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Anti-stress Effect of Lactic Acid Bacteria (*Lactobacillus acidophilus*, *Pediococcus pentosaseus*, *Bifidobacterium longum* SPM1205)

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유산균(*Lactobacillus acidophilus*, *Pediococcus pentosaseus*, *Bifidobacterium longum* SPM1205)의 항스트레스 효과

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요 약

이 연구의 목적은 유산균(LAB)의 항스트레스 효과를 알아보기 위함이다. 본 실험에서는 ICR 마우스에게 구속 스트레스를 가하면서 혼합유산균과 홍삼추출물을 경구투여 하였다. 5일간 Normal 그룹을 제외한 saline (control), LAB100, LAB200, RGE200에게 구속 스트레스를 주었다. 구속 스트레스를 가한 후에 ICR 마우스에게 kg당 100 mg (3.0×10^{11} CFU/g) 또는 200 mg (3.0×10^{11} CFU/g)의 혼합유산균(*Lactobacillus acidophilus*, *Pediococcus pentosaseus*, *Bifidobacterium longum* SPM1205)을 투여하였으며 대조물질로는 홍삼 추출물(Red ginseng extract) 200 mg (홍삼추출물)/kg (마우스)을 투여하였다. 마지막 실험일에는 locomotor와 elevated plus-maze 실험을 통하여 마우스의 행동변화를 측정하였다. 그 결과, 혼합유산균을 투여한 그룹에서는 스트레스를 일부 억제하는 효과를 보였다. 특히 Elevated plus-maze 실험에서, 스트레스를 받은 마우스는 open zone에서보다 closed zone에서 더욱 많은 시간을 보냈다. 그렇지만 혼합유산균을 투여한 그룹에서는 open zone에서 더욱 오래 시간을 보냈으며 그 시간은 saline과 RGE200 그룹에 비해서도 길었다. 그것은 또한 아무것도 처리하지 않은 Normal 그룹과도 비슷한 결과이다. 그리고 마우스의 분변에서 유산균수를 측정하였는데 스트레스를 가하고 혼합유산균은 투여하지 않은 saline 그룹에서는 유산균수가 감소했지만 혼합유산균을 투여한 LAB100, LAB200 그룹에서는 유산균수가 증가하였다.

Key words : anti-stress, lactic acid bacteria, locomotor activity, plus-maze, red ginseng extract

INTRODUCTION

Stress is the plague of the modern society as well as the unavoidable consequence of life. As Seyle

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noted, "without stress, there would be no life" (Selye, 1993). A moderate amount of stress can be positive, making individuals more alert, motivated, and thereby allowing for better performance. However, too much pressure can cause physical damage to the gastrointestinal tract, endocrinal system, skin and cardiovascular system (Hurst *et al.*, 1976; Breier *et al.*, 1987; Chrousos and Gold, 1992). Also, stress can bring about feelings of distrust, rejection, anger, and depression, which in turn can lead to health problems such as headaches, stomach upsets and ulcers, liver disease, rashes, insomnia, hypertension, heart disease, stroke, diabetes, immune disorders, and sexual disorders (Hurst *et al.*, 1976; Glass, 1977; Breier *et al.*, 1987; Selye, 1993; Dimsdale *et al.*, 2000).

In order to eliminate stress, many functional foods such as Ginseng extract, Docosahexaenoic acid (DHA), and Gingko biloba extract were introduced and tested (Yuan *et al.*, 1989; Ward *et al.*, 2002; Kim *et al.*, 2003b; Takeuchi *et al.*, 2003). Panax ginseng and its constituents have been shown to exhibit both anti-stress and antioxidant activity, and to exert various benefits relating to stress and the immune system (Kim *et al.*, 1970; Takahashi *et al.*, 1992; Simsek *et al.*, 2007). Red ginseng is popularly used as an anti-stress medication and is known to exert more pharmacologically active benefits than white ginseng (Kaneko and Nakanishi, 2004; Oh *et al.*, 2006).

