

ORIGINAL ARTICLE

## 과민성 장증후군 쥐 모델에서의 신 유산균의 효과

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### Effects of Novel Probiotics in a Murine Model of Irritable Bowel Syndrome

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**Background/Aims:** Dysbiosis is an important factor in the pathogenesis of irritable bowel syndrome (IBS). Several studies have reported promising results using probiotics for the treatment of IBS. This study evaluated the efficacy of novel probiotics isolated from Kimchi, a Korean fermented food, and the feces of healthy Vietnamese people in a murine model of IBS.

**Methods:** *Lactobacillus paracasei* DK121 was isolated from Kimchi, and *L. salivarius* V4 and *L. plantarum* V7 were isolated from the feces of healthy Vietnamese people residing in Korea. Forty rats were allocated to receive one of the study strains, a mixture of the strains, or the vehicle. After 5 days of administration, the rats were restrained in a cage to induce IBS. The effects of the probiotics on IBS were analyzed by evaluating the stool weights and stool consistency scores.

**Results:** The primary outcome was analyzed upon the completion of a three-week experiment. The rats in the V7 group showed lower stool weights than those in the control group at week 2 (median: 1.10 [V7] vs. 2.35 [control],  $p=0.04$ , Mann-Whitney U-test) and week 3 (median: 1.10 [V7] vs. 2.80 [control],  $p=0.017$ ). The rats in the DK121 (median: 2.00,  $p=0.007$ ), V7 (median: 2.00,  $p=0.004$ ), and mixture (median: 1.50,  $p=0.001$ ) groups showed better stool consistency scores at week 2 than the control group (median: 3.00).

**Conclusions:** The novel probiotics have beneficial effects on defecation in a murine model of IBS. Human studies confirming the efficacy are warranted. (Korean J Gastroenterol 2020;75:141-146)

**Key Words:** Probiotics; Irritable bowel syndrome; Defecation

## INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional bowel disorder characterized by recurrent abdominal pain associated with defecation or a change in bowel habits without organic disease.<sup>1</sup> The prevalence varies according to the geographical region, ranging from 4.0-21.0%.<sup>2,3</sup> IBS is reported

more frequently in adolescents and young adult females, posing a substantial socioeconomic burden.<sup>4</sup> The pathogenesis of IBS is heterogeneous; both environmental factors (e.g., psychosocial stressors, food intolerance, and enteric infection) and host factors (e.g., visceral hypersensitivity, altered pain perception, and brain-gut interaction) can contribute to its development.<sup>2</sup> Thus, both pharmacological and psychological

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interventions are considered to be therapeutic for IBS patients.<sup>5</sup> Dysbiosis also plays a role in IBS by increasing the intestinal permeability, altering the intestinal motility, increasing the intestinal sensitivity, and dysregulating the immune system.<sup>6,7</sup> Several studies have reported the therapeutic benefits of probiotic therapy in both animal models and human studies.<sup>8</sup> One study showed that the administration of *Lactobacillus paracasei* NCC 2461 could prevent antibiotic-induced visceral hyperalgesia and reverse the stress-mediated alterations of the gut permeability and sensitivity in mice.<sup>9</sup> Two large systemic reviews highlighted the positive effects of probiotics on the IBS symptoms and quality of life.<sup>10,11</sup> This study examined the effects of novel probiotics isolated from the feces of healthy Vietnamese subjects and the traditional Korean fermented food, Kimchi, in a murine model of IBS.

## SUBJECTS AND METHODS

### 1. Animals

Male Wistar rats, which were purchased from Central Lab Animal Inc., Seoul, Korea (weighing 337-416 g, 10 weeks old), were used in the study. The animals were acclimatized to the facility and handled daily for 3 weeks before being used in the experiment. All animals were given access to the same dietary food and water *ad libitum* and were housed in a temperature- ( $22\pm 2^\circ\text{C}$ ) and light-controlled (12 hours light:dark) environment. The Institutional Animal Care and Use Committee of Samsung Biomedical Research Institute reviewed and approved this study. The Samsung Biomedical Research Institute is an Association for the Assessment and Accreditation of Laboratory Animal Care International accredited facility, and the study abided by the Institute of Laboratory Animal Resources guidelines (IACUC No. 20170216002).

### 2. Restrain stress model

A restrain stress rat model has been established as a model for human IBS and used for more than three decades.<sup>12</sup> The restraint method has been proven to cause the stimulation of large intestinal activity, an increase in fecal excretion,<sup>12</sup> and visceral hypersensitivity.<sup>13</sup> The restrain stress method was used in all study animal groups individually on day 6 of each week using a cylinder container (diameter, 70 mm; height, 220 mm), forcing the immobilization of the animal for 1 hour during the experiment.

