Anti-inflammatory Activities of *Lactococcus lactis* subsp. *cremoris* FC in *In Vitro* and *In Vivo* Gut Inflammation Models

Yosuke NISHITANI1* and Masashi MIZUNO2

1 Organization of Advanced Science and Technology, Kobe University, 1–1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan
2 Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, 1–1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

Received for Publication, August 11, 2010

Many food factors such as probiotics are effective against human gastrointestinal disorders including inflammatory bowel disease. However, it remains unclear how probiotics act to protect against intestinal inflammation. Here, we describe a novel *in vitro* gut inflammation model for evaluating the anti-inflammatory activity of food factors, and *in vitro* and *in vivo* inflammation models for assessment of the gut anti-inflammatory activities of *Lactococcus lactis* subsp. *cremoris* FC (strain FC). A coculture system with intestinal epithelial Caco-2 cells and RAW264.7 macrophages can be used to assess the anti-inflammatory activity of food factors. Stimulation of RAW264.7 cells with lipopolysaccharide (LPS) increases tumor necrosis factor (TNF)-α production from RAW264.7 cells and interleukin (IL)-8 mRNA expression in Caco-2 cells and decreases the transepithelial electrical resistance of Caco-2 monolayers. The increases in TNF-α and IL-8 mRNA are suppressed by anti-TNF-α antibodies or budesonide. This indicates that this coculture model can imitate gut inflammation *in vivo*. Strain FC significantly downregulates IL-8 mRNA expression in Caco-2 cells and inhibits nuclear factor-κB nuclear translocation in RAW264.7 cells. A mouse model of dextran sulfate sodium (DSS)-induced colitis has been used to assess the anti-inflammatory activity of strain FC, which significantly ameliorates shortening of the colon and improves colon histology, especially in inflammatory cell infiltration, and proinflammatory cytokine and chemokine mRNA expression in inflamed tissue. These results indicate that oral administration of strain FC improves DSS-induced colitis through inhibition of inflammatory cell infiltration and that Caco-2/RAW264.7 cells stimulated with LPS can be used for screening anti-inflammatory factors and elucidating the mechanism of anti-inflammatory activity.

Key words: animal models; anti-inflammatory activity; colitis; dextran sulfate sodium; gastrointestinal inflammation; *Lactococcus lactis* subsp. *cremoris* FC

INTRODUCTION

The pathogenic mechanism of inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn’s disease, remains unknown but is assumed to be a dysregulation of the gut immune response to intestinal antigens such as intestinal microflora (6, 7, 20). In various studies, typical features of these diseases have shown that intestinal epithelial cells and macrophages secrete large amounts of chemokines and proinflammatory cytokines in the inflamed intestine of IBD patients (2, 13). It has been reported that modulation of the mucosal epithelial barrier plays a crucial role in the initiation and propagation of IBD. It seems that compromised intestinal barrier function is a specific feature of IBD (13). Interleukin (IL)-8 is a proinflammatory CXC chemokine that is associated with the promotion of neutrophil chemotaxis and degranulation (51). IL-8 protein is secreted excessively by a variety of cell types at the site of inflammation, such as intestinal epithelial cells, in IBD (10). *IL-8 causes excessive recruitment and transmigration of neutrophils into inflamed tissues following injury of the epithelium* (51). It has also been shown that lamina propria mononuclear cells (LPMCs) from patients with Crohn’s disease spontaneously secrete tumor necrosis factor (TNF)-α and induce epithelial barrier destruction (59). Although it is well-known that the secretion of inflammatory cytokines like IL-8 and TNF-α might be an important part of the immune response, the dysregulation of these cytokines is implicated in the pathogenesis of IBD (16, 36).

Probiotics have been used to treat a variety of human gastrointestinal disorders including IBD (19), pouchitis (17, 18) and irritable bowel syndrome (38), as well as antibiotic- and virus-associated diarrhea (4, 21, 32). Although little is known about their mechanisms of action, it seems that probiotics have protective, trophic
and anti-inflammatory effects on intestinal mucosa. Proposed mechanisms include the production of ammonia, hydrogen peroxide (1, 25, 42) and bacteriocins (14, 45), which inhibit the growth of pathogenic bacteria, the competition for adhesion sites on intestinal epithelia (27, 28) and adjuvant-like stimulation of the immune system against pathogenic organisms (30). However, the exact mechanisms by which probiotics act to prevent intestinal inflammation are not known.

