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Lactobacillus HY2782 and *Bifidobacterium* HY8002 Decrease Airway Hyperresponsiveness Induced by Chronic PM2.5 Inhalation in Mice

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ABSTRACT Epidemiological studies have shown that exposure to particulate matter (PM) is associated with adverse health effects. Inhalation of fine particulate matter (PM2.5) is associated with elevated pulmonary diseases. However, the molecular mechanism underlying the initiation of lung inflammation following inhalation is unclear. In this study, we investigated the beneficial effects of two probiotics, *Lactobacillus casei* HY2782 and *Bifidobacterium lactis* HY8002, against PM-induced pulmonary inflammation. Model mice were subjected to chronic exposure of PM2.5. The results showed that PM2.5 enhanced oxidative stress and led to Th2 cytokine responses in the mice. PM2.5-exposed mice were orally administered with HY2782 and HY8002 from the day of first exposure to the end point of the study. The results showed that HY2782 ameliorated PM 2.5 exposure-enhanced leukocyte migration and activation of proinflammatory cytokines. HY2782 and HY8002 also prevented exacerbation of eosinophil and neutrophil infiltration in the bronchoalveolar lavage fluid. HY2782 and HY8002 significantly increased scavenging of PM2.5-induced reactive oxygen species and activated superoxide dismutase and catalase activity in the blood. These results indicate that the probiotics HY2782 and HY8002 protect against PM-induced pulmonary inflammation.

KEYWORDS: • **Bifidobacterium lactis** ***HY8002*** • **Lactobacillus casei** ***HY2782*** • ***particulate matter*** • ***probiotics*** • ***pulmonary disease***

INTRODUCTION

Air pollution that causes *harm* to the natural environment, humans, and other organisms is an important environmental issue worldwide. Particulate matter (PM), a major component of air pollution, is a fine pollutant with a particle size of 100 μm or less. It is mainly generated by fossil fuel combustion in furnaces and power plants, waste incineration processes, blast furnaces during the steelmaking process, heat treatment facilities, petroleum refining processes, and petrochemical manufacturing process. PM is classified as PM10, PM2.5, or PM0.1 when the aerodynamic particle diameter is ≤10, ≤2.5, or ≤0.1 μm, respectively.[1] PM is a complex mixture of secondary inorganic molecules, carbon-centered combustion particles, and crustal-derived particles from diverse sources, including biomass combustion, waste incineration, traffic, industrial processes, and transported air pollution.[1,2]

Many studies of the correlation between PM and health effects have been performed and showed that PM has negative effects in humans, such as causing cardiovascular and respiratory diseases.[3,4] PM2.5 has a small diameter and thus can carry a variety of toxic substances. These particles can pass through the filter of nose hairs, reaching the end of the respiratory tract via air flow. Breathing air polluted with PM2.5 can lead to damage in various parts of the body through air exchange in the lungs.[5]

Lactic acid bacteria, which produce lactic acid, include *Lactobacillus, Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. Among these bacteria, *Lactobacillus* and *Bifidobacterium* are important components of commercial fermented foods. *Lactobacillus* species are facultative anaerobic, heterotypic fermentation bacteria found in traditional fermented foods and dairy products such as yogurt.[6] *Lactobacillus* species are found humans and animals, particularly in the digestive system. These species are important parts of the human intestinal microbiota, and the probiotic effects of *Lactobacilli* in the gastrointestinal tract of human and animals have been widely studied. *Lactobacilli* have important health-promoting functions such as reducing inflammatory bowel disease and maintaining intestinal homeostasis in the human gut.[7,8]

Bifidobacterium species are gram-positive and belong to the Actinobacteria phylum. These species are commonly found in the human vagina and gastrointestinal tract. They

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can utilize indigestible human milk oligosaccharide in human breast milk and thus *Bifidobacterium* species are a dominant component of the intestinal microbiota of breast-fed infants.[9,10] Numerous species, including the genera *Lactobacillus* and *Bifidobacterium*, are probiotic microorganisms; *Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum, L. gasseri*, *L. helveticus*, *L. paracasei*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. salivarius*, *Bifidobacterium animalis subsp. lactis*, *B. bifidum*, *B. breve*, and *B. longum* have been approved for commercial use by the Korean Ministry of Food and Drug Safety (KFDA).

