Lactic Acid Bacteria Improves Peyer’s Patch Cell-Mediated Immunoglobulin A and Tight-Junction Expression in a Destructed Gut Microbial Environment

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Introduction

The intestinal mucosa is the first line of host defense. Orally administered antigens interact with the gut-associated lymphoid tissue, which is a well-developed immune network that is involved not only in protection of the host from pathogens but also in preventing unusual reactions to ingested proteins [50]. This immune response is mainly a humoral immune response mediated by immunoglobulin A (IgA)-producing cells and results in the secretion of IgA, which constitutes almost 80% of all the antibodies produced in mucosal-associated tissue [19, 26]. This antibody inhibits microbial adherence and also prevents the absorption of antigens into mucosal surfaces. Antigen uptake occurs...
through a specialized system that includes the membranous epithelial (M) cells overlying lymphoid follicles of Peyer’s patches, which is the major site of the intestinal immune response involving macrophages, dendritic cells, and T and B lymphocytes [16, 17, 35, 45, 54]. Secretory IgA (SIgA) plays a critical role as an immunological barrier in the intestine. It is the predominant intestinal immunoglobulin and acts as the first line of defense for the intestinal mucosa. SIgA adheres mainly to bacteria or viruses on the intestinal epithelial surface. It guarantees both immune exclusion and neutralization of translocated bacteria, thus preserving the integrity of the intestinal barrier by preventing bacterial-induced inflammation. The anti-inflammatory properties of SIgA have been well characterized [7, 9, 20]. SIgA secreted into the intestinal mucus is an important regulatory factor for maintaining the intestinal barrier [36].

The tight-junction complex, which consists of ZO-1 and occludin proteins, creates a regulated paracellular barrier to the movement of water, solutes, and immune cells across both epithelial and endothelial cell layers. It has been previously demonstrated that alveolar epithelial cells express tight-junction proteins. The tight-junction proteins ZO-1 and ZO-2 are bound to the surface of the cytoplasmic membrane, but damage to the endothelium or epithelium causes dysfunction of the tight-junction proteins and disruption of pulmonary barriers, leading to edema and atopic disease during acute lung injury [6, 51].

Lactic acid bacteria (LAB) are the most commonly used probiotics, and are defined as live microbial food supplements that can beneficially affect the host by improving the intestinal microbial balance [12]. It is well known that LAB can also improve the intestinal immune system, and the immunological properties of probiotics have been extensively studied. Certain strains belonging to some species of *Lactobacillus*, such as *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum*, enhance both systemic and mucosal immunity [34] by efficiently adhering to the gut mucosa and stimulating the phagocytic cells [39].

Among the food microorganisms, bifidobacteria also have potent immune potentiating activity. In particular, *Bifidobacterium adolescentis*, derived from human intestinal microflora, has the strongest mitogenic activity on splenocytes and Peyer’s patch cells [15].

As previously reported, kanamycin decreases the intestinal microbial population and their diversity, and oral administration of kanamycin resulted in a decrease in sennoside-hydrolyzing bacteria that are already present in the intestine [25]. The administration of kanamycin also increased the levels of total IgG1 and IgE, while decreasing the level of IgG2a and IgA in the serum. Because the production of IgE and IgG1 has been demonstrated to be dependent on the Th2 cells, whereas the production of IgG2a and IgA requires the participation of Th1 cells, these results suggest that such elimination of microflora by kanamycin treatment may prevent the Th1-mediated immune response and drive a shift in the Th1/Th2 balance toward Th2 dominant immunity [44]. Therefore, the present study used kanamycin to induce destruction of the gut microbial environment in mice.

The aim of this work was to analyze the effects of oral administration of LAB on Peyer’s patch cell-mediated IgA response and the integrity of the tight junction in the gut after the microbial environment has been disturbed by a high dose of the antibiotic kanamycin.

### Materials and Methods

#### Reagents

The human Caco-2 cell line was obtained from the KTCC (Korea Types Culture Collection, Korea). DMEM, RPMI1640 media, and fetal bovine serum (FBS) were from Gibco (CA, USA). Lipopolysaccharide (LPS) and kanamycin were from Sigma (MO, USA).