To assess the anti-inflammatory properties of food factors against intestinal inflammation, animal intestinal inflammation models that are induced by administration of either 2,4,6-trinitrobenzene sulfonic acid (35) or dextran sodium sulfate (DSS) (43) and IL-7 transgenic mice (58), IL-2 knockout mice (49) and IL-10 knockout mice (24) have been used as IBD models (12). The intake of food factors such as resveratrol, curcumin, vitamin D and probiotics results in the arrest of weight loss, increase in stool consistency and improvement of mucosal appearance in these model mice (12). However, the anti-inflammatory activity of these factors is still poorly understood at the cellular level. To evaluate anti-inflammatory effects and mechanisms of the action exhibited by probiotics at the cellular level, in vitro models in a single culture of epithelial or immunocompetent cells with proper stimulants have been used (29, 33, 46, 61). It has been shown that treatment of various probiotic strains including Lactobacillus casei Shirota, Lactobacillus rhamnosus GG, Lactobacillus reuteri and VSL#3 (composed of Streptococcus thermophilus and several species of Lactobacillus and Bifidobacterium) results in inhibition of TNF-α-induced IL-8 production from epithelial cell lines (29, 61) or lipopolysaccharide (LPS)-induced proinflammatory cytokine production in these models (33, 46). However, epithelial cells that line the intestinal tract are considered to participate in the initiation and regulation of the mucosal immune response to bacteria by interacting with immunocompetent cells such as macrophages and lymphocytes (22). Therefore, a more precise in vitro assessment model of anti-inflammatory effects is required to elucidate this mechanism. As part of this requirement, we have developed a novel gut inflammatory model (54).

In this review, in addition to describing an in vitro gut inflammation model, in which an intestinal epithelial cell line, Caco-2, is placed in transwells at the apical side and a macrophage cell line, RAW264.7, is placed at the basolateral side, we also describe a mechanism of anti-inflammatory activity by which Lactococcus lactis subsp. cremoris FC (strain FC) contributes to amelioration of colitis.

**DEVELOPMENT OF AN IN VITRO GUT INFLAMMATION MODEL (54)**

An unusual immunological response of the intestinal immune cells to gut microflora and any food substances is an important factor in IBD in genetically susceptible hosts (25, 26). It has been reported that the inflamed tissue in IBD patients contains a large number of immune cells that secrete proinflammatory cytokines in excess (31). IL-8 is produced excessively by a variety of cells at the site of inflammation, such as intestinal epithelial cells in IBD (51). LPMCs from patients with Crohn’s disease spontaneously secrete TNF-α (59), and newly produced TNF-α induces epithelial barrier destruction (52), which results in reduction of transepithelial electrical resistance (TER) (15). These parameters (IL-8, TNF-α and TER) are characteristic of the pathology of IBD. As shown in Fig. 1, a coculture model that uses a combination of intestinal epithelial Caco-2 cells and RAW264.7 macrophages has been developed as an in vitro intestinal model. LPS is added to the basolateral compartment to imitate gut inflammation. The TER of Caco-2 cells shows no changes for 24 hr and then starts to decrease at 36 hr after stimulation of RAW264.7 cells with LPS (54). However, no drastic change is seen without LPS stimulation. This result suggests that the Caco-2 cell monolayer is damaged by LPS treatment in the coculture model. Similar results have been observed in other in vitro gut inflammation models (49) and in IBD patients (51). As shown in Fig. 2-1A, the mRNA expression level of IL-8 in the coculture system with LPS treatment maintains the basal level at 1 hr, then increases rapidly to approximately 5.5 times the initial level at 3 hr, remains steady to 6 hr and thereafter decreases to 3-fold at 12 hr. Satsu et al. (49) also have shown a high IL-8 mRNA expression level in intestinal epithelial cells in their in vitro gut inflammation model. In our study, IL-8 was mainly secreted into a basolateral compartment of the coculture system (Fig. 2-1B). TNF-α production in the

![Fig. 1. Coculture system constructed with Caco-2 cells and RAW264.7 cells. To imitate the gut inflammation, LPS was added to the basolateral compartment of this coculture system for various incubation times.](image-url)
c coculture model rapidly increased at 1 hr after LPS stimulation and thereafter remained at almost the same level during prolonged incubation (Fig. 2-2A). Mizuno et al. (34) also have reported the same finding of TNF-α production in RAW264.7 cells that are stimulated by purified polysaccharide from Agaricus brasiliensis.