In this study, we investigated the effects of the probiotics *Lactobacillus casei* HY2782 (HY2872) and *B. animalis* spp. lactis HY8002 (HY8002). We hypothesized that HY2782 and HY8002 have beneficial effects against PM-induced pulmonary injury. A mouse model was used to evaluate the effects of the probiotics, HY2782 and HY8002, on pulmonary histology, lung inflammation, and oxidative stress after PM2.5 exposure.

MATERIALS AND METHODS

Reagents and equipment

Diesel PM (NIST1650B; Sigma Aldrich, St. Louis, MO, USA), *L. casei* (HY2782; Korea Yakult Co., Ltd., Seoul, Korea), Mouse IL-4 Quantikine ELISA kit (M4000B; R&D Systems, Minneapolis, MN, USA), Mouse CCL2/JE/MCP-1 Quantikine ELISA kit (MJE00B; R&D Systems), Mouse CXCL2/MIP-2 Quantikine ELISA kit (MM200; R&D Systems), Mouse IL-5 Quantikine ELISA kit (M5000; R&D Systems), OxiSelect™ Superoxide Dismutase Activity Assay (STA-340; Cell Biolabs, Inc., San Diego, CA, USA), OxiSelect Catalase Activity Assay (STA-341; Cell Biolabs, Inc.), Easy-spin™ RNA kit (17221; iNtRON Biotechnology, Gyeonggi-do, Korea), Lysing Matrix D (6913-500; MP Biomedicals, Santa Ana, CA, USA), Fast-prep®-24 (MP Biomedicals), Omniscript® RT Kit (205113; Qiagen, Hilden, Germany), Gene Expression Master Mix (4639016; Applied Biosystems, Foster City, CA, USA), and QuantStudio™ 6 Flex Real-Time Instrument (Thermo Fisher Scientific, Waltham, MA, USA) were used as reagents and equipment.

In vivo *assay*

Male C57BL/6 mice aged 6 weeks were purchased from DooYeol Biotech (Seoul, Korea). The mice were divided into groups of 10 mice each. PM at 200 μg/20 μL was administered intranasally to induce lung disease in the mice for 30 days. The PM was suspended in phosphate-buffered saline (PBS) at 10 mg/mL and sonicated at 70°C for 2 h. *L. casei* HY2782 was added to the feed at 1×10^8 CFU/day mouse. After 30 days, the mice were sacrificed and the bronchoalveolar lavage fluid (BALF), blood, liver, and lungs were extracted. The obtained organs and blood were stored at −70°C until cytokine and gene expression analyses. The experimental procedures were approved by the Ethics Review Committee of the Korea Yakult Company Limited R&D Center, Korea (AEC-2019-00066-Y)

Preparation of BALF

The bronchial sections of the sacrificed mice were cut in half vertically. A feeding needle for oral administration was inserted, and 1 mL PBS was added and collected again. The collected BALF was centrifuged, and the supernatant was used for cytokine analysis. The remaining pellet was resuspended in 100 μL PBS and used for cell analysis.

Analysis of immunocytes and cytokines in BALF and serum

The BALF was analyzed using the Mindray BC-5000 Vet. The number of cells was determined as $\times 10^3$ cell/μL.

Secreted cytokines in BALF were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit. To separate the serum, blood from the mice was left at room temperature for 30 min and then centrifuged at 2000 *g* for 10 min. Subsequent steps to detect interleukin (IL)-4, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-2, and IL-5 were performed according to the ELISA kit instructions. The BALF was centrifuged at 3000 *g* for 10 min. Cytokine secretion from the supernatant of the BALF was analyzed.

Analysis of antioxidant enzyme activity in serum

For cytokine analysis in the serum, separated serum was analyzed using the OxiSelect Superoxide Dismutase Activity Assay Kit and OxiSelect Catalase Activity Assay Kit. The results are shown in units of IU/mL.

Gene expression analysis of liver and lung tissue

Total RNA was extracted using the Easy-spin RNA kit. The liver and lung tissue along with lysis buffer were added to Matrix D tubes and pulverized through Fast-prep-24. After grinding the tissue, the remaining protocol of the Easy-spin RNA Kit was followed. Total RNA was stored at −20°C until gene expression analysis. The extracted RNA was reverse-transcribed into cDNA using an Omniscript RT Kit. cDNA was amplified with Gene Expression Master Mix and a TaqMan Probe in a QuantStudio 6 Flex Real-Time Instrument. Table 1 shows the names and catalog numbers of the genes.