Lactic acid bacteria (LAB) are well-known probiotic organisms in humans. These organisms have roles in the improvement of diarrhea or constipation, synthesis of vitamins, improvement of lactose utilization, and lowering of blood cholesterol levels. LAB, which are typically associated with the human gastrointestinal tract, have been reported to suppress the growth of pathogens and stabilize the digestive system by increasing intestinal barrier functions. Also, LAB strengthens immunity through the propagation of macrophages (Perdigon *et al.*, 1986) and it prevents cancer (Lee *et al.*, 1993). However, no reports on the anti-stress effects of LAB have been published as yet.

The main aim of this study was to investigate the anti-stress effects of mixed LAB (*Lactobacillus acidophilus*, *Pediococcus pentosaceus*, *Bifidobacterium longum* SPM1205) as a candidate for anti-stress-related functional supplements, by comparing its effect to that of red ginseng extract, which is already used to treat stress.

MATERIALS AND METHODS

1. Animals and diets

Male ICR mice (4-week-old) used in this study were obtained from Hanlim experimental animal Co. (Hwasung, Korea). We used mixed lactic acid bacteria (LAB) (*Lactobacillus acidophilus*, *Pediococcus pentosaseus*, *Bifidobacterium longum* SPM1205), which was provided by Cellbiotech Co. Red ginseng extract (RGE) was produced by Korean Ginseng Corp. (Seoul Korea). Mice were housed in a temperature (22 ± 2°C) and humidity (55 ± 5%)-controlled animal room on a 12 hr/12 hr light/dark (6 A.M.-6 P.M.) schedule. They had free access to food and water throughout the experiments. The animals were divided into five groups (normal, saline, LAB100, LAB200, RGE200) after stabilizing them for 1 week in the animal room. And the mice were orally administered with the test sample (0.1 mL/10 g body weight) and exposed to stress. Mice belonging to the normal group were not exposed to any stress. Normal group were no treated. And saline group were administered saline (0.9% NaCl) as control. LAB group were orally administered once a day with 100 mg or 200 mg of LAB (3.0×10^{11} CFU/g)/kg body weight and exposed to stress. The RGE200 group were orally administered with 200 mg of RGE/kg body weight and exposed to stress. Mice were orally administered each samples for 5 days, and then mice were given samples with stress for 5 days.

2. Induction of stress

Mice were given supplementary materials before exposing them to stress. The mice were usually subjected to restraint stress by keeping them in well-ventilated conical plastic tubes (3 cm in diameter and 7

cm in length) for 30 minutes each day. During the restraining period, the mice did not have any access to food and water.

3. Behavioral apparatus

The equipment was located in the animal room, allowing the observer to view and observe the animals through a computer outside the room. After inducing terminal stress (in the manner described above), behavioral changes of animals were monitored automatically using a computerized EthoVision system (Noldus IT b.v., Netherlands). In the locomotor activity and elevated plus-maze tests, the behavioral parameters were analyzed by an automatic video-tracking system.

4. Locomotor activity

The apparatus consisted of 9 black plastic boxes (47 × 47 cm), and the field was bordered by 42-cm-high sidewalls. The total distance moved and the total movement time was monitored for 20 minutes after terminal stress (Noldus *et al.*, 2001; Tan-Lee *et al.*, 2004).

5. Elevated plus-maze test

The elevated plus-maze box and arms were made of plastic. The apparatus consisted of two open arms (30 × 6 cm in mice), alternating at right angles, with two arms enclosed by high walls (20 cm in mice). The four arms delimited a central area of 10 × 10 cm. The whole apparatus was placed 50 cm above the floor. The animals were placed in the central square and allowed to explore the maze freely for 5 minutes. The parameters measured were the times spent in open and closed areas (Noldus *et al.*, 2001; Tan-Lee *et al.*, 2004).