Analysis of mRNA expression by RT-PCR has revealed that RAW264.7 cells mainly express TNF-α in the coculture system (Fig. 2-2B). The levels of IL-8 mRNA expression and TNF-α production peak at 3 hr after LPS stimulation; therefore, the incubation time was fixed at 3 hr after LPS stimulation in subsequent experiments.

It is well-known that LPS treatment of RAW264.7 cells enhances TNF-α production via Toll-like receptor 4 signaling (47). When the Caco-2/RAW264.7 coculture model is stimulated with 100 ng/ml LPS on the basolateral side, approximately 90 ng/ml of TNF-α is produced in the basolateral compartment (54) but not in the apical compartment. It has been reported that TNF-α induces IL-8 expression in almost all types of cells including intestinal epithelial cells (5), and as indicated in Fig. 2-1A, IL-8 mRNA expression is slower at 2 hr than TNF-α production. Therefore, it has been postulated that TNF-α secretion is necessary for upregulation of IL-8 mRNA expression in Caco-2 cells in this model. To prove this hypothesis, TNF-α antibody, which is used as for treatment of Crohn’s disease (3), has been applied simultaneously with LPS into the basolateral compartment in the Caco-2/RAW264.7 coculture system. As expected, IL-8 mRNA expression is completely suppressed by TNF-α antibody treatment (Fig. 3A). Moreover, TNF-α production is not detected (Fig. 3B). Budesonide is also employed as an oral drug for Crohn’s disease (9), and its effect is due to inhibition of the function of nuclear factor (NF)-κB and, consequently, the transcription of proinflammatory genes such as TNF-α (11).

To establish whether this coculture system is sufficiently accurate as a gut inflammation model, the
effect of budesonide on TNF-α production and IL-8 mRNA expression has been tested in the Caco-2/RAW264.7 coculture system. Addition of budesonide (1 μM) into the apical compartment significantly downregulates expression of IL-8 mRNA (Fig. 3A) and reduces TNF-α production, compared with the LPS-stimulated controls (Fig. 3B). Pahl et al. (44) also have reported the inhibitory effect of budesonide on TNF-α production from monocytes. Moreover, direct simultaneous application of budesonide and LPS to RAW264.7 cells inhibits mRNA expression and production of TNF-α. Thus, it is thought that the downregulating effect of budesonide on IL-8 mRNA expression in Caco-2 cells is due to suppression of TNF-α production from RAW264.7 cells stimulated with LPS. This indicates that the inhibitory effect of budesonide on the Caco-2/RAW264.7 coculture system stimulated with LPS shows a similar anti-inflammatory effect in Crohn’s disease patients. Together with the results in Figs. 2–3, we suggest that gut inflammation like IBD can be imitated by a coculture system that comprises Caco-2 and RAW264.7 cells with LPS stimulation.

**ANTI-INFLAMMATORY EFFECT OF STRAIN FC ON IL-8 mRNA EXPRESSION IN THE GUT INFLAMMATION MODEL OF CACO-2 CELLS AND LPS-ACTIVATED RAW264.7 CELLS (40)**