#### Cell Culture

The Caco-2 cells were maintained in RPMI1640 supplemented with 10% FBS and 2 mM L-glutamine at 37°C in a humidified atmosphere of 5% CO₂. Cells were reseeded in 6-well plates (for real-time PCR) at a density of 2 × 10⁶ cells/ml. Cells were co-treated with LPS (1 µg/ml) and probiotics over a time course of 24 h.

#### Isolation of Lactic Acid Bacteria and Preparation of the Dead Microbial Cells

LAB were isolated from the fecal matter of a 7-day-old infant (vaginal delivery, breast-fed) by enrichment culture using transgalactosylated oligosaccharide (TOS)-propionate agar base medium (or MRS medium) (Yakult Pharmaceutical Industry Co., Ltd., Japan) [46]. The isolate was subcultured on TOS-propionate agar (or MRS agar) [23] and was routinely cultured on BL broth (BBL) at 37°C under anaerobic conditions using the GasPak EZ Anaerobe Pouch System (BD Biosciences, CA, USA) (or MRS broth at 37°C under aerobic conditions). The biological properties of the LAB were characterized using various biochemical and physiological tests including the F6PPK test [31] and API kit (bioMerieux, France), and identification was confirmed using 16S rRNA gene sequencing.

#### Preparation of Dead Lactic Acid Bacteria Cells

In brief, after LAB were grown anaerobically in BL medium (or...
MRS medium) at 37°C for 24 h, the cells were collected by centrifugation and washed twice with phosphate-buffered saline (PBS). The cells were counted and suspended in PBS at 10^7 CFU/ml, followed by boiling at 100°C. Dead LAB cells were stored at −20°C until use.

**Peyer’s Patch Cell Isolation**

Briefly, five to six mice were sacrificed by cervical dislocation and Peyer’s patches were removed and transferred to a beaker containing cold PBS. Then, the Peyer’s patches were stirred in 20 ml of RPMI1640 supplemented with 300 mg of dispase (Grade II; Boehringer-Mannheim, Germany) and 1 g of DNase (Sigma) for 30 min at 37°C. The medium containing dissociated cells was collected by aspiration. This procedure was repeated four times. Finally, harvested cells were washed twice with HBSS (Sigma) and suspended in complete medium (RPMI1640 with 10% FBS).

**Animals, Diets, and Experimental Design**

Male ICR mice aged 6 weeks were purchased from the Jackson Laboratories (ME, USA). All mice were individually housed at a constant temperature and humidity (22 ± 1°C, 55 ± 10% humidity) with a 12 h light/dark cycle. All mice were fed AIN-76A diets. The probiotics for the LAB (E20, L84, M49, HY8301) groups were obtained from the orbital plexus at mouse sacrifice, and the plasma was kept at −80°C until antibody levels were measured. The spleen and Peyer’s patch cells were also removed for further analysis, as described below. All procedures were approved by the Animal Ethics Committee of Korea Yakult Company Limited R&D Center (KYIACUC-2014-00022-Y).

**Table 1. Composition of AIN-76A diet.**

<table>
<thead>
<tr>
<th>Diet ingredient</th>
<th>AIN-76A diet (g/kg)</th>
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<tbody>
<tr>
<td>Casein</td>
<td>200</td>
</tr>
<tr>
<td>Corn starch</td>
<td>150</td>
</tr>
<tr>
<td>D,L-Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Fiber (cellulose)</td>
<td>50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>250</td>
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<tr>
<td>Dextrose</td>
<td>250</td>
</tr>
<tr>
<td>AIN mineral mix 76A</td>
<td>35</td>
</tr>
<tr>
<td>AIN vitamin mix 76A</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
</tbody>
</table>

**Total Cell Count in Peyer’s Patch**

Briefly, Peyer’s patch total cells collected from 10 mice in the normal, kanamycin-treated group and seven mice in the LAB (E20, L84, M49, HY8301) groups were pooled, respectively. The Peyer’s patches were homogenized in RPMI1640 medium containing 10% defined FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cell suspensions were then washed three times in this medium and counted using the cell counter equipment (Bio-Rad, CA, USA).