Preparation of lung tissue slides and measurement of mucosa thickness

Lung tissues fixed in 10% formalin solution were used to prepare hematoxylin and eosin-stained slides by KPC (Gwangju-si, Korea). The thickness of the mucosa was measured under an Olympus CK2 microscope (Tokyo, Japan) at 200× magnification. The mucosa thickness was analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Data are shown as the mean ± SD of independent experiments. Statistical comparisons between groups were performed by the Student's *t* test.

Table 1. Gene Names and Symbols Used in Gene Expression Analysis

Gene symbol	*Gene name*	*Catalog number*	*Reference sequence*
CAT	Catalase	Mm00437992_m1	NM_0098404.2
SOD-1	Superoxide dismutase 1	Mm01344233_g1	NM_011434.1
SOD-2	Superoxide dismutase 2	Mm01313000_m1	NM_013671.3
GPX-1	Glutathione peroxidase 1	Mm00656767_g1	NM_008160.6
GPX-2	Glutathione peroxidase 2	Mm00850074_g1	NM_030677.2
AHR	Aryl hydrocarbon receptor	Mm00478932_m1	NM_013464.4
NQO1	NAD(P)H quinone oxidoreductase 1	Mm01253561_m1	NM_008706.5
NRF2	Nuclear factor erythroid 2-related factor 2	Mm00477784_m1	NM_010902.3

RESULTS

Increased cytokine and chemokine production in PM2.5-exposed mice

Stimulation by PM2.5 introduced through the mucosa led to the secretion of chemokines such as MCP-1 and MIP-2. MCP-1 secretion was increased to 361.44 ± 9.53 pg/mL in the BALF of PM-induced mice. In contrast, in probiotics-fed mice, MCP-1 recovered to normal levels (Fig. 1). The secretion of MIP-2 was increased by 126% by PM exposure compared to normal-fed mice. The increased levels of MIP-2 were found to be decreased to 0.99 ± 0.29 pg/mL (HY2782), not detectable (N.D.) (HY8002), and N.D. (HY2782+HY8002) by probiotics treatment. Secretion of MCP-1 and MIP-2 into the serum showed the same pattern as that in the BALF, and the increased levels of chemokines induced by PM treatment were decreased by the administration of both probiotics (Fig. 1).

MCP-1 secretion is associated with the activation of Th2 responses and enhanced secretion of cytokines