Satsu et al. (49) have reported that activated macrophages induce disruption of the intestinal epithelial monolayer in an in vitro intestine model. However, there have been few studies on the establishment of in vitro gut inflammation models to search for anti-inflammatory agents such as drugs and food substances. To test whether this Caco-2/RAW264.7 gut inflammation model is applicable for searching for anti-inflammatory factors in foods, the anti-inflammatory effects of strain FC have been assessed. Strain FC was isolated from Caspian Sea yogurt and shown to have probiotic activities such as improving defecation frequency and fecal microflora in healthy elderly volunteers (55). UV-inactivated strain FC (1×10⁹ CFU/ml) was added to the apical compartment of the Caco-2/RAW264.7 coculture system for 3 hr, LPS was then added to the basolateral side at a final concentration of 500 pg/ml and the cells were incubated for an additional 3 hr. As shown in Fig. 4A, treatment with strain FC resulted in downregulation of IL-8 mRNA expression in Caco-2 cells. Similar results have been described in other studies, which indicates that direct treatment of probiotic bacterial strains, including L. rhamnosus GG and L. reuteri, blunt TNF-α-induced IL-8 production in human epithelial cell lines through reduction of the nuclear translocation of NF-κB and inhibition of IκB degradation (29, 61). In addition, soluble factors produced by VSL#3 inhibit the chymotrypsin-like activity of the proteasome in murine gut epithelial cells (46). A member of the CXC chemokine family, IL-8, is secreted excessively by a variety of cells at the site of inflammation, such as intestinal epithelial cells, in human IBD (10). IL-8 causes excessive recruitment and transmigration of neutrophils.
ANTI-INFLAMMATORY ACTIVITY OF Lactococcus lactis subsp. cremoris FC

Therefore, oral administration of strain FC could have preventive or curative activity in human IBD. As shown in Fig. 4B, strain FC decreases TNF-α production from RAW264.7 cells compared with control cells, although not significantly. Moreover, immunostaining of NF-κB p65 in RAW264.7 cells has revealed that treatment with strain FC results in significant inhibition of NF-κB nuclear translocation in RAW264.7 cells stimulated by 5 ng/ml LPS (Fig. 5). These results suggest that anti-inflammatory molecules are produced by intestinal epithelial cells in response to direct stimulation with strain FC. Zeuthen et al. have reported that thymic stromal lymphopoietin (TSLP) and transforming growth factor (TGF)-β production from Caco-2 cells increases upon microbial stimulation in a strain-dependent manner and that TSLP and TGF-β cooperate in inducing the tolerogenic dendritic cell phenotype (60). Further studies are required to elucidate the mechanism of the crosstalk between epithelial and immune cells. We consider that the oral administration of strain FC might exert anti-inflammatory activity in the intestine through inhibition of excessive IL-8 secretion by suppressing the production of TNF-α from intestinal immune cells.
ANTI-INFLAMMATORY EFFECT OF STRAIN FC ON DSS-INDUCED COLITIS IN MICE (40)

To evaluate anti-inflammatory activity of strain FC in vivo, a DSS-induced colitis mouse model has been used. DSS was mixed with drinking water at a concentration of 3% and administered ad libitum for 5 days. During the experimental period, no significant differences in body weight loss between strain FC-treated and untreated mice were observed. However, a significant improvement in colon shortening was observed in strain FC-treated mice (Fig. 6A). In addition, the histological scores for inflammation severity, thickness of inflammatory cell infiltration and extent of inflammatory lesions were significantly lower in strain FC-treated mice compared with untreated mice (Fig. 6B) (40). It has been hypothesized that neutrophil-mediated inflammation is an important factor in the development of DSS-induced colonic mucosal injury (8, 37, 53). Recent studies have presented the following evidence to support this hypothesis. First, enhanced expression of colonic mucosal endothelial adhesion molecule 1 (ICAM-1) is observed at an early stage in the inflammatory process of DSS-induced colitis (8). Second, selective depletion of neutrophils using specific monoclonal antibody improves colitis in rats (37). Third, colonic mucosal injury and neutrophil accumulation in rats are significantly attenuated by immunoneutralization of ICAM-1 on endothelial cells (53). From this point of view, we consider that the inhibition of inflammatory cell accumulation by strain FC might be one of the protective factors that help to decrease DSS-induced colonic mucosal injury. This hypothesis is supported by previous studies that have demonstrated that probiotic organisms, including L. plantarum, L. brevis and L. reuteri, significantly inhibit increased myeloperoxidase activity, an index of tissue-associated neutrophil accumulation, in a mouse model of DSS-induced colitis (26, 57).

