, *S. typhimurium* might be well received in the treatment of tissue disorders in the caecum and proximal colon as well (Fig. 4).

DSS stimulated growth of S. typhimurium in the proximal colon tissue

In general, there was higher consistency of data in homogenates of the DSS-treated group than in the control group. The presence of DSS slightly reduced the growth of both investigated strains in the contents samples, with the exception of growth of *S. typhimurium* in proximal small intestine. The reduction was more visible in the tissue of caecum, where the growth of *S. typhimurium* dropped to the levels seen with PBS cultivation. On the other hand, DSS stimulated growth of *S. typhimurium* in the proximal colon tissue (Fig. 5). In contrast to *S. typhimurium*, the growth of *E. coli* was mainly inhibited in both contents and tissues under the DSS treatment. However, in homogenate samples, the variance in growth rates of *E. coli* was lower.

Conclusion

S. typhimurium growth rates were considerably higher in the investigated samples compared to PBS. However, even the highest values of growth rates of *S. typhimurium* were smaller compared to the *E. coli* growth rates. Taking into account that the *E. coli* growth rate was inhibited at 6-h time point, we conclude that *E. coli*, even though more aggressive and exhibiting higher replication rate, is not a suitable candidate for the vector therapy. *S. typhimurium*, on the other hand, despite its lower replication capabilities was not eliminated at any time point.

Our findings support the studies previously performed in our laboratory confirming the use of *S. typhimurium* SL7207 as a nucleic acid carrier in experimental treat-

