Journal of Functional Foods 47 (2018) 107–115

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Effects of lactobacilli with different regulatory behaviours on tight junctions in mice with dextran sodium sulphate-induced colitis

Gang Wang[a,b,c], Qi Xu[a,c], Xing Jin[a,c], Feng Hang[b,*], Zhenmin Liu[b], Jianxin Zhao[a,c,d,e,*], Hao Zhang[a,c,e,f], Wei Chen[a,c,f,g]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China
[b] State Key Laboratory of Dairy Biotechnology, Shanghai Engineering Research Center of Dairy Biotechnology, Dairy Research Institute, Bright Dairy & Food Co., Ltd., Shanghai 200436, PR China
[c] School of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China
[d] International Joint Research Laboratory for Probiotics, Jiangnan University, Wuxi 214122, PR China
[e] (Yangzhou) Institute of Food Biotechnology, Jiangnan University, Yangzhou 225004, PR China
[f] National Engineering Research Center for Functional Food, Jiangnan University, Wuxi 214122, PR China
[g] Beijing Innovation Centre of Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing 100048, PR China

ARTICLE INFO

Keywords:
Lactobacilli
Inflammatory bowel disease
Colitis
Intestinal barrier
Tight junction

ABSTRACT

In this study, six strains of *Lactobacillus* exhibiting different regulatory effects on the tight junction (TJ) proteins of mammalian cells *in vitro* were used in the treatment of mice with colitis induced by dextran sodium sulphate (DSS). Consistent with the results *in vitro*, different strains displayed different effects on repairment of TJs in the intestinal barrier, as well as alleviation on symptoms of colitis including disease activity index, tissue damage and inflammation. The lactobacilli showed significant inter- and intra-species differences in their ability to relieve the syndrome of colitis in mice, which were highly correlated with their effects on TJ protein recovery, although the impacts of bacteria on immune system and SCFAs should also be considered. Therefore, probiotics associated with TJ recovery are potential candidates for the treatment of colitis. The capacity for TJ recovery should be one of the important targets in the screening of probiotics for colitis treatment.

1. Introduction

Inflammatory bowel disease (IBD) is a common autoimmune disease of the digestive system with a high incidence in many countries (Burisch, Jess, Martinato, Lakatos, & EpiCom, 2013; Prideaux, Kamm, De Cruz, Chan, & Ng, 2012). Its most common forms are Crohn's disease and ulcerative colitis (Levesque et al., 2015; Molodecky et al., 2012; Sairenji, Collins, & Evans, 2017). A strong irritable inflammatory response occurs in the bowels of IBD patients, whose risk of colorectal cancer is six times greater than that of healthy individuals (Summers et al., 2003). In addition, IBD patients experience symptoms including weight loss, repeated abdominal pain, acute diarrhoea and bloody stools. Delayed treatment can lead to organ failure (Ashida, Ogawa, Kim, Mimuro, & Sasakawa, 2011; de Lange & Barrett, 2015). Although the pathogenesis of IBD is still unclear, it seems that changes in intestinal microbiota lead to immune system dysregulation in the bowel (Abraham & Cho, 2009). Researchers have developed many animal models for the study of IBD. Among the various models, mice with colitis induced by dextran sodium sulphate (DSS) may be the most widely used because of the model's simplicity and many similarities with ulcerative colitis in humans, although it is not believed to be an accurate mimic of human IBD (Chassaing, Aitken, Malleshappa, & Vijay-Kumar, 2014; Kiesler, Fuss, & Strober, 2015). The mechanism by which DSS induces colitis is believed to be via dissemination of intestinal contents because of damage to the intestinal barrier, which also occurs in patients with IBD (Eichele & Kharbanda, 2017).