6. Change of fecal lactic acid bacteria counts

To compare the numbers of fecal LAB between the

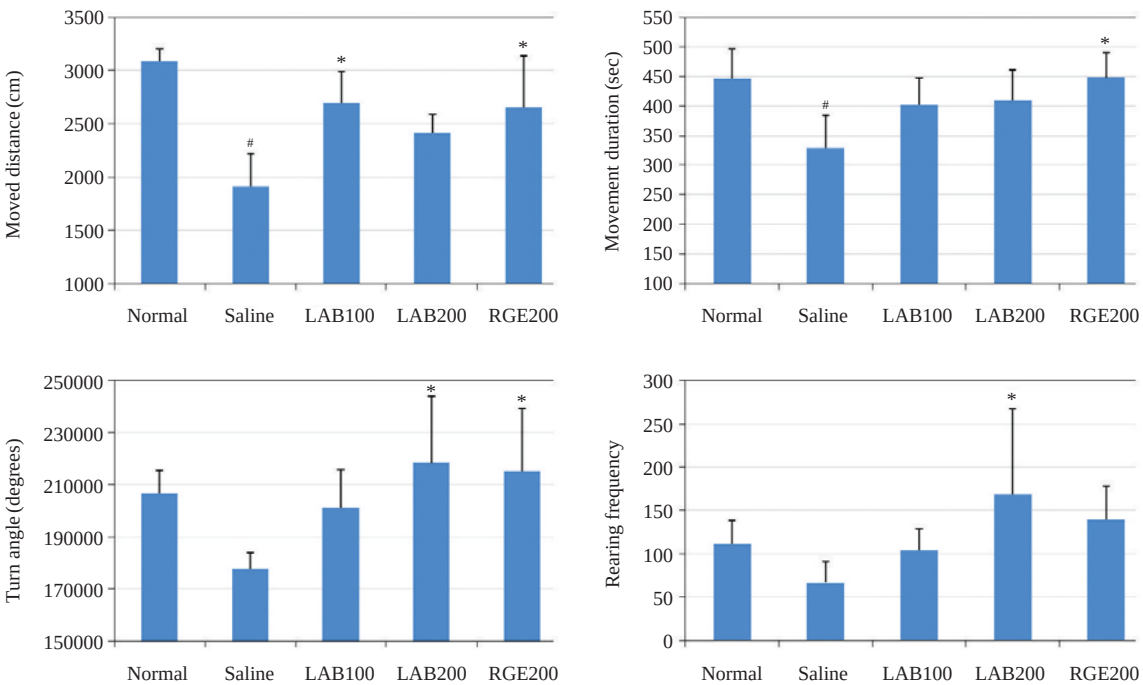

Fig. 1. Effects of lactic acid bacteria (LAB) on locomotor activity test in ICR mice (n=8). Saline: only stress without LAB, LAB100: 100 mg/kg of LAB, LAB200: 200 mg/kg of LAB, RGE200: 200 mg/kg of red ginseng extract. The data are presented as means ± SEM. $^{\#}P < 0.05$ compared to normal group, $^{*}P < 0.05$ compared to saline group.

LAB fed groups and the saline (control) group, fecal samples were periodically collected. 0.1 g of feces was suspended in 0.9 mL of 0.1 M phosphate buffer (pH 6.8 containing 0.5% cystein) and serially diluted 10-fold from 10^{-1} to 10^{-7}. 100 µL of appropriate dilutions was spread onto the Lactobacilli MRS agar (Difco, USA). After 48 h of incubation under anaerobic conditions (Bactron Anaerobic Chamber, Sheldon MFG. Inc., USA), the colonies were counted.

7. Statistical analysis

The data are expressed as the mean ± standard error of the mean (SEM). For comparison between groups, one way analysis of variance (ANOVA) and Tukey's post-hoc test were performed and differences between groups were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

1. Effects of lactic acid bacteria (LAB) on locomotor activity in ICR mice

Locomotor activities, measured as the distance moved and duration of movement, were significantly different between mice that were or were not exposed to stress. Exposure to stress resulted in a significant decrease in distance moved and duration of movement (Fig. 1). These results were comparable to other published studies (Glass, 1977; Fanselow, 1980; Morimoto *et al.*, 1993; Djordjevic *et al.*, 2003; Kim *et al.*, 2003b; Takeuchi *et al.*, 2003). LAB and red ginseng extract (RGE) suppressed this stress-induced locomotor behavior. Also, LAB and RGE suppressed stress-induced behavioral changes such as rearing frequency and turned degree which are behavioral factor that decrease in stressed mice. Exposure to stress resulted in a decrease in rearing frequency and turn angle, but these changes were restored by treatment of the animals with LAB and RGE (Fig. 1). Ginseng extract and its constituents including ginsenosides, exert anti-stress activity on animals subjected to stressful stimuli such as footshock, cold, and heat (Yuan *et al.*, 1989; Takahashi *et al.*, 1992; Kaneko *et al.*, 1996; Choi *et al.*, 2003; Kim *et al.*, 2003a). Although LAB has never been known to counteract stress, the effects of LAB were similar to that of Ginseng in this study.