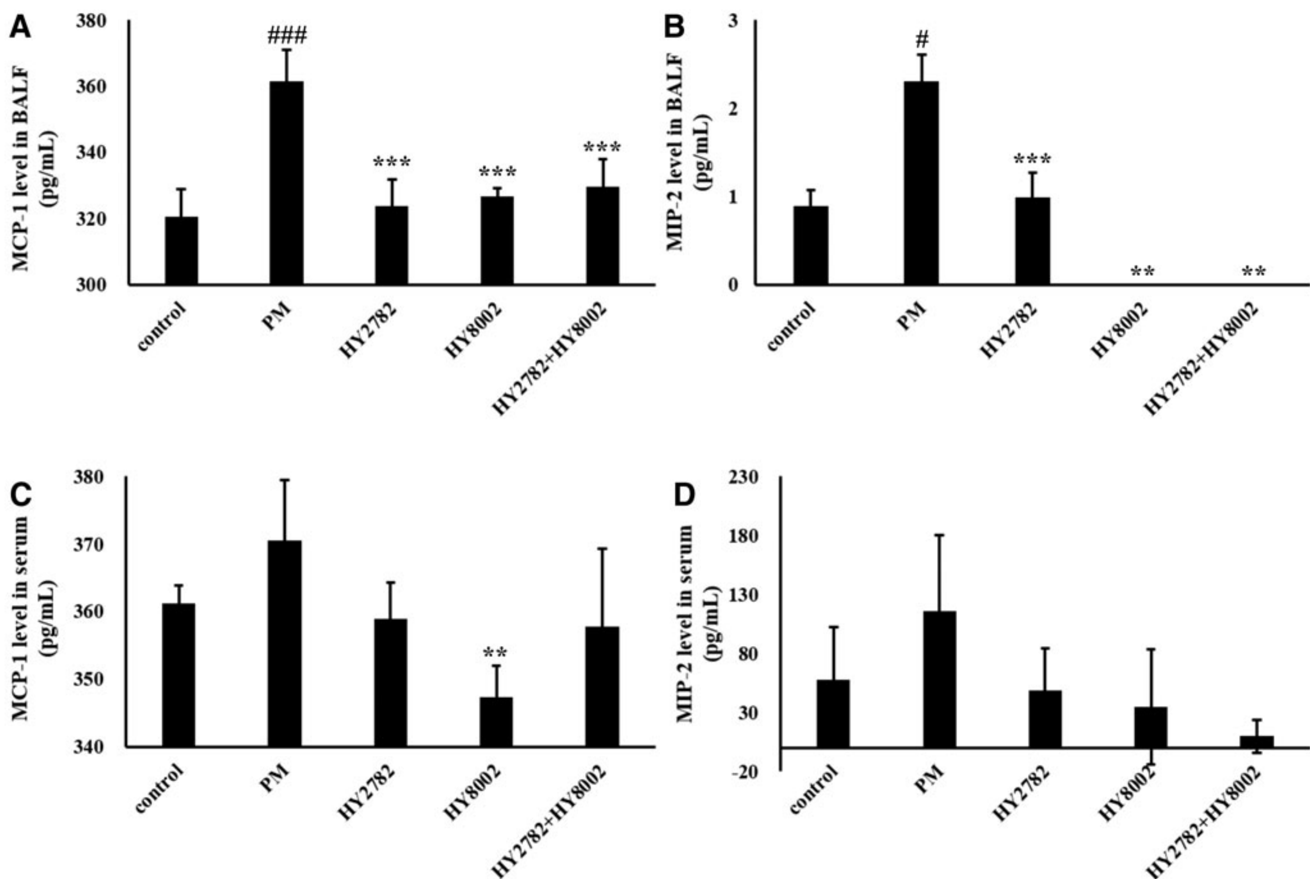

FIG. 1. Chemokine levels in BALF and serum. **(A, B)** BALF was collected and analyzed to determine MCP-1 and MIP-2 levels. **(C, D)** Decreased serum chemokine levels in probiotics-fed mice. Data shown as the mean ± SD. Significant differences are indicated as $^{\#}P<.05$ and $^{\#\#\#}P<.001$ when compared with the normal group, and $^{**}P<.01$, and $^{***}P<.001$ when compared with the PM2.5 exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. BALF, bronchoalveolar lavage fluid; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein; PM, particulate matter.

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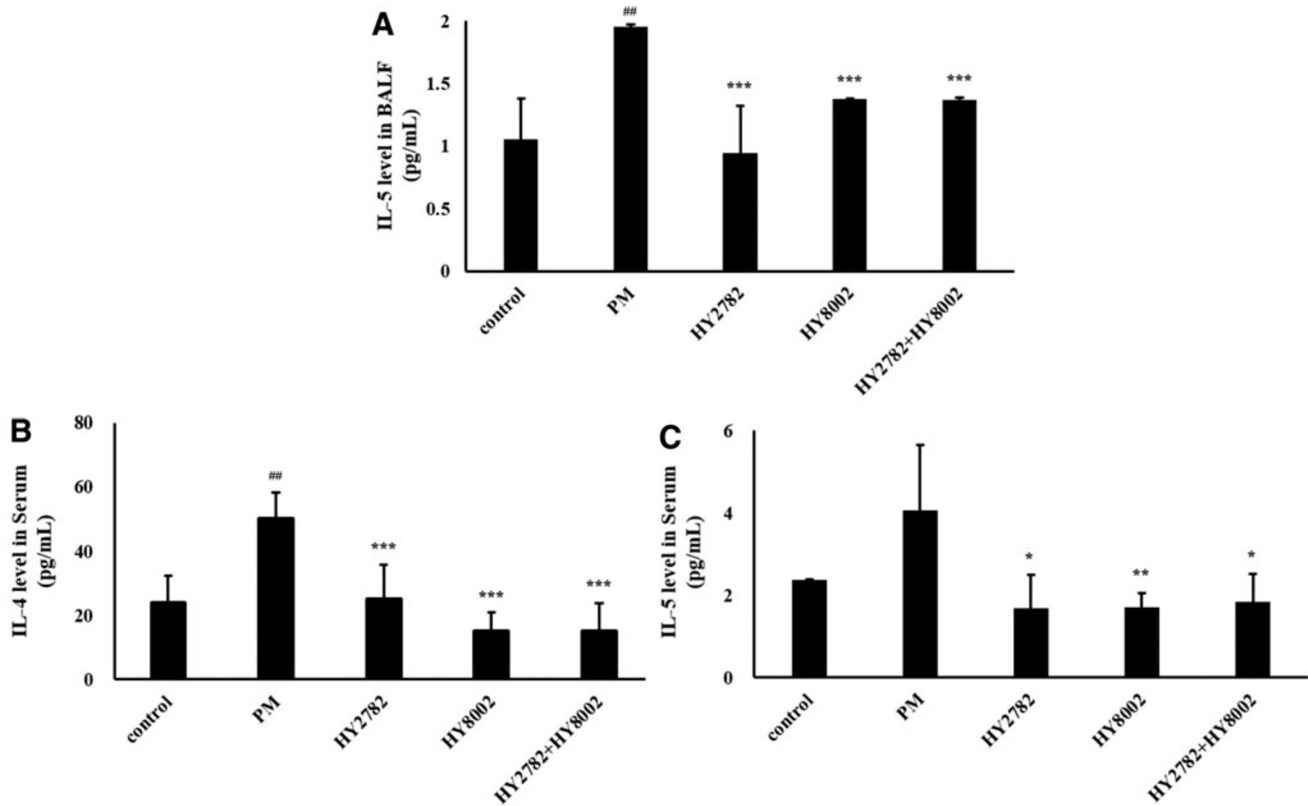