It is thought that DSS is a physical agent with an inherent property to disrupt the epithelial barrier, which causes normal intestinal microfloral substances to activate mucosal macrophages, which in turn produce proinflammatory cytokines (23). The macrophage-induced inflammation and tissue damage is accompanied by a cellular cytotoxic-mediated inflammatory response along with the progression of colitis (50). To investigate the reason why DSS-induced colitis is less severe in strain FC-treated mice, semi-quantitative RT-PCR analysis of several cytokines and inducible nitric oxide synthase (iNOS) has been performed (Fig. 7). In DSS-treated mice, the mRNA expression of interferon (IFN)-γ and macrophage-derived proinflammatory cytokines such as TNF-α, IL-6 and iNOS is dramatically induced, but IL-12 p40 mRNA is only weakly induced (Fig. 7A–D) (40). In contrast, Th2-type cytokine IL-4 mRNA expression is slightly decreased by DSS treatment (40). As shown in Fig. 7A and 7D, strain FC attenuates the aberrant mRNA expression induced by DSS treatment near the control.

![Figure 6](image-url)

Fig. 6. Strain FC improved the macroscopic and microscopic appearance of the colon in DSS-colitis mice. (A) The colon lengths of wild-type (WT) and DSS-colitis mice with or without administration of strain FC (1×10⁸ CFU/day). Values represent the means ± SE (n = 3 mice for the untreated WT group, n = 2 mice for the strain FC administration WT group and n = 6 mice for the DSS-treated groups). **P<0.01. (B) Histological scores of the colon sections of DSS-colitis mice with or without administration of strain FC. Values represent the means ± SE (n = 6). *P<0.05.
level through significant suppression of TNF-α and IFN-γ mRNA expression. Moreover, the mRNA expression of IL-6, IL-12 p40 and iNOS tends to decrease in strain FC-treated mice compared with untreated mice, although the difference is not significant (Fig. 7B and C). The blockade of chemokine receptors or inhibition of chemokine production prevents inflammatory cell infiltration into the intestinal mucosa in murine experimental colitis (39, 57). It is well known that mice possess functional homologs such as macrophage inflammatory protein (MIP)-2 in substitution for human IL-8. Therefore, to investigate whether strain FC inhibits chemokine production, the mRNA expression of MIP-2 in colon tissues has been analyzed using real-time PCR. The mRNA expression of CXC-chemokine MIP-2 is markedly induced in DSS-treated mice (40). The excessive mRNA expression of MIP-2 is decreased by administration of strain FC, although not significantly (40). Moreover, a tendency towards increased mRNA expression for anti-inflammatory cytokine IL-10 has been found in strain FC-treated mice compared with untreated mice (40). These results suggest that strain FC induces a suppressive effect on in vivo colitis via modulation of mRNA expression for proinflammatory cytokines in the gut.

**CONCLUSION**

In conclusion, an in vitro gut inflammation model using Caco-2 cells and LPS-stimulated RAW264.7 cells has been established, and this coculture model can imitate gut inflammation, as seen in IBD. With this model, apical treatment of strain FC downregulates IL-8 mRNA expression in Caco-2 cells through inhibition of TNF-α secretion from LPS-stimulated RAW264.7 cells. In an in vivo model of DSS-induced colitis, strain FC significantly inhibits colonic injury, especially in inflammatory cell infiltration. In addition, we have shown that expression of macrophage-derived proinflammatory cytokines, such as TNF-α, IL-6 and iNOS mRNA, is enhanced in DSS-induced intestinal inflammation and that these increases are reversed by strain FC. The above results indicate that a gut inflammation model with Caco-2/RAW264.7 cells stimulated with LPS is applicable for screening of anti-inflammatory factors and elucidating the mechanism of anti-inflammatory activity against intestinal inflammation.
ACKNOWLEDGMENTS

We are grateful to the Laboratory of Glyco-chain Biochemistry, Division of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Japan, for its cooperation.