**IgA ELISA Assay**

The IgA enhancement effects of LAB on Peyer’s patch cells were determined using a mouse ELISA assay kit (Bethyl, TX, USA). In brief, isolated Peyer’s patch cells (5 × 10^7 cells/ml) in a 96-well plate were treated with LPS (1 µg/ml) and LAB (E20, L84, M49, HY8301) for 24 h. For IgA measurement, the mouse IgA assay kit was used following the manufacturer’s instructions.

**RNA Preparation and Real-Time Polymerase Chain Reaction (RT-PCR)**

The total cellular RNA or total RNA, which was extracted from the ileum (100 mg) tissues, was isolated using the Qiagen RNA prep kit (Qiagen, CA, USA), according to the manufacturer’s instructions. From each sample, 2 µg of RNA was reverse-transcribed using MuLV reverse transcriptase, 1 mM dNTP, and 0.5 µg/µl oligo d(T) (12–18 dT). RT-PCR analysis was performed on aliquots of the cDNA preparations to detect occludin, ZO-1, IL-8, IL-1β, and TNF-α (using GAPDH as an internal standard) according to the manufacturer’s protocol under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The PCR primers used in this study were purchased from Applied Biosystems Inc. (MA, USA). After amplification, data were analyzed using the 7500 RT-PCR software (Applied Biosystems Inc., CA, USA).

**Blood Analysis**

Blood samples were centrifuged (1,000 × g for 15 min at 4°C) and the plasma was stored at −80°C until use. Plasma IgA and IgE concentrations were determined using the mouse IgA assay kit (Bethyl, TX, USA) and mouse IgE assay ELISA kit (Japan), respectively, according to the manufacturer’s instructions.

**Measurement of Fecal IgA Content**

The total IgA extracted from fecal material (100 mg) was isolated using the mouse IgA assay kit (Bethyl), according to the manufacturer’s instructions.
Statistical Analysis
All experiments were performed at least three times. The data shown are representative results of the means ± SD of triplicate experiments. Significant differences between groups were determined using the unpaired Student’s t-test. Differences were judged to be statistically significant when the P value was <0.05.

Results
Effects of Lactic Acid Bacteria on Peyer’s Patch Cell-Mediated Immunoglobulin A and Tight-Junction Expression in Caco2 Cells
To examine the Peyer’s patch cell-mediated IgA enhancement of Lactobacillus and Bifidobacterium species isolated from fecal matter of a 7-day-old infant, we first investigated IgA production using murine Peyer’s patch cells. Murine Peyer’s patch cells were treated with dead microbial cells (0.01% of dead cells per well) of Lactobacillus and Bifidobacterium species. After 24 h, we measured the IgA production using an IgA ELISA assay kit. As shown in Fig. 1, results reveal that dead LAB (E20, L84, M49, HY8301, 4-9, C9-5 & L84, Lactobacillus gasseri; KY7-2 & E20, Lactobacillus casei; CH7-2 & M49, Lactobacillus plantarum).

Fig. 1. Effect of LAB on IgA production in Peyer’s patch cells. LPS from the microbial cell wall was used as the positive control treatment. The IgA concentration in the culture supernatant was determined using the ELISA system. Each value is expressed as the means ± SD of three independent experiments. *p < 0.05, **p < 0.01 in comparison with the normal control group. 4-9, Leuconostoc mesenteroides; HY8301, Bifidobacterium bifidum; C9-5 & L84, Lactobacillus gasseri; KY7-2 & E20, Lactobacillus casei; CH7-2 & M49, Lactobacillus plantarum.

LAB (E20, L84, M49, HY8301) treatment, as it did in response to LPS (positive control), whereas treatment with dead microbial cells of other species of Lactobacillus and Bifidobacterium did not induce IgA production. As a result, a total of four LAB (E20, L84, M49, HY8301) that activate the Peyer’s patch cells and produce IgA were selected for additional experiments.

Biological properties of the LAB (E20, L84, M49, HY8301) strains were characterized with various biochemical and physiological tests, including the Api kit test, F6PPK test, confirmation tests for the genus Lactobacillus and Bifidobacterium [31], and 16S rDNA sequencing. Based on the BLAST search using the resulting 16S rDNA sequence, we identified the
As shown in Fig. 2, LPS treatment significantly reduced the mRNA levels of ZO-1 and occludin in comparison with the normal control. However, the treatment of HY8301 (and E20) improved the expression of both ZO-1 and occludin in the LPS-induced Caco-2 cell environment.