FIG. 2. Cytokine levels in BALF and serum. **(A)** IL-5 levels in BALF. **(B)** IL-4 levels in serum. **(C)** IL-5 levels in serum. Data are shown as the mean ± SD. Significant differences are indicated as $^{\#\#}P < .01$ when compared with the normal group, and $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ when compared with the PM2.5-exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. IL, interleukin.

such as IL-4.[11] Increased MCP-1/MIP-2 protein activates the Th2 response. IL-5 secretion in the BALF of PM-exposed mice was increased by 84.6% (1.95 ± 0.02 pg/mL) compared to that in normal mice (1.06 ± 0.33 pg/mL). The level in the probiotics-fed groups was reduced to 0.95 ± 0.38 pg/mL (Fig. 2). Measurement of IL-4 and IL-5 in the serum revealed increases of 231.5% and 71.0%, respectively. IL-4 and IL-5 in the serum were decreased to normal levels following probiotics intake (Fig. 2).

Tumor necrosis factor-α (TNFα) production in the serum and BALF tended to increase with PM exposure, but not significantly ($P > .05$). There was no significant change following probiotics intake (Fig. 3).

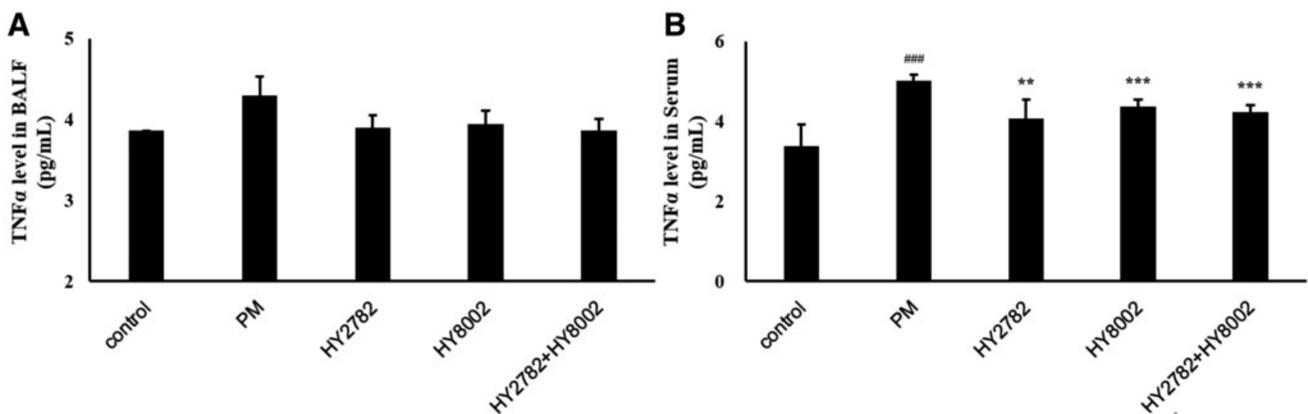

FIG. 3. TNFα levels in serum and BALF. **(A)** TNFα levels in BALF. **(B)** Serum level. Data are shown as the mean ± SD. Significant differences are indicated as $^{\#\#\#}P < .001$ when compared with the normal group, and $^{**}P < .01$, and $^{***}P < .001$ when compared with the PM2.5 exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. TNFα, tumor necrosis factor-α.