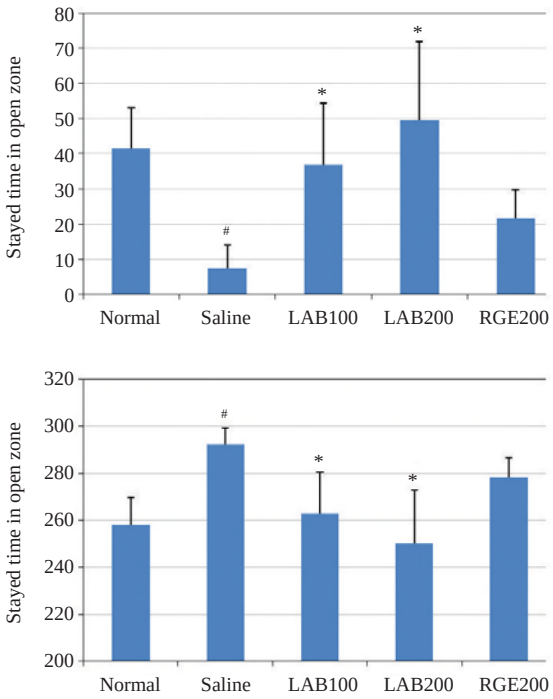

Fig. 2. Effects of lactic acid bacteria (LAB) on elevated plus maze test in ICR mice (n=8). Saline: only stress without LAB, LAB100: 100 mg/kg of LAB, LAB200: 200 mg/kg of LAB, RGE200: 200 mg/kg of red ginseng extract. The data are presented as means ± SEM. $^{\#}P < 0.05$ compared to normal group, $^{*}P < 0.05$ compared to saline group.

2. Effects of lactic acid bacteria (LAB) on elevated plus-maze test in ICR mice

Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms), and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms). Total number of arm entries and number of closed-arm entries are usually employ-

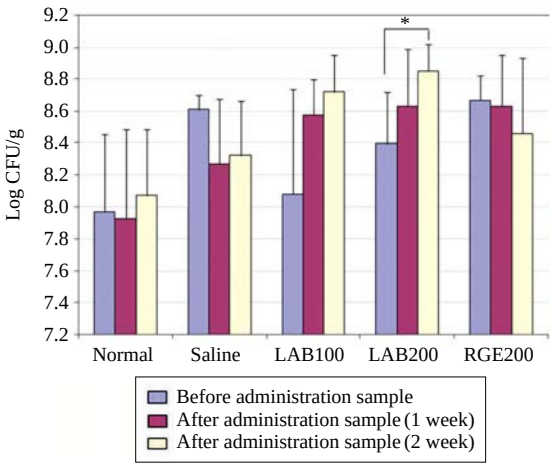

Fig. 3. Change in LAB cell counts in faeces of mice (n=8). The data are presented as means±SEM. *$P<0.05$.

ed as measures of general activity (Hogg, 1996). Mice exposed to stress spent less time in the open arm and more time in the closed arm than the unexposed ones. Stress conditions (saline group) significantly decreased the time spent in the open zone while increasing the time spent in the closed zone. LAB and red ginseng extract (RGE) significantly reversed the stress-induced response in elevated plus maze test (Fig. 2). The anti-stress effect of LAB was higher than that of RGE in the elevated plus-maze test. These results revealed that LAB and RGE partially protected the animals from psychological stress.

3. Change of fecal lactic acid bacteria (LAB) counts

During the administration of LAB, the number of LAB in faeces was significantly increased in the LAB200 group (Fig. 3). As shown in Fig. 3, stress conditions decreased LAB numbers in the gastrointestinal tract, but LAB-fed groups were increased about 3 times after 2 weeks of administration. This result was attributed to the LAB feeding. Also, these organisms may offer health benefits by effecting positive changes to the host. Probiotic bacteria are defined as 'live microorganisms which, when administered in adequate amount, confer a health benefit on the host' (Araya *et al.*, 2002). So, our results showed that LAB might be beneficial as a probiotic strain.

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