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ORIGINAL ARTICLE

The effect of selected *Lactobacillus* strains on dextran sulfate sodium-induced mouse colitis model

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ABSTRACT

Inflammatory bowel disease (IBD) comprises two major illnesses: Crohn's disease (CD) and ulcerative colitis (UC). Dextran sulfate sodium (DSS) mouse colitis model has been used in understanding the mechanism of IBD. This study was conducted to examine selected *Lactobacillus* spp. as potential IBD treatment in the DSS-induced animal model. Balb/c mice were used and colitis was induced by adding 5% dextran sodium sulfate into the drinking water for 8 days. Colon length, disease activity index (DAI) and histological analysis were measured as markers of inflammation in DSS colitis mice. The majority of the *Lactobacillus* species significantly prevented the shortening of the colon length compared with the DSS group. The DAI scores of mice were significantly reduced following usage of four *Lactobacillus* strains included: *Lactobacillus plantarum* 03 and 06, *Lactobacillus brevis* 02 and *Lactobacillus rhamnosus* 01. The histological analysis exhibited that oral administration of *Lactobacillus* strains had therapeutic effects on mice colitis. *L. plantarum* and *L. brevis* showed better therapeutic effect against DSS-induced acute colitis mice. The probiotic activities of these three isolates indicated that the probiotic effects were strain specific and none of these useful bacteria could exhibit all of the valued probiotic properties simultaneously.

KEYWORDS

inflammatory bowel disease (IBD), dextran sulfate sodium (DSS), *Lactobacillus*, probiotic

INTRODUCTION

Inflammatory bowel disease (IBD) comprises as two major illnesses; Crohn's disease (CD) and ulcerative colitis (UC). Genetic, immunological responses and environmental factors are effective in creation of IBD [1].

The many studies in variety of countries demonstrate that the incidence of IBD in increasing, especially in adolescence [2]. Recently, the frequency of Crohn's disease (CD) and ulcerative colitis (UC) are increasing globally. The annual incidence of IBD is greatly varied ranged from 24.3 per 100,000 person-years for UC, and 12.7 per 100,000 person-years for CD in Europe to 6.3 per 100,000 person-years for UC and 5.0 per 100,000 person-years for CD.in Asia and Middle East [3].

Dextran sulfate sodium (DSS) mouse colitis model is a suitable in understanding of the mechanism of IBD. This model, which is similar to human IBD symptoms, includes weight

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loss, biological and pathological effects on the large intestine, colon cancer, mucosal ulcer, diarrhea and fecal blood [4].

Gut microbiome has an essential role in maintaining the gut's hemostasis. The probiotics could be used as a replaced treatment for the intestinal diseases instead of steroids and chemical immunomodulators by balancing the intestinal flora [5]. One of the most common probiotic bacteria used in the commercial products is *Lactobacillus* strains. The safety and beneficial effects of *Lactobacillus* strains in intestinal disorders have been evaluated by several investigators [6, 7].

Based on the results of our previous studies [8, 9], thirteen *Lactobacillus* strains showed some probiotic effects such as bacteriocins production, attachment to epithelial tissue, anti-proliferation of the tumor cell lines and the bactericidal effects against pathogenic bacteria. These bacteria were selected and this study was conducted to examine these bacteria as the potential treatment for IBD in the DSS-induced animal model.

MATERIALS AND METHODS

Experimental animals

Seventy five Balb/c mice (Female, 8-weeks-old and weighing 25–30 g) were used and placed in 15 groups of five. Mice were maintained under standard conditions (12/12-h light/dark cycle and enviromental temperature of 20–25 °C) during the experiment period. DSS colitis was induced in the mice as described previously [10]. Briefly, 8-weeks old BALB/c were treated by adding 5% dextran sodium sulfate into the drinking water for 8 days. All experiments were performed by supervision and approval of the animal experimentation ethics committee of Pasteur Institute of Iran.