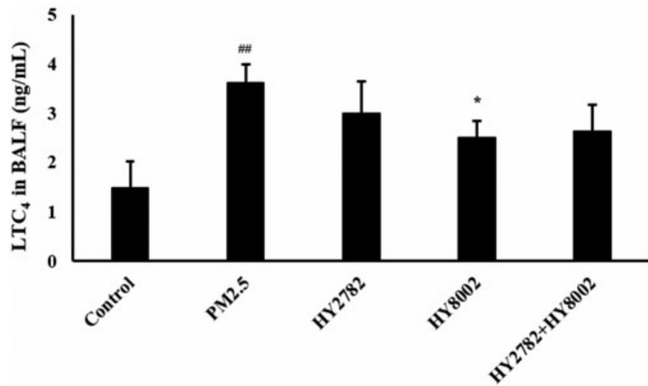

FIG. 4. LTC_4 secretion in BALF. Reduced LTC_4 levels in probiotics-fed mice. Data are shown as the mean ± SD. $^{\#\#}P < .01$ when compared with the normal group, and $^{*}P < .05$ when compared with the PM2.5-exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. LTC_4, leukotriene C4.

In immune cells stimulated by IL-4, secretion of the leukotriene C4 (LTC_4) was increased. LTC_4 is a representative airway hypersensitivity trigger. Measurement of LTC_4 in the BALF of mice exposed to PM showed an increase of 126% (3.61 ± 0.39 ng/mL) compared to normal mice (1.47 ± 0.55 ng/mL) (Fig. 4). Mice fed probiotics showed lower secretion of LTC_4 compared to those exposed to PM (Fig. 4).

Changes in mice immune cell composition by PM2.5 exposure

To confirm the inflammatory impact of PM2.5 on the lungs, the mice were exposed to PM2.5 and immune cells in the BALF were counted. Neutrophils, lymphocytes, monocytes, and eosinophils in the BALF were significantly increased in the PM2.5 group compared to in normal control mice (Fig. 5). The number of neutrophils in the BALF was $0.13 \pm 0.01 \times 10^3$ cells/μL in PM2.5-exposed mice, which was significantly decreased to $0.06 \pm 0.01 \times 10^3$ cells/μL after HY2782 intake (Fig. 5, $P < .01$). Ingestion of probiotics has been shown to reduce lung inflammation via PM2.5 exposure.

Reduction in reactive oxygen species after probiotics ingestion

It has been suggested that reactive materials in fine dust can directly induce oxidative stress.[12] In addition, reactive oxygen species (ROS) induced by PM increases the levels of chemokines such as MCP-1 and MIP-2. Therefore, the regulation of ROS is an important factor controlling airway inflammation caused by PM exposure. Probiotics have been shown in many studies to have antioxidant effects. Therefore, inhibition of ROS by probiotics may attenuate airway inflammation.

The gene expression of nuclear factor erythroid 2-related factor 2 (NRF2), a representative oxidative stress regulator, was slightly increased in PM2.5-exposed mice and increased significantly after ingestion of probiotics (Fig. 6). In contrast, aryl hydrocarbon receptor, another oxidative stress