### 3. Probiotics

Three novel probiotic strains isolated and characterized by Kim et al. at Dankook University were used. *L. paracasei* DK121 was isolated from homemade and commercial Kimchi, which is a lactic acid-fermented vegetable product from several regions in Korea that is consumed raw. *L. salivarius* V4 and *L. plantarum* V7 strains were isolated from the database of the gut microbiota for Vietnamese patients with IBS established at the Samsung Medical Center, which was comprised of the feces of 60 healthy Vietnamese adult volunteers residing in Korea. The Institutional Review Board at the Samsung Medical Center approved the collection of feces from Vietnamese people (IRB No. SMC 2016-08-173). This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET) through High Value-Added Food Technology Development Program and was funded by the Ministry of Agriculture, Food and Rural Affairs of Korea (MAFRA) (316061-3) to develop novel probiotic strains for the treatment of Vietnamese people with IBS. For this reason, feces were collected from Vietnamese people with limited environmental exposure outside of Vietnam, specifically, less than 6 months of residence in Korea. The Vietnamese volunteers

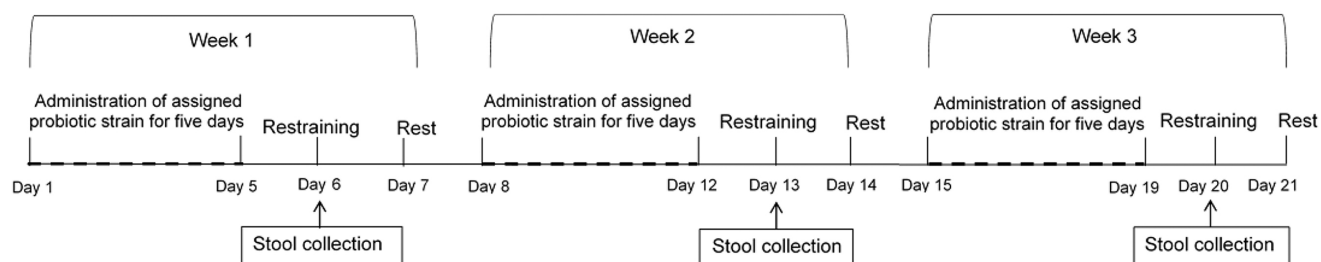


Fig. 1. Study design.

were excluded if they had any disease, recent treatment with antibiotics, a medical history of gastrointestinal surgery, or any gastrointestinal symptoms before screening and feces collection. The probiotic strains were reconstituted from freeze-dried frozen stocks stored at  $-72^{\circ}\text{C}$  in sterile tap water prior to use.

#### 4. Experimental design

nForty Wistar rats were divided randomly into five groups of eight rats each, including the control, DK121, V4, V7, and mixture groups. All rats in the treatment groups were treated with the assigned lactic acid bacillus strains (DK121, V4, V7, or a mixture of all three) at the same time each day by an oral gavage at a dose of  $2 \times 10^9$ , for five consecutive days (Fig. 1). Lactose with dextrose (vehicle) was administered to the control group. On day 6, all rats were confined to a cylindrical cage without anesthesia and restrained for 1 hour at the same time between 1 PM to 4 PM (Fig. 2), after which the rat stool was collected. On day 7, the rats were allowed to rest without any manipulation. This 1-week experiment was repeated 3 times for a total study period of 3 weeks. The body weight of each rat was measured daily throughout the

experimental period.

#### 5. Assessments

The stool weights and stool consistency scores were measured. The collected stool was contained in a tube and weighed with an electronic scale. The stool consistency was scored as follows: 0 (normal, Bristol stool form scale 3 or 4), 1 (slightly loose, Bristol stool form scale 5), 2 (loose, Bristol stool form scale 6), 3 (severely loose, Bristol stool form scale between 6 and 7), and 4 (diarrhea or watery, Bristol stool form scale 7). The primary outcomes were the stool weight and consistency.

#### 6. Statistical analysis

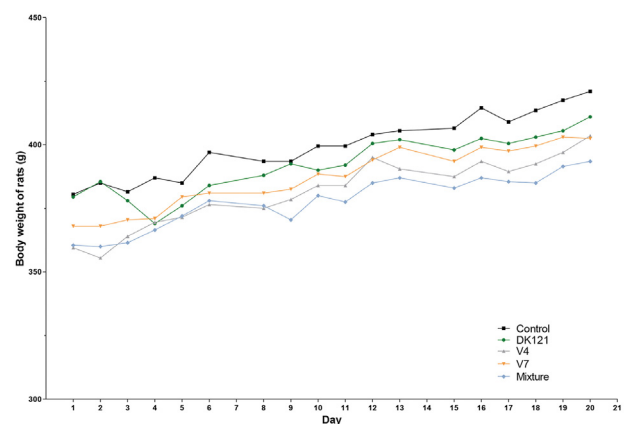
The results are expressed as the median and interquartile range. The statistical significance of the differences observed in the experimental groups compared to the control group was analyzed using a Mann-Whitney U-test on SPSS 24.0 statistical software (IBM Corp., Armonk, NY, USA). A p-value less than 0.05 was considered significant.