Preparation of *Lactobacillus* strains

The thirteen *Lactobacillus* strains which had some potentially probiotic effect such as bacteriocins production and bactericidal effects, attachment to epithelial tissue and anti-proliferation of the tumor cell lines against pathogenic bacteria were selected for this study. These strains were included 6 *Lactobacillus plantarum*, 3 *Lactobacillus brevis*, 2 *Lactobacillus rhamnosus*,1 *Lactobacillus delbruecki* and 1 *Lactobacillus reuteri*.

Thirteen experimental groups received 0.5 mL of 10^9 colony forming units/mL (daily via gavage) of each selected *Lactobacillus* spp. for eight days. Two control groups included a healthy control group and a positive (DSS) group received water and DSS solution respectively under the same condition.

Colon length and disease activity index (DAI)

The length of the colon was measured as a marker of inflammation in DSS colitis mice. Three features included; body weight, stool consistency and occult blood were determined by scoring based on Hamamoto et al. [11] study as the DAI (Table 1).

Histological analysis

The colon tissues were extracted, fixed in 10% phosphate-buffered formalin and fixed in paraffin for followed the process. The sections of the colon were stained with Haemotoxylin and Eosin (H&E) for light microscopic examination. Histological scores were calculated based on Cooper et al. study [12] (Table 2).

Statistical analysis

Statistical analysis was carried out using SPSS (version 16, SPSS Inc., Chicago, IL) softwarePackage. All data are expressed as the means ± SEM and analyses with one-way analysis of variance (ANOVA) method and Differences of $P < 0.05$ were considered statistically significant.

RESULTS

The colon length of mice in the DSS group was significantly reduced (6.9 ± 1.1 cm) as compared with the healthy control group (9.4 ± 1.1 cm, $P < 0.01$).

All of the *Lactobacillus* species significantly prevented the shortening of the colon length compared with the DSS group except *L. plantarum* 04 which intensified the length of colon shortening (7.9 ± 1.1 cm) compared with the healthy control group (9.4 ± 1.1 cm, $P < 0.01$) (Fig. 1).

L. plantarum 05 and *L. brevis 02* which had the better probiotic effect in our previous studies showed more inhibitory effect on colon length reduction. In addition, *L. plantarum* 01 and *L. reuteri* showed less ability to reduce shortening of the colon length compared to other strains ($P < 0.05$) (Table 3) (Fig. 1).

Table 1. Disease activity index

Score	Weight loss (%)	Stool consistency	Occult bleeding
0	None	Normal	Normal
1	1–5		
2	5–10	Loose stools	Guiac +
3	10–15		
4	>15	Diarrhea	Gross bleeding

Table 2. Histological scores of colon sections

Score	Description
0	Normal
1	Shortening and loss of the basal 1/3 of the crypts
2	Loss of the basal 2/3 of the crypts
3	Crypt loss, mild inflammatory cell infiltration of lamina propria and submucosa
4	Erosions and marked cell infiltration

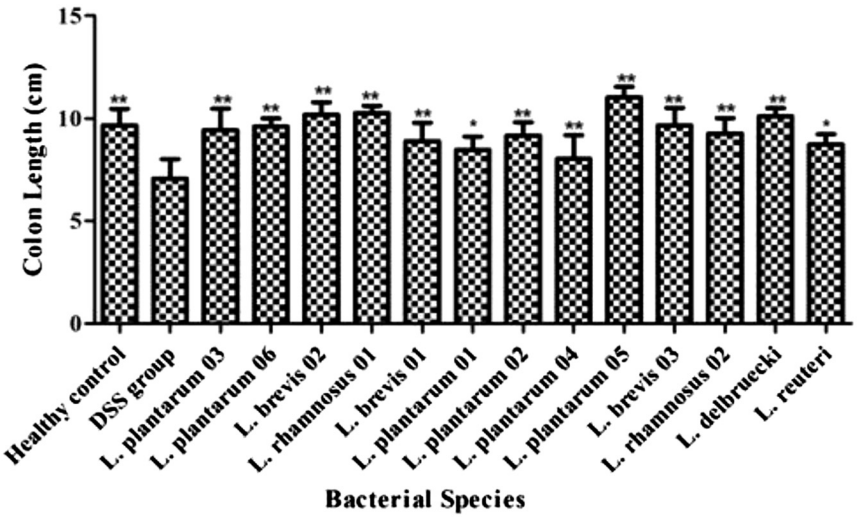

Figure 1. Inhibitory effects of *Lactobacillus* strains on shortening of the colon length

DAI score was determined based on weight loss, stool consistency and occult blood. DSS in mice resulted in shortened colon length and a significant increase in DAI scores as compared to the healthy control group ($P < 0.01$).