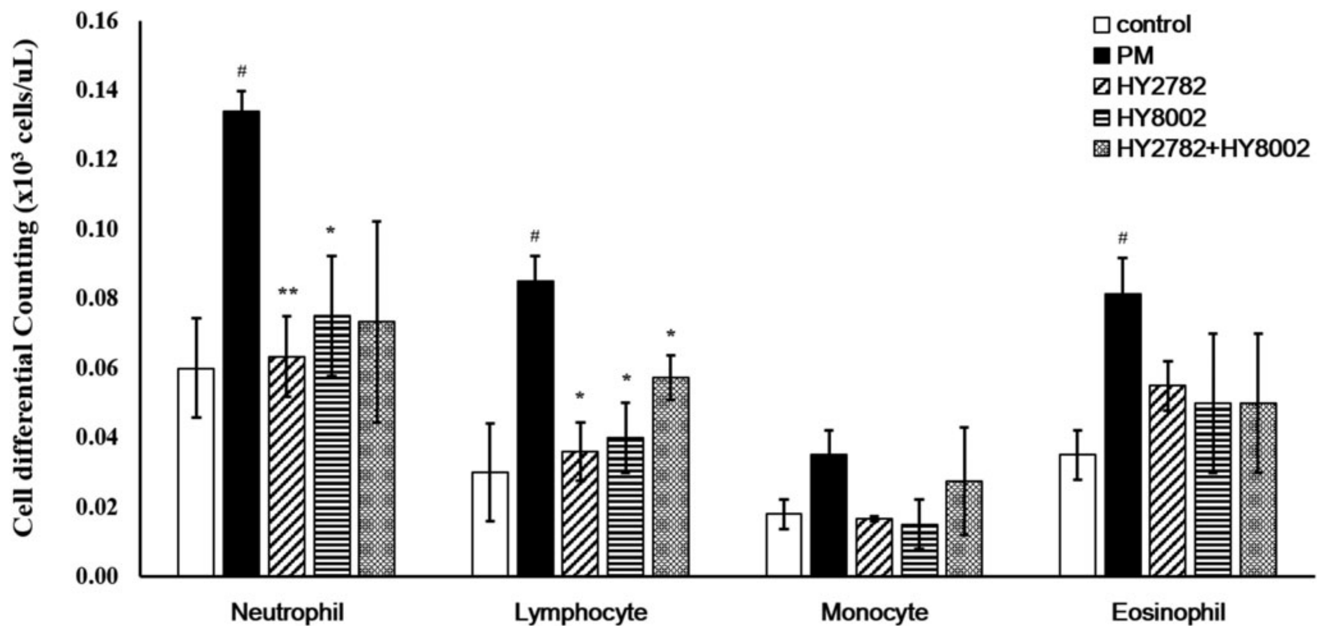

FIG. 5. Immune cell composition in the BALF. The immune cell count was increased by PM2.5 exposure and was decreased by probiotic intakes. Data are shown as the mean ± SD. $^{\#}P < .05$ when compared with the normal group, and $^{*}P < .05$, and $^{**}P < .01$ when compared with the PM2.5 exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice.

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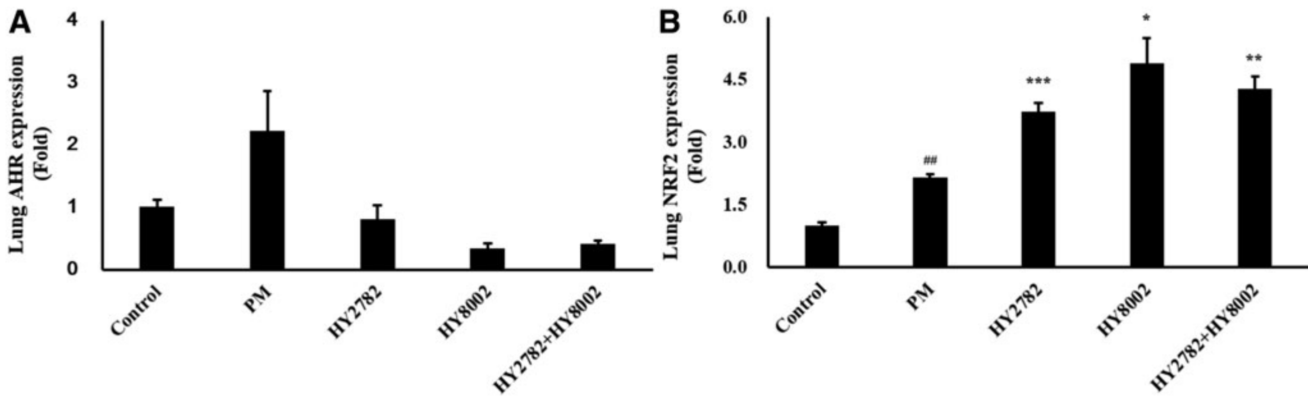

FIG. 6. Antioxidant gene expression levels in organs. Changes in expression levels of **(A)** AHR and **(B)** NRF2 gene in the lung. Data are shown as the mean ± SD. $^{\#\#}P<.01$ when compared with the normal group, and $^{*}P<.05$, $^{**}P<.01$ and, $^{***}P<.001$. when compared with the normal control group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. AHR, aryl hydrocarbon receptor; NRF2, nuclear factor erythroid 2-related factor 2.