## RESULTS

All rats grew well during the 3-week experimental period, except for two rats, one in the DK121 group, which lost 14 g, and the other in the V7 group, which lost 6 g of weight by the end of the study period (Fig. 3).



**Fig. 2.** Cylindrical cage used to confine the rats during the experiment (diameter, 70 mm; height, 220 mm). Each rat was restrained in this cage for 1 hour on days 6, 13, and 20, provoking stress-induced defecation.



**Fig. 3.** Body weights of rats during the 3-week study period. Each dot represents the median body weight (g) of the rats in the assigned group.

## 1. Stool weight

Fig. 4 shows the stool weights of the rats in each study group during the 3-week experiment. The rats in the V7 group had lower stool weights than the rats in the control group at week 2 (V7: 1.10 g [0.70-1.40 g] vs. control: 2.35 g [1.20-2.83 g],  $p=0.040$ ) and week 3 (V7: 1.10 g [0.60-1.80 g] vs. control: 2.80 g [1.55-3.35 g],  $p=0.017$ ). In addition, the rats in the DK121 group had lower stool weights than those in the control group at week 3, with marginal statistical significance (DK121: 1.00 g [0.40-2.00 g] vs. control: 2.80 g [1.55-3.35 g],  $p=0.073$ ).

## 2. Stool consistency

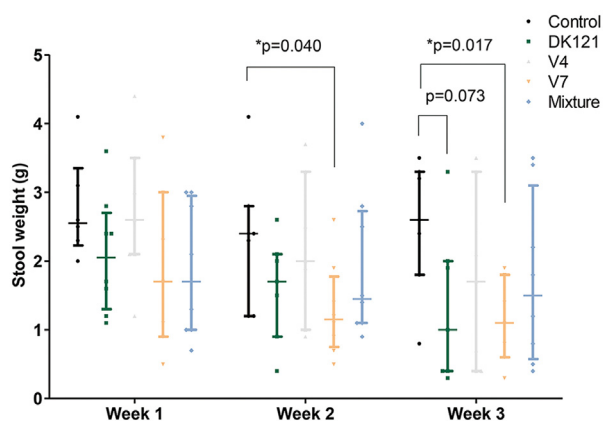
Fig. 5 presents the stool consistency scores of the rats in each study group during the 3-week experiment. The stool consistency scores of the rats in the DK121 group were lower than in the control group at week 2 (DK121: 2.00 [2.00-3.00] vs. control: 3.00 [3.00-4.00],  $p=0.007$ ). The rats in the V7 group had lower stool consistency scores than in the control group (V7: 2.00 [1.00-3.00] vs. control: 3.00 [3.00-4.00],  $p=0.004$ ). The rats in the mixture group showed better (lower) stool consistency scores than the control group at week 2 (mixture: 1.50 [1.00-2.00] vs. control: 3.00 [3.00-4.00],  $p=0.001$ ). At week 3, however, the stool consistency scores of the rats in all groups (treatments and control) were

comparable.

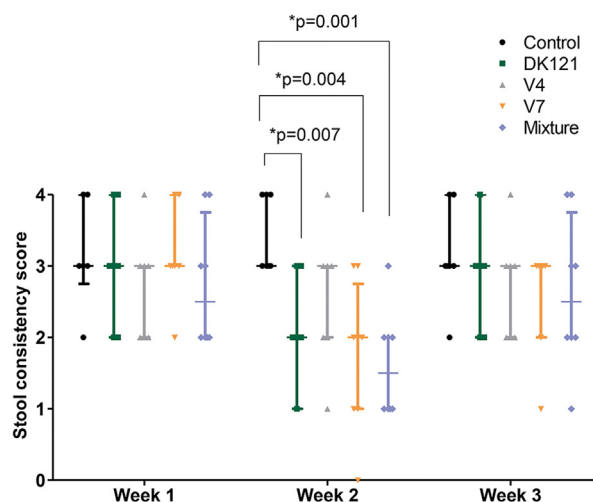
## DISCUSSION

Dysbiosis is important in the etiology of IBS, and several studies have established the utility of probiotic treatments, which modulates the gut microbial composition and activity.<sup>7,10,14-17</sup> Therefore, the development of novel probiotics that improve the symptoms of IBS is crucial. This study examined the effects of three novel probiotics isolated from the Korean fermented food Kimchi and the feces of Vietnamese people residing in Korea in a murine IBS model. All study strains, including a mixture of all three strains, were previously tested for acid tolerance, bile acid tolerance, heat resistance, mucous layer-binding activity, and functional probiotic properties, such as the 1,1-diphenyl-2-picrylhydrazine radical and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activities, which confirmed their properties as probiotics. All strains had outstanding properties compared to commercially used strains.<sup>18,19</sup>