The DAI scores of mice were significantly reduced in the four *Lactobacillus* strains included: *L. plantarum* 03 and 06, *L. brevis* 02 and *L. rhamnosus* 01 (Table 3). On the other side, the results indicated no significant difference in DAI scores once they were fed with other *Lactobacillus* isolates (Fig. 2).

Histological examination for all experimental and control groups was determined as previously described. The colon sections of the DSS mouse colitis model showed intense mucosal damage which included neutrophil infiltration, disturbance of epithelial layer and crypt structure compared with the control group.

Table 3. The effect of *Lactobacillus* species on disease activity index (DAI) and colon length

Mice group	DAI	Colon Length
Control	0.1 ± 0.04**	9.4 ± 1.1***
DSS	1.9 ± 0.2	6.9 ± 1.1
L. plantarum 01	2.4 ± 0.2	8.7 ± 0.6*
L. plantarum 02	1.8 ± 0.6	9.4 ± 0.8**
L. plantarum 03	0.1 ± 0.5**	9.2 ± 1.4**
L. plantarum 04	1.6 ± 0.3	7.9 ± 1.1
L. plantarum 05	1.2 ± 0.6	11.1 ± 0.5**
L. plantarum 06	0.4 ± 0.5**	9.4 ± 0.6**
L. brevis 01	1.4 ± 0.7	9 ± 0.3**
L. brevis 02	0.1 ± 0.6**	10.4 ± 0.6**
L. brevis 03	1.2 ± 0.5	9.3 ± 0.9**
L. rhamnosus 01	0.8 ± 0.9*	10.2 ± 0.8**
L. rhamnosus 02	1.4 ± 0.3	9.3 ± 0.7**
L. delbruecki	1.4 ± 0.4	10.1 ± 0.3**
L. reuteri	1.8 ± 0.6	8.8 ± 0.6*

The results analyzed with unpaired *t*-test. $P < 0.05$ (*), $P < 0.001$ (**).

The results exhibited that administration of *Lactobacillus* isolates had therapeutic effects such as lowering the inflammatory degree, reducing infiltration and intestinal mucosa damage, but no significant difference was observed compared to control group ($P < 0.05$).

DISCUSSION

The many etiological aspects of human intestinal diseases such as IBD remain unknown. The high rate of treatment failure suggests that there are no definitive therapeutic procedures for IBD [13]. Several studies [14, 15] on different probiotic bacteria have suggested that these bacteria can be used for the treatment of intestinal disorders. This study was conducted to examine a selected lactobacillus as a potential IBD treatment in the DSS-induced animal model.

In our study, the distinguished clinical symptoms of acute colitis in mice was induced with administered 5% DSS in the drinking water. Colon length is one of the colitis symptoms, indicating intestinal inflammation. Our results showed that all of the *Lactobacillus* species significantly prevented the shortening of the colon length compared with the DSS group. interestingly, the strains which had better probiotic effect during the our previous research showed more inhibitory effect on the colon length reduction of colitis mice. These results are in accordance with Cui et al. [16] who demonstrated that *L. plantarum* and *Lactobacillus fermentum* had significantly reduced shortening of the colon length. Chen et al. [17] have reported that *L. fermentum* had the ability to effectively alleviate the colon length.