The physical barrier comprising intestinal epithelial cells, tight junctions (TJs) and mucus is believed to be the key to maintaining the integrity of the intestinal wall. Once this physical barrier is damaged, bowel inflammation will occur and may lead to organ failure and bowel dysfunction syndrome (Turner, 2009; Watson, Duckworth, Guan, & Montrose, 2009). Although most of the physical barrier comprises intestinal epithelial cells, the TJs between these cells ensure the integrity of the intestinal barrier maintaining normal bowel function and overall health (Beisner, Stange, & Wehkamp, 2010). Existing studies show that the expression levels of TJ proteins in patients with IBD and in animal

* Corresponding authors at: State Key Laboratory of Dairy Biotechnology, Shanghai Engineering Research Center of Dairy Biotechnology, Dairy Research Institute, Bright Dairy & Food Co., Ltd., Shanghai 200436, PR China (F. Hang). State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China (J. Zhao).
E-mail addresses: hangfeng@brightdairy.com (F. Hang), zhaojianxin@jiangnan.edu.cn (J. Zhao).

https://doi.org/10.1016/j.jff.2018.05.044
Received 12 March 2018; Received in revised form 14 May 2018; Accepted 21 May 2018

models of IBD are abnormal. This leads to a loss of function of the intestinal barrier and susceptibility to invasion by harmful bacteria (Pithadia & Jain, 2011).

Traditional medicine mainly treats IBD by inhibiting inflammation-related signalling pathways and by clearance of free radicals and oxides (Silosi et al., 2014). However, long-term use of chemosynthetic drugs may create a greater burden on the body and more side effects. In recent years, probiotics have been suggested to be beneficial in the process of IBD interference and to have potential in the clinical treatment of IBD (Fujiya, Ueno, & Kohgo, 2014; Shadnoush et al., 2015). For example, VSL#3, a probiotic mixture including four strains of *Lactobacillus*, three strains of *Bifidobacterium* and *Streptococcus salivarius* subsp. *thermophiles*, has clinical potential in the treatment of IBD (Carnero-Gregorio, Molares-Vila, Corbalan-Rivas, Villaverde-Taboada, & Rodriguez-Cerdeira, 2018; Fedorak et al., 2015; Lee et al., 2012). Lactobacilli are regarded as important probiotics and have been shown to be beneficial to overall health in many ways, including IBD alleviation. However, although studies have revealed some of the signalling pathways involved in the protective effect of lactobacilli on intestinal epithelial cells, the specific mechanism by which lactobacilli regulate the intestinal barrier remains unclear. Furthermore, how lactobacilli relieve IBD through intestinal barrier repair is also not clear. Although it has been reported that some strains of probiotics can make efforts on the repairment of TJs in recent years (Alvarez, Badia, Bosch, Gimenez, & Baldoma, 2016; Blackwood et al., 2017; Cui et al., 2017; Jariwala, Mandal, & Bagchi, 2017; Orlando, Linsalata, Notarnicola, Tutino, & Russo, 2014), few studies have compared the differences among species of *Lactobacillus* in restoring TJs to relieve intestinal injury. In our previous study, a cellular model was used to judge the different effects of lactobacilli on TJs *in vitro* (Xu et al., 2016). To investigate the real effects of *Lactobacillus* strains on the intestinal barrier *in vivo*, mice models with DSS-induced colitis were treated with these bacteria. Six strains of *Lactobacillus* were found to have different alleviating effects on colitis. Mitigation of colitis was highly positively correlated with the ability of the bacteria to repair TJs.

2. Materials and methods

2.1. Lactobacillus strains and culture conditions

Six strains of *Lactobacillus* were used in this study. Their culture conditions, characteristic and source were as described in our previous study (Xu et al., 2016). Except LGG, all the strains were obtained from fermented foods and included in the Culture Collection of Food Microorganisms (CCFM) of Jiangnan University (Wuxi, China). The CCFM number for each strain are shown below: *L. casei* C1 (CCFM9), *L. fermentum* F1 (CCFM437), *L. plantarum* P1 (CCFM200), *L. plantarum* P2 (CCFM8610) and *L. plantarum* P3 (CCFM47).