None of the rats that were administered the three novel probiotics experienced significant adverse events. The rats in the DK121 and V7 groups showed lower stool weights and



**Fig. 4.** Stool weights of the rats in each study group during the 3 weeks of probiotics administration. The rats in the V7 group had lower stool weights than those in the control group at weeks 2 ( $p=0.040$ ) and 3 ( $p=0.017$ ). The rats in the DK121 group had lower stool weights than the control group at week 3 with marginal statistical significance ( $p=0.073$ ). \*The lines are the median and interquartile range of the eight animals in each group. Each value is shown as a scatter dot. Lines and scatter dots are shown for the control, DK121, V4, V7, and the mixture groups.



**Fig. 5.** Stool consistency scores of the rats in each study group during the 3-week probiotics treatment experiment. The rats in the DK121 ( $p=0.007$ ), V7 ( $p=0.004$ ), and the mixture ( $p=0.001$ ) groups showed better (lower) stool consistency scores than the control group at week 2. No significant difference was observed among the study groups at week 3. \*The lines represent the median and interquartile range of the eight animals in each group. Each value is shown as a scatter dot. The lines and scatter dots are shown for the control, DK121, V4, V7, and the mixture groups.

transient improvement of the stool consistency scores than the control group.

According to the Food and Drug Administration guidance statement on clinical trial end points for IBS with diarrhea, the defecation component of the proposed primary end point should be an assessment of the stool consistency, with abdominal pain intensity as a second component of the primary endpoint via pain assessment.<sup>20</sup> Following these recommendations, this study assessed stool consistency and total stool weight as the primary outcomes. The abdominal pain intensity could not be assessed as it was an animal study, which could be a limitation of this study. Further studies assessing the visceral hypersensitivity or mucosal cytokines could be helpful in evaluating the efficacy of probiotics on IBS.

A restraint method was used for this murine IBS model. Williams et al.<sup>12</sup> initially suggested this restraint model using masking tapes in 1988. Similar models have been used in many studies with modification.<sup>21,22</sup> Although no animal model is considered to mimic human IBS fully, the restraint stress model was effective in developing immediate hyperalgesia, stimulation of large intestinal activity, and increased fecal excretion.<sup>23</sup> In addition, rats subjected to restraint stress presented with mucosal inflammation, with an increase in the number of mast cells and eosinophilic granulocytes,<sup>24</sup> which was similar to the colonic biopsy results of IBS.<sup>25</sup> These rats also showed changes in the levels of neurotransmitters in glial cells, which are considered to be accountable for the dysmotility and hypersensitivity in IBS patients. In this manner, it was concluded that the restraint stress rat model could be suitable to represent human IBS.

The adequacy of such “short-period subacute stress-induced” models for studying IBS is a limitation because IBS is a chronic disorder that is diagnosed only if symptoms persist for at least 6 months. Chronic stress models, such as maternal separation and water avoidance stress, have been proposed and used in studies validating the efficacy of probiotics.<sup>26-29</sup> On the other hand, because no single model has been proven to possess the best predictive validity, this study implemented a restraint method at the research facility. In addition, ramosetron, a 5-HT<sub>3</sub> receptor antagonist, has demonstrated the effects both in a restraint model, similar to the one used in the present study<sup>23</sup> and in humans.<sup>30</sup> Therefore, the restraint method could still be used for a

short-term experiment.

Another limitation of the study was that only a relatively small number of rats were used. On the other hand, even with these limitations, the effects of two novel probiotics strains, DK121 and V7, were determined. This could be attributed to thorough screening of the isolated probiotic strains using characteristic laboratory tests. Finding specific strains that provide the greatest benefits is important because the effectiveness of probiotics varies among species and strains.<sup>31-33</sup>

In conclusion, the probiotic strains tested, DK121 and V7, reduced the stool weight significantly and partially improved the stool consistency in a murine model of IBS. With further studies confirming their efficacy in humans, these new probiotics could be a potential treatment option for IBS.

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