REFERENCES

(1) Annuk H, Shchepepetova J, Kullisaar T, Songisepp E, Zilmer M, Mikelsaar M. 2003. Characterization of intestinal lactobacilli as putative probiotic candidates. J Appl Microbiol 94: 403–412.
(2) Arai F, Takahashi T, Furukawa K, Matsushima K, Asakura H. 1999. Mucosal expression of interleukin-6 and interleukin-8 messenger RNA in ulcerative colitis and Crohn’s disease. Dig Dis Sci 43: 2071–2079.
(3) Ardizzone S, Pooro GB. 2005. Biologic therapy for inflammatory bowel disease. Drugs 65: 2253–2286.
(4) Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, Isolauri E. 1999. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 104: e64.
(5) Baggioolini M, Loetscher P, Moser B. 1995. Interleukin-8 and the chemokine family. Int J Immunopharmacol 17: 103–108.
(6) Benno P, Leijonmarck CE, Monsen U, Uribe A. 1993. Functional alteration of the microflora in patients with ulcerative colitis, Scand J Gastroenterol 28: 839–844.
(7) Berrebi D, Languelin J, Ferkadji L, Foussat A, De Lagausie P, Paris R, Emilie D, Mougenot JF, Cezard JP, Navarro J, Peuchmair M. 2003. Chemokine receptors, and homing molecule distribution in the rectum and stomach of pediatric patients with ulcerative colitis, J Pediatr Gastroenterol Nutr 37: 300–308.
(8) Breider MA, Eppinger M, Gough A. 1997. Intercellular adhesion molecule-1 expression in dextran sodium sulfate-induced colitis in rats. Vet Pathol 34: 598–604.
(9) Chan EP, Lichtenstein GR. 2006. Chemoprevention: risk reduction with medical therapy of inflammatory bowel disease. Gastroenterol Clin North Am 35: 675–712.
(10) Chin AC, Parkos CA. 2006. Neutrophil transepithelial migration and epithelial barrier function in IBD: potential targets for inhibiting neutrophil trafficking. Ann NY Acad Sci 1072: 276–287.
(11) Clark AR. 2007. Anti-inflammatory functions of glucocorticoid-induced genes. Mol Cell Endocrinol 275: 79–97.
(12) Clarke JO, Mullin GE. 2008. A review of complementary and alternative approaches to immunomodulation. Nutr Clin Pract 23: 49–62.
(13) Clayburgh DR, Shen L, Turner JR. 2004. A porous defense: the leaky epithelial barrier in intestinal disease. Lab Invest 84: 282–291.
(14) Cleveland J, Montville TJ, Nes IF, Chikindas ML. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol 71: 1–20.
(15) Dongmei Y, Iris M, Thomas YM. 2006. Molecular mechanism of tumor necrosis factor-a modulation of intestinal epithelial tight junction barrier. Am J Physiol Gastrointest Liver Physiol 290: 496–504.
(16) Gerard C, Rollins BJ. 2001. Chemokines and disease. Nat Immunol 2: 108–115.
(17) Gionchetti P, Rizzello F, Helwig U, Ventura A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. 2003. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterol 124: 1202–1209.
(18) Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. 2000. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterol 119: 305–309.
(19) Gionchetti P, Rizzello F, Venturi A, Campieri M. 2000. Probiotics in infective diarrhea and inflammatory bowel disease. J Gastroenterol Hepatol 15: 489–493.
(20) Gorbach SL, Nahas L. 1968. Studies of intestinal microflora. V. Fecal microbial ecology in ulcerative colitis and regional enteritis, relationship to severity of disease and chemotherapy, Gastroenterol 54: 575–587.
(21) Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. 1991. A human Lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 88: 90–97.
(22) Kagnoff MF, Eckmann L. 1997. Epithelial cells as sensors for microbial infection. J Clin Invest 100: 6–10.
(23) Kitajima S, Takuma S, Morimoto M. 1999. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. Exp Anim 48: 137–143.
(24) Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75: 263–274.
(25) Kullisaar T, Zilmer M, Mikelsaar M, Vihalem T, Annuk H, Kairane C, Kilik A. 2002. Two antioxidative lactobacilli strains as promising probiotics. Int J Food Microbiol 72: 215–224.
(26) Lee HS, Han SY, Bae EA, Huh CS, Ahn YT, Lee JH, Kim DH. 2008. Lactic acid bacteria inhibit proinflammatory cytokine expression and bacterial glycosaminoglycan degradation activity in dextran sulfate sodium-induced colitic mice. Int Immunopharmacol 8: 574–580.
(27) Lee YK, Lim CY, Teng WL, Ouwehand AC, Tuomola EM, Salminen S. 2000. Quantitative approach in the
study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. Appl Environ Microbiol 66: 3692–3697.