2.2. Animal model

The mouse model with DSS-induced colitis treated with probiotics is described in our previous study (Xu et al., 2016). The experimental procedures are shown in Table 1. Briefly, 2.5% (w/v) DSS (molecular weight 36,000–50,000, MP Biomedicals, Aurora, OH, USA) was added to the drinking water of the mice to induce colitis; 27.8 mg/mL of mesalazine (INN; Ethypharm Pharmaceutical Co., Ltd., Shanghai, China) in vehicle (3% sucrose solution) was used as a positive control (0.2 mL/d). A dose of 1×10^9 CFU/mL of each strain of lactobacilli in vehicle was given to each mouse in the lactobacilli groups once a day by gavage. All experimental procedures (approval number: JN. No. 20160301–20160320[12]) were approved by the Animal Ethics Committee of Jiangnan University, China, and strictly followed the ethical guidelines of European Community Directive 2010/63/EU. On the 14th day, all of the mice were anaesthetised (100 mg/kg bw ketamine) and sacrificed.

2.3. Extraction of total RNA and quantitative polymerase chain reaction (qPCR)

Cellular model for analysis of tight junctions enhancement, total isolation of RNA from cultured cells and colonic tissue sample, qPCR were performed as described previously (Xu et al., 2016).

2.4. Disease activity index evaluation

After DSS was added to the drinking water, the body weights of the mice were recorded each day. In addition, a faecal occult blood tests kit (Baso Diagnostics Co., Ltd., Zhuhai, China) was used to test for occult blood or gross bleeding of the mice. The faeces of the mice were observed each day. If the blood in the faeces was visible to the naked eye, this character was recorded. Faecal character was defined according to three levels: 1, normal granular stool; 2, viscosity of stool increased but dispersed easily and did not adhere to the anus; 3, watery or unformed stool adhering to the anus (Murthy et al., 1993). Following Park et al. (2015) the disease activity index (DAI) was evaluated according to weight loss, stool consistency and occult blood or gross bleeding. Grading standards were designed according to the report by Wang et al. (2016).

2.5. Blood serum analysis

After the mice were sacrificed, blood samples were collected from their eyeballs. Serum for biochemical analysis was obtained by centrifugation (3000*g*, 10 min). The levels of serum inflammatory markers TNF-α, IL-1β, IL-2, IL-6, IL-10 and IL-17a were determined with Luminex® equipment using a MILLIPLEX® MAP Kit.

2.6. Tissue collection, colon length, immunofluorescence and histopathological analysis

After the mice were sacrificed, the entire colon was dissected (anus to the end of the caecum). The length of the colon was measured and recorded. The colonic contents were collected for analysis of short-

Table 1
Animal model experimental design.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	vehicle once a day (day1-day7)							water + vehicle once a day (day8-day14)						
DSS	vehicle once a day (day1-day7)							2.5% (w/v) DSS in water + vehicle once a day (day8-day14)						
DSS + INN	INN once a day (day1-day7)							2.5% (w/v) DSS in water + INN once a day (day8-day14)						
DSS + C1	CCFM9 once a day (day1-day7)							2.5% (w/v) DSS in water + CCFM9 once a day (day8-day14)						
DSS + F1	CCFM437 once a day (day1-day7)							2.5% (w/v) DSS in water + CCFM437 once a day (day8-day14)						
DSS + P1	CCFM200 once a day (day1-day7)							2.5% (w/v) DSS in water + CCFM200 once a day (day8-day14)						
DSS + P2	CCFM8610 once a day (day1-day7)							2.5% (w/v) DSS in water + CCFM8610 once a day (day8-day14)						
DSS + P3	CCFM47 once a day (day1-day7)							2.5% (w/v) DSS in water + CCFM47 once a day (day8-day14)						
DSS + LGG	LGG once a day (day1-day7)							2.5% (w/v) DSS in water + LGG once a day (day8-day14)						