DAI clinical symptoms including weight loss, stool consistency and occult blood are suitable criteria that could be used to determine the severity of the IBD disease. Increase or decrease scores of the symptoms is considered as the fail or success in the treatment process. In the present study, the DAI scores were significantly reduced in mice, treated with *L. plantarum* 03 and 06, *L. brevis* 02 and *L. rhamnosus* 01 and

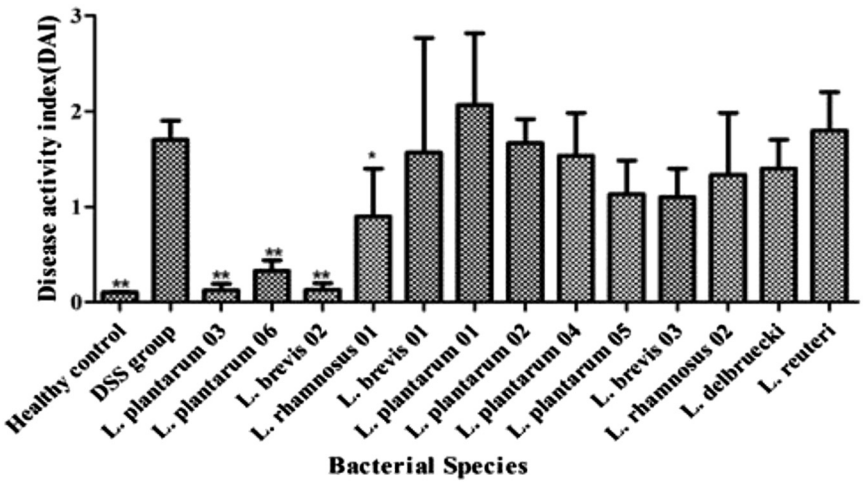

Figure 2. The effect of *Lactobacillus* species on disease activity index

significantly lower than the DSS-group. Other investigators such as Geier et al. [18] have demonstrated that mice with colitis treated with *L. fermentum* showed improved survival rate and DAI score. Toumi and colleagues [19] described that certain strains of *Lactobacillus* and *Bifidobacterium* significantly improved the DAI score in the DSS-induced colitis model. The results collectively suggested that the usage of the lactobacillus as a candidate for IBD is species-specific.

The histopathological assessment has an essential role in the diagnosis and follow-up treatment process of intestinal disorder. The current study showed that all of *Lactobacillus* species exhibited therapeutic effects, including reduced colonic damages and severity of the illness in DSS colitis mice. Many reports [20, 21] have revealed that probiotic bacteria, such as *Lactobacillus*, *Bifidobacterium* and other species could reduce the DAI and negative histological scores in colitis mice.

CONCLUSION

In short, our results described that *L. plantarum* and *L. brevis* showed more therapeutic effective against DSS-induced acute colitis mice with potential probiotic properties. Furthermore, the results suggested that the probiotic effect of these isolates was strain-specific and none of these useful bacteria could exhibit all of the valued probiotic properties simultaneously. The interactions between probiotic bacteria with colonic epithelium by using a combination of probiotic bacteria are the aim of the future study.

Conflicts of interest: There are no conflicts of interest.

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REFERENCES

1. Sheil B, Shanahan F, O'mahony L. Probiotic effects on inflammatory bowel disease. J Nutr. 2007; 137: 819–24.
2. Ye Y, Pang Z, Chen W, Ju S, Zhou C. The epidemiology and risk factors of inflammatory bowel disease. Int J Clin Exp Med. 2015; 8: 22529–42.
3. Bassaganya-Riera J, Viladomiu M, Pedragosa M, De Simone C, Hontecillas R. Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. PloS One 2012; 7: e34676.
4. Jacouton E, Chain F, Sokol H, Langella P, Bermudez-Humaran LG. Probiotic strain *Lactobacillus casei* Bl23 Prevents colitis-associated colorectal cancer. Front Immunol. 2017; 8: 1553.
5. Orel L, Kamhi TT. Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. World J Gastroenterol. 2014; 20: 11505–24.
6. Liu YW, Su YW, Ong WK, Cheng TH, Tsai YC. Oral administration of *Lactobacillus plantarum* K68 ameliorates DSS-induced ulcerative colitis in BALB/c mice via the anti-inflammatory and immunomodulatory activities. Int J Immunopharmacol. 2011; 11: 2159–66.
7. Ao X, Zhang X, Shi L, Zhao K, Yu J, Dong L, et al. Identification of lactic acid bacteria in traditional fermented yak milk and evaluation of their application in fermented milk products. J Dairy Sci. 2012; 95: 1073–84.
8. Bibalan MH, Eshaghi M, Rohani M, Esghaei M, Darban-Sarokhalil D, Pourshafie MR, et al. Isolates of *Lactobacillus plantarum* and *L. reuteri* display greater antiproliferative and antipathogenic activity than other Lactobacillus isolates. J Med Microbiol. 2017; 66: 1416–20.
9. Bibalan MH, Eshaghi M, Rohani M, Pourshafie MR, Talebi M. Determination of Bacteriocin Genes and Antibacterial Activity of Lactobacillus Strains Isolated from Fecal of Healthy Individuals. Int J Mol Cell Med. 2017; 6: 50–5.
10. Matsumoto S, Hara T, Hori T, Mitsuyama K, Nagaoka M, Tomiyasu N, et al. Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. Clin Exp Immunol. 2005; 140: 417–26.