(28) Lee YK, Puong KY, Ouwehand AC, Salminen S. 2003. Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli. J Med Microbiol 52: 925–930.

(29) Ma D, Forsythe P, Bienenstock J. 2004. Live Lactobacillus reuteri is essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8 expression. Infect Immun 72: 5308–5314.

(30) Maassen CB, van Holten-Neelen C, Balk F, Boersma WJ, Claassen E. 2000. Strain-dependent induction of cytokine profiles in the gut by orally administered Lactobacillus strains. Vaccine 18: 2613–2623.

(31) MacDermott RP. 1999. Chemokines in the inflammatory bowel diseases. J Clin Immunol 19: 266–272.

(32) Majamaa H, Isolauri E, Saxelin M, Vesikari T. 1995. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 20: 333–338.

(33) Matsumoto S, Hara T, Hori T, Mitsuyama K, Matsumoto M, Tomiyasu N, Suzuki A, Sata M. 2005. 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 140: 417–426.

(34) Mizuno M, Kawakami S, Sakamoto Y, Fujitake N. 2003. Macrophages stimulated by polysaccharide purified from Agaricus brasiliensis S. Wasser et al. (Agaricomycetideae) enhance mRNA expression of Th1 cytokine including IL-12 and 18. Int J Med Mycos 5: 383–389.

(35) Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. 1989. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterol 96: 795–803.

(36) Murch SH, Braegger CP, Walker-Smith JA, MacDonald TT. 1993. Location of tumour necrosis factor α by immunohistochemistry in chronic inflammatory bowel disease. Gut 34: 1705–1709.

(37) Natsui M, Kawasaki K, Takizawa H, Hayashi SI, Matsuda Y, Sugimura K, Seki K, Narisawa R, Sendo F, Asakura H. 1997. Selective depletion of neutrophils by a monoclonal antibody, RP-3, suppresses dextran sulphate sodium-induced colitis in rats. J Gastroenterol Hepatol 12: 801–808.

(38) Niedzielin K, Kordecki H, Birkenfeld B. 2001. A controlled, double-blind, randomized study on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome. Eur J Gastroenterol Hepatol 13: 1143–1147.

(39) Nishihara T, Matsuda M, Araki H, Oshima K, Kihara S, Funahashi T, Shimomura I. 2006. Effect of adiponectin on murine colitis induced by dextran sulfate sodium. Gastroenterol 131: 853–861.

(40) Nishitani Y, Tanoue T, Yamada K, Ishida T, Yoshida M, Azuma T, Mizuno M. 2009. Lactococcus lactis subsp. cremoris FC alleviates symptoms of colitis induced by dextran sulfate sodium in mice. Int Immunopharmacol 9: 1444–1451.

(41) Noursadeghi M, Tsang J, Haustein T, Miller RF, Chain BM, Katz DR. 2008. Quantitative imaging assay for NF-kappaB nuclear translocation in primary human macrophages. J Immunol Methods 329: 194–200.

(42) Ocana VS, Pesce de Ruiz Holgado AA, Nader-Macias ME. 1999. Selection of vaginal H2O2-generating Lactobacillus species for probiotic use. Curr Microbiol 38: 279–284.

(43) Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. 1990. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis. Gut 98: 694–702.

(44) Pahl A, Bauhofer A, Peltzold U, Cnota PJ, Maus J, Brune K, Szenenyi S. 2006. Synergistic effects of the anti-cholinergic R,R-glycopyrrolate with anti-inflammatory drugs. Biochem Pharmacol 72: 1690–1696.

(45) Paraje MG, Albesa I, Eraso AJ. 2000. Conservation in probiotic preparations of Lactobacillus with inhibitory capacity on other species. New Microbiol 23: 423–431.