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regulator, was increased in PM2.5-exposed mice, but decreased after probiotics ingestion (Fig. 6).

The antioxidant enzymes glutathione peroxidase 1 (GPX-1) and glutathione peroxidase 2 (GPX-2) showed similar patterns. Mice exposed to PM2.5 exhibited slightly increased expression levels of GPX-1/2, which was increased significantly after ingestion of probiotics. Another antioxidant enzyme, NAD(P)H dehydrogenase quinone-1, was slightly increased after exposure to PM2.5, regardless of probiotic intakes (Fig. 7).

The expression levels of superoxide dismutase 1 (SOD-1), superoxide dismutase 2 (SOD-2), and catalase (CAT), which are representative antioxidant enzymes in the body, were measured in the mouse lung tissue. SOD-1 and SOD-2 showed similarly increased patterns after exposure to PM2.5. After ingestion of probiotics, the expression level was higher than after exposure to PM2.5 alone. In contrast, CAT gene expression was decreased after ingestion of probiotics, which was similar to that in normal mice (Fig. 8).

Finally, the activity of antioxidant enzymes in serum was confirmed. Both SOD and CAT were increased after PM2.5 exposure. Consumption of probiotics with PM2.5 exposure increased SOD, but decreased CAT (Fig. 9). These results confirm that the enzyme activity in the serum showed a similar pattern as gene expression in the lung tissue (Fig. 9).

Taken together, ROS increases induced by PM2.5 are regulated by NRF2, the expression of which is increased by probiotics, and SOD enzyme activity.

Probiotics attenuate lung inflammation in PM2.5-exposed mice

We found that more inflammatory infiltration occurs in the lung tissue of mice exposed to PM2.5. In contrast,

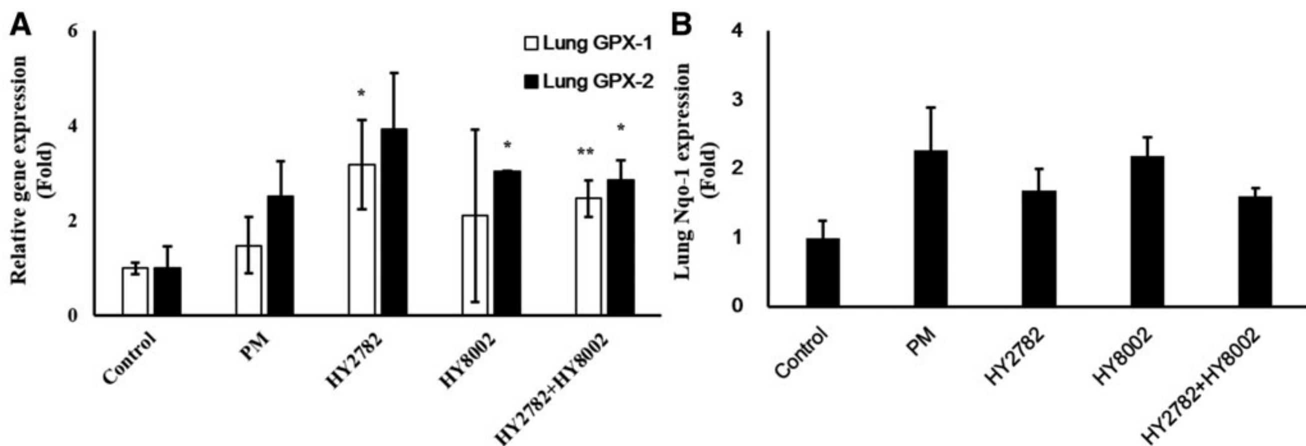

FIG. 7. Antioxidant gene expression in organs. Changes in expression levels of **(A)** GPX-1/2 and **(B)** NAD(P)H dehydrogenase quinone-1 (Nqo-1) in lung. Data are shown as the mean ± SD. $^{*}P<.05$ and $^{**}P<.01$ when compared with the normal control group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. GPX-1/2, glutathione peroxidase 1/2.