11. Hamamoto N, Maemura K, Hirata I, Murano M, Sasaki S, Katsu K. Inhibition of dextran sulphate sodium (DSS)-induced colitis in mice by intracolonically administered antibodies against adhesion molecules (endothelial leucocyte adhesion molecule-1 (ELAM-1) or intercellular adhesion molecule-1 (ICAM-1)). Clin Exp Immunol. 1999; 117: 462–8.
12. Cooper HS, Murthy S, Shah R, Sedergran D. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 1993; 69: 238–49.
13. Pandurangan AK, Mohebali N, Esa NM, Looi CY, Ismail S, Saadatdoust Z. Gallic acid suppresses inflammation in dextran sodium sulfate-induced colitis in mice: Possible mechanisms. Int J Immunopharmacol. 2015; 28: 1034–43.
14. Macfarlane S, Furrie E, Cummings JH, Macfarlane GT. Chemotaxonomic analysis of bacterial populations colonizing the rectal mucosa in patients with ulcerative colitis. Clin Infect Dis. 2004; 38: 1690–9.
15. Kokešová A, Frolova L, Kverka M, Sokol D, Rossmann P, Bartova J, et al. Oral administration of probiotic bacteria (*E. coli* Nissle, *E. coli* O83, *Lactobacillus casei*) influences the severity of dextran sodium sulfate-induced colitis in BALB/c mice. Folia Microbiol. 2006; 51: 478–84.
16. Cui Y, Wei H, Lu F, Liu X, Liu D, Gu L, et al. Different effects of three selected Lactobacillus strains in dextran sulfate sodium-induced colitis in BALB/c mice. PloS One 2016; 11: e0148241.
17. Chen X, Zhao X, Wang H, Yang Z, Li J, Suo H. Prevent effects of *Lactobacillus fermentum* HY01 on dextran sulfate sodium-induced colitis in mice. Nutrients. 2017; 9: 545.
18. Geier MS, Butler RN, Giffard PM, Howarth GS. *Lactobacillus fermentum* BR11, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulfate sodium (DSS) in rats. Int J Food Microbiol. 2007; 114: 267–74.
19. Toumi R, Soufli I, Rafa H, Belkhelfa M, Biad A, Touil-Boukoffa C. Probiotic bacteria lactobacillus and bifidobacterium attenuate inflammation in dextran sulfate sodium-induced experimental colitis in mice. Int J Immunopathol Pharmacol. 2014; 27: 615–27.
20. Peran L, Sierra S, Comalada M, Lara-Villoslada F, Bailón E, Nieto A, et al. A comparative study of the preventative effects exerted by two probiotics, *Lactobacillus reuteri* and *Lactobacillus fermentum*, in the trinitrobenzenesulfonic acid model of rat colitis. Br J Nutr. 2007; 97: 96–103.
21. Hudcovic T, Štěpánková R, Kozakova H, Hrnčíř T, Tlaskalova-Hogenova H. Effects of monocolonization with *Escherichia coli* strains O6K13 and nissle 1917 on the development of experimentally induced acute and chronic intestinal inflammation in germ-free immunocompetent and immunodeficient mice. Folia Microbiol. 2007; 52: 618–26.