(46) Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, De Simone C, Chang EB. 2004. Probiotics inhibit nuclear factor-kappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterol 127: 1474–1487.

(47) Royle MC, Tötemeyer S, Alltridge LC, Maskell DJ, Bryant CE. 2003. Stimulation of Toll-like receptor 4 by lipopolysaccharide during cellular invasion by live Salmonella typhimurium is a critical but not exclusive event leading to macrophage responses. J Immunol 170: 5445–5454.

(48) Sadlack B, Merz H, Schorle H, Simimpl A, Feller AC, Horak I. 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell 75: 253–261.

(49) Satsu H, Ishimoto Y, Nakano T, Mochizuki T, Iwana T, Shimizu M. 2006. Induction by activated macrophage-like THP-1 cells of apoptotic and necrotic cell death in intestinal epithelial Caco-2 monolayers via tumor necrosis factor-alpha. Exp Cell Res 312: 3909–3919.

(50) Strober W, Fuss IJ, Blumberg RS. 2002. The immunology of mucosal models of inflammation. Annu Rev Immunol 20: 495–549.

(51) Struyf S, Gouwy M, Dillen C, Proost P, Opdenakker G, Damme JV. 2005. Chemokines synergize in the
recruitment of circulating neutrophils into inflamed tissue. Eur J Immunol 35: 1583–1591.

(52) Suenaert P, Bulteel V, Lemmens L, Noman M, Geypens B, Assche GV, Geboes K, Ceuppens JL, Rutgeerts P. 2002. Anti-tumor necrosis factor treatment restores the gut barrier in Crohn’s disease. Am J Gastroenterol 97: 2000–2004.

(53) Taniguchi T, Tsukada H, Nakamura H, Kodama M, Fukuda K, Saito T, Miyasaka M, Seino Y. 1998. Effects of the anti-ICAM-1 monoclonal antibody on dextran sodium sulphate-induced colitis in rats. J Gastroenterol Hepatol 13: 945–949.

(54) Tanoue T, Nishitani Y, Kanazawa K, Hashimoto T, Mizuno M. 2008. In vitro model to estimate gut inflammation using co-cultured Caco-2 and RAW264.7 cells. Biochem Biophys Res Commun 374: 565–569.

(55) Toda T, Kosaka H, Terai M, Mori H, Benno Y, Yamori Y. 2005. Effects of fermented milk with Lactococcus lactis subsp. cremoris FC on defecation frequency and fecal microflora in healthy elderly volunteers. J Jpn Soc Food Sci Technol 52: 243–250.

(56) Tokuyama H, Ueha S, Kurachi M, Matsushima K, Moriyasu F, Blumberg RS, Kakimi K. 2005. The simultaneous blockade of chemokine receptors CCR2, CCR5 and CXCR3 by a non-peptide chemokine receptor antagonist protects mice from dextran sodium sulfate-mediated colitis. Int Immunol 17: 1023–1034.

(57) van der Kleij H, O’Mahony C, Shanahan F, O’Mahony L, Bienenstock J. 2008. Protective effects of Lactobacillus reuteri and Bifidobacterium infantis in murine models for colitis do not involve the vagus nerve. Am J Physiol Regul Integr Comp Physiol 295: R1131–1137.

(58) Watanabe M, Ueno Y, Yajima T, Okamoto S, Hayashi T, Yamazaki M, Iwao Y, Ishii H, Habu S, Uehira M, Nishimoto H, Ishikawa H, Hata J, Hibi T. 1998. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. J Exp Med 187: 389–402.

(59) Zareie M, Singh PK, Irvine EJ, Sherman PM, McKay DM, Perdue MH. 2001. Monocyte/macrophage activation by normal bacteria and bacterial products: implications for altered epithelial function in Crohn’s disease. Am J Pathol 158: 1101–1109.

(60) Zeuthen LH, Fink LN, Frokiaer H. 2008. Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. Immunology 123: 197–208.

(61) Zhang L, Li N, Caicedo R, Neu J. 2005. Alive and dead Lactobacillus rhamnosus GG decrease tumor necrosis factor-alpha-induced interleukin-8 production in Caco-2 cells. J Nutr 135: 1752–1756.