To determine whether LAB (E20, L84, M49, HY8301) exhibit protection against LPS in Caco-2 cells, the cells were co-treated with E20, L84, M49, or HY8301 and LPS for 24 h. LPS stimulation significantly increased the mRNA expression of the three pro-inflammatory factors TNF-α, IL-1β, and IL-8 (Figs. 3A–3C). However, co-treatment with LPS and dead LAB (E20, L84, HY8301) cells significantly down-regulated the expression of TNF-α, IL-1β, and IL-8.

**Fig. 3.** The anti-inflammatory effect of LAB on LPS-infected Caco-2 cells. Effect of LAB on TNF-α expression (A), IL-1β expression (B), and IL-8 expression (C) in LPS-infected Caco-2 cells. Each value is expressed as the mean ± SD of three independent experiments. LAB affected the expression of anti-inflammatory cytokine-related genes (TNF-α, IL-1β, and IL-8) in LPS-infected Caco-2 cells. **p < 0.01 in comparison with the normal control group; *p < 0.05 in comparison with the LPS-treated group. HY8301, Bifidobacterium bifidum; L84, Lactobacillus gasseri; E20, Lactobacillus casei; M49, Lactobacillus plantarum.**

**Effect of LAB Treatment on Peyer’s Patch Cell-Mediated Immunoglobulin A and Tight-Junction Expression in Mice Treated with a High Dose of Antibiotic**

During the 7-week experimental period, body weight and food intake were measured weekly. There were no significant differences in body weight or food intake among the normal group (ICR mice, AIN-76A feed), kanamycin-injected control group (kanamycin-treated ICR mice, AIN-76A feed), or the LAB (E20, L84, M49, HY8301) groups (kanamycin-treated ICR mice, AIN-76A feed including LAB) (Figs. 4A–4B). Likewise, there were no significant differences in the weight of spleen tissue among the normal, kanamycin-injected control, or the LAB (E20, L84, M49, HY8301) groups (Fig. 4C).

As shown in Figs. 5A and 5B, the number of Peyer’s patch structures in the kanamycin-treated mice was significantly decreased in comparison with the normal mice. However, the LAB (E20, L84, M49, HY8301)-treated mice had the comparable number of Peyer’s patch structures as the mice in the normal group. The same trend was observed in the total number of Peyer’s patch cells.

Because the number of Peyer’s patch structures in the kanamycin-treated mice significantly decreased in comparison with the normal mice, we compared the difference in serum IgE and IgA levels among the animals. As previously reported, administration of kanamycin increased the serum IgG1 and IgE levels while decreasing the serum IgG2a and IgA levels [44].

As shown in Figs. 6A and 6B, the serum IgE levels in the kanamycin-treated mice were significantly higher than in the normal mice at 7 weeks after the cessation of kanamycin administration. This elevation was significantly suppressed in the LAB (E20, L84, M49, HY8301)-treated mice. Kanamycin treatment reduced the serum IgA level, but the LAB (E20, L84, M49, HY8301) strains (data not shown).
L84, M49, HY8301)-treated mice had a serum IgA level similar to the normal mice. The same trend was observed for the fecal IgA level (Fig. 6C).

We measured gene expression for tight-junction proteins ZO-1 and occludin in the ileum tissue via RT-PCR (Fig. 7). The kanamycin-treated group produced significantly lower expression of the ZO-1 and occludin genes in the ileum tissue. We found that the ZO-1 and occludin expression levels in the ileum tissue were higher in the LAB (E20, L84, M49, HY8301)-treated group than in the kanamycin-treated control group.

**Discussion**

The human intestine is more densely populated with microorganisms than any other organ and is a site where...
The microflora may have a profound impact on immune function, nutrient processing, and a broad range of other host activities [27]. Recent studies have shown that *Bacteroides thetaiotaomicron*, a common commensal microorganism, modulates the expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation. In addition, it has been also suggested that exposure to commensal microflora may be a key modulator of the immune system that works against allergic diseases and prevents some of the risk factors underlying the development of metabolic syndrome [55].