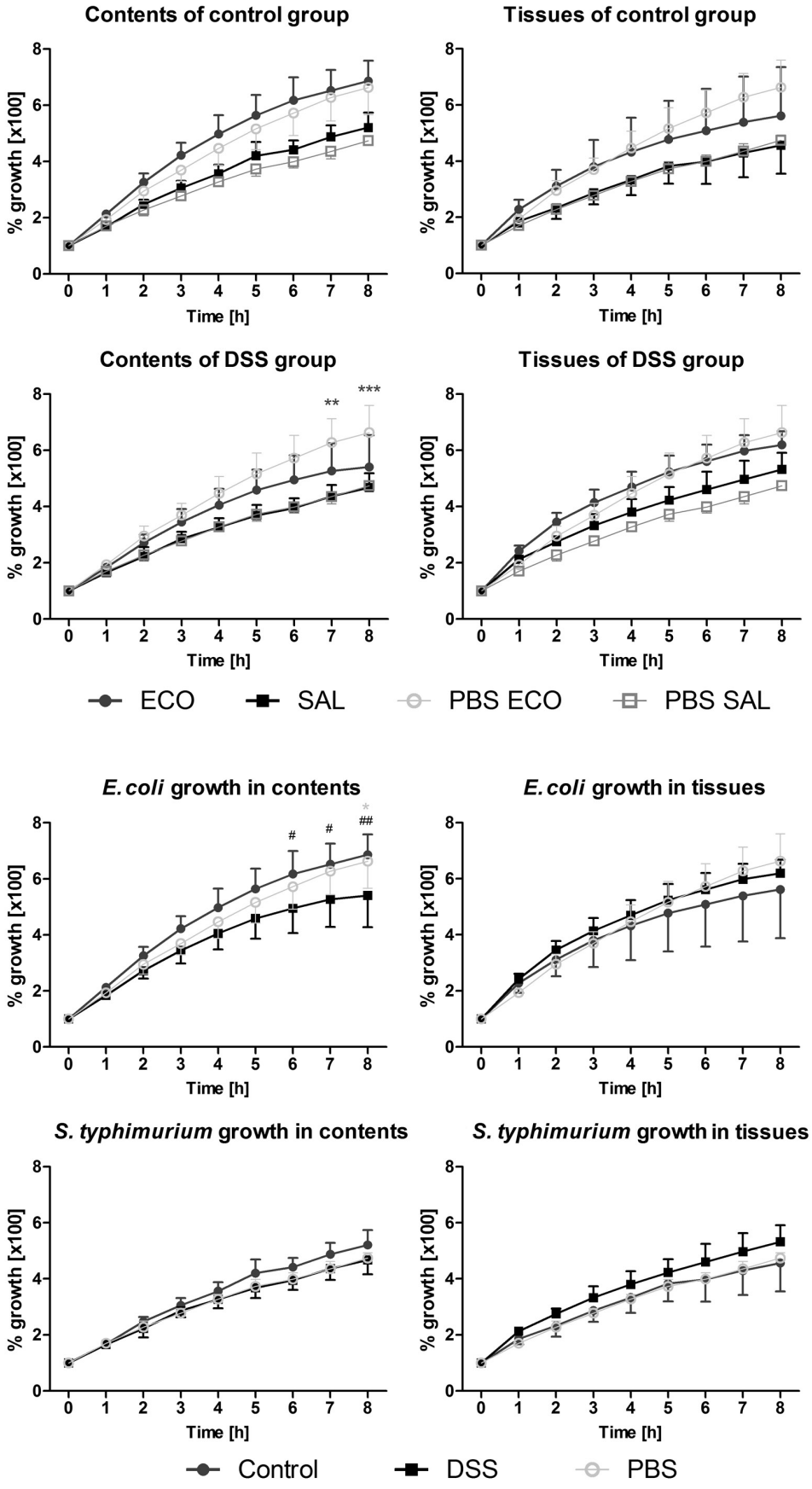

Fig. 4. Bacterial growth in the contents and tissues of proximal colon. SAL – *S. typhimurium* SL7207, ECO – *E. coli* Nissle 1917, PBS – PBS as a negative control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to PBS. $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ DSS-treated group compared to the control group. All data are presented as mean ± SD.

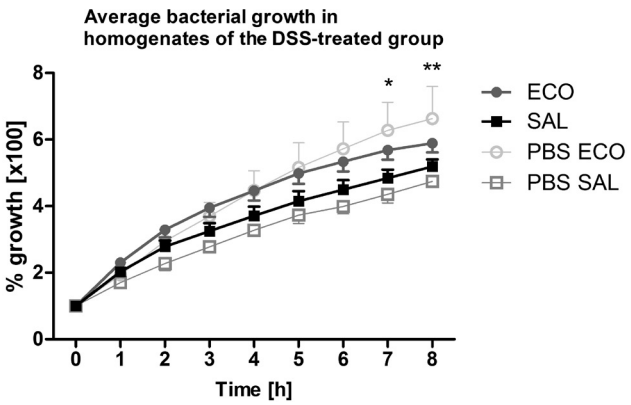

Fig. 5. Average bacterial growth in tissue homogenates of DSS-treated animals. Shown are pooled data of tissue samples during 8 h. Bacteria were added to homogenized tissues of the stomach, proximal and distal small intestine, caecum, and proximal and distal colon at time 0 and OD was measured during 8 h. SAL – *S. typhimurium* SL7207, ECO – *E. coli* Nissle 1917, PBS – PBS as a negative control. * $P < 0.05$, ** $P < 0.01$ growth of *E. coli* compared to PBS. All data are presented as mean ± SD.

ment of murine colitis (Palffy et al., 2011; Gardlik et al., 2011, 2012, 2013). Considering our reported results, we can conclude that despite *E. coli* being repeatedly used as a bacterial vector for gene therapy, there are other, maybe more promising candidates and possibilities for the treatment of intestinal diseases (Westendorf et al., 2005). We are convinced that *S. typhimurium* SL7207 has a therapeutic potential for the treatment of IBD and disorders of the small intestine and further studies will be beneficial in exploring the full potential of our findings.

Acknowledgement

The animal experiments were approved by the institutional review board and Ethics Committee of the Faculty of Medicine, Comenius University in Bratislava, Slovakia on June 16, 2014 under the number 00201435.

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