The gastrointestinal tract is the most common site of entry for infectious agents at the mucosal surfaces. Secretory immunity is the basis for specific protection at the mucosal level, where IgA is the main immunoglobulin. Mucosal IgA prevents the adhesion of bacteria or viruses, and reduces the absorption of food antigens in the intestine. Recently, studies reported that certain lactic acid bacteria, which generate large quantities of IgA, could prevent rotavirus infections [3, 41, 43]. In the present study, we demonstrated that LAB (E20, L84, M49, HY8301) enhanced IgA production in Peyer’s patch cells in vitro.

The intestinal barrier is the largest interface between humans and the external antigens, including commensals and opportunistic pathogens, and the maintenance of its integrity has an important role in preserving health. When the intestinal barrier is compromised, it can become “leaky,” allowing pathogens and toxins to enter the body. The function of the intestinal barrier is compromised in human conditions such as inflammatory bowel diseases including Crohn’s disease and ulcerative colitis [8] and irritable bowel syndrome [5], and some food-borne infections [13]. Moreover, the intestinal barrier function can be temporarily impaired during times of stress [14], and it inevitably deteriorates with aging [28]. In addition, increased intestinal permeability can also result in pathological changes in distant organs and tissues, which can lead to further complications such as asthma [18], chronic heart failure [38], type 1 diabetes [48], chronic fatigue syndrome [21], and depression [22] in susceptible individuals.

A critical component of the intestinal barrier is the intercellular junctional complexes between adjacent intestinal epithelial cells, which form a semi-permeable diffusion barrier. These intercellular complexes consist of tight junctions, adherens junctions, desmosomes, and gap junctions [10]. Tight junctions are directly involved in the intestinal epithelial barrier against the paracellular penetration

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**Fig. 6.** Effect of LAB treatment on serum IgE (A), IgA (B), and fecal IgA (C) in the kanamycin-treated mice.

The results are expressed as the mean ± SE. Significant differences between the kanamycin-injected control group (kanamycin-treated ICR mice, fed with AIN-76A, *n* = 10) and the normal control (ICR mice, fed with AIN-76A, *n* = 10) are indicated as *p* < 0.05 and **p** < 0.01. Significant differences between the LAB group (kanamycin-treated ICR mice, fed with AIN-76A + LAB, *n* = 7) and the kanamycin-injected control group are indicated as *p* < 0.05 and **p** < 0.01.
of endotoxins, exogenous pathogens, and toxic luminal antigens. ZO-1 and occludin are the major components of TJs, which are located on the cytoplasmic membrane surface of the intercellular tight junctions [11]. They provide a physical linkage between integral membrane proteins and the cellular cytoskeleton, and affect intercellular adhesion and signal transduction. Interactions between ZO-1 and occludin, as well as a cascade of regulatory factors, are involved in the tight-junction structure and remodeling [52]. LPS treatment disrupts intestinal barrier function and increases tight-junction permeability by down-regulating protein expression in tight junctions and by altering the cellular localization of tight junctions. LPS can also initiate a signal transduction cascade by binding to the extracellular domain of TLR4, which enhances pro-inflammatory cytokine (TNF-α, IL-1β, and IL-8) transcription through the NF-kB signaling pathway [2]. The functional mechanism of pro-inflammatory cytokines probably includes instability and weakening of the intestinal tight-junction barrier [4]. In this study, LPS stimulation significantly decreased the mRNA expression of ZO-1 and occludin. However, LAB (E20, HY8301) treatment improved the expression of ZO-1 and occludin in LPS-treated Caco-2 cells.

The gut harbors the most dense and complex microbiota of the human body, which contributes importantly to several basic physiological functions, including nutrition, defense against pathogens, and metabolic and immune homeostasis. Consequently, disturbances in the composition and function of the gut microbiota (i.e., dysbiosis) can have severe consequences for health at several different levels [40]. In particular, evidence is mounting for the involvement of dysbiosis in the broad variety of health problems associated with immune and metabolic malfunctions. Antibiotics are one of the main factors causing dysbiosis and therefore play a significant role in generating the associated suite of undesirable health effects.

The impact of antibiotics on the gut microbiota has short- and long-term effects on the development and operation of the immune system that can generate a variety of health problems. Such problems relate mainly to a decreased resistance to infection or to a disrupted immune homeostasis, which may result in atopic, inflammatory, or autoimmune disease. Drastic alterations of gut microbiota composition and function can affect the immediate risk for intestinal infection owing to the acquisition and spread of incoming pathogens or to the opportunistic pathogenic behavior of some resident members of the gut microbiota. An important example of this problem is the prevalence of antibiotic-associated diarrhea [37, 42, 53, 56].

The effects of antibiotics on the capacity of the immune system to battle infection probably proceed through several related ways, involving both innate and adaptive immunity. Antibiotic-induced gut microbiota changes can alter the type and diversity of microbial-associated molecular patterns that are encountered by receptors such as the cytosolic nucleotide-binding oligomerization domain-containing protein 1 and the membrane-spanning Toll-like receptors, present in various intestinal epithelial cells and innate immunity cells. In turn, the altered stimulation of these receptors can impact numerous processes, from the development of intestinal lymphoid tissues to the differentiation of T cell subtypes, the priming of neutrophils for bacterial killing, the production of antibacterial molecules, and the release of cytokines and pro-cytokines with a variety of functions [47].

Fig. 7. Effect of LAB treatment on tight-junction-related gene expression in the ileum tissue of kanamycin-treated mice. The results are expressed as the mean ± SE. Significant differences between the kanamycin-injected control group (kanamycin-treated ICR mice, fed with AIN-76A, n = 10) and the normal control group (ICR mice, fed with AIN-76A, n = 10) are indicated as *p < 0.05 and **p < 0.01. Significant differences between the LAB group (kanamycin-treated ICR mice, fed with AIN-76A + LAB, n = 7) and kanamycin-injected control group are indicated as *p < 0.05 and **p < 0.01.
Beyond increasing the risk for infection, antibiotic-induced alterations of the gut microbiota can affect basic immune homeostasis with body-wide and long-term repercussions. Atopic, inflammatory, and autoimmune diseases have been linked to gut microbiota dysbiosis [24, 49]. At the cellular and molecular levels, the mechanisms by which gut microbiota species interact with components of the immune system to impact the development of immune tolerance are currently debated [33].

Until recently, the most critical factor in maintaining immune homeostasis was thought to be the balance between the adaptive immunity Th1 and Th2 helper cell subsets. Indeed, excessive Th1 or Th2 activation results in chronic inflammatory and autoimmune disease or in allergic disease, respectively [1, 30].

As previously reported, the administration of kanamycin increased the levels of total IgG1 and IgE, while decreasing the levels of IgG2a and IgA in the serum. Because IgG1 and IgE production is dependent on Th2 cells, whereas the production of IgG2a and IgA is required for the participation of Th1 cells, it can be speculated that kanamycin treatment may prevent the Th1-mediated immune response and drive a shift in the Th1/Th2 balance toward Th2 dominant immunity [44].

To evaluate the effects of LAB (E20, L84, M49, HY8301) in kanamycin-treated mice, we orally administered kanamycin (1,000 mg/ml per day) for 7 consecutive days to eliminate bacteria from the intestine. Consistent with previous work, we found that kanamycin treatment reduced the number of Peyer’s patches and cells within these patches [29], but LAB (E20, L84, M49, HY8301) treatment caused an increase in both the number of patches and the number of cells in each. Our findings clearly demonstrated that oral administration of LAB (E20, L84, M49, HY8301) effectively decreased the level of serum IgE and increased the level of serum IgA, fecal IgA, and Peyer’s patch formation in kanamycin-treated mice, without causing significant changes in body weight, food intake, or spleen weight. In the ileum tissue, 7 weeks of LAB (E20, L84, M49, HY8301) administration significantly increased the mRNA levels of ZO-1 and occludin relative to the kanamycin-treated control group.

We conclude that LAB enhance the intestinal immunity by improving the integrity of the intestinal barrier and increasing the production of IgA in Peyer’s patches; however, more studies are needed to explore the mechanism through which LAB exert these effects. We conclude that LAB isolated from fecal matter of a 7-day-old infant could be considered as potential probiotic candidates for improving intestinal immunity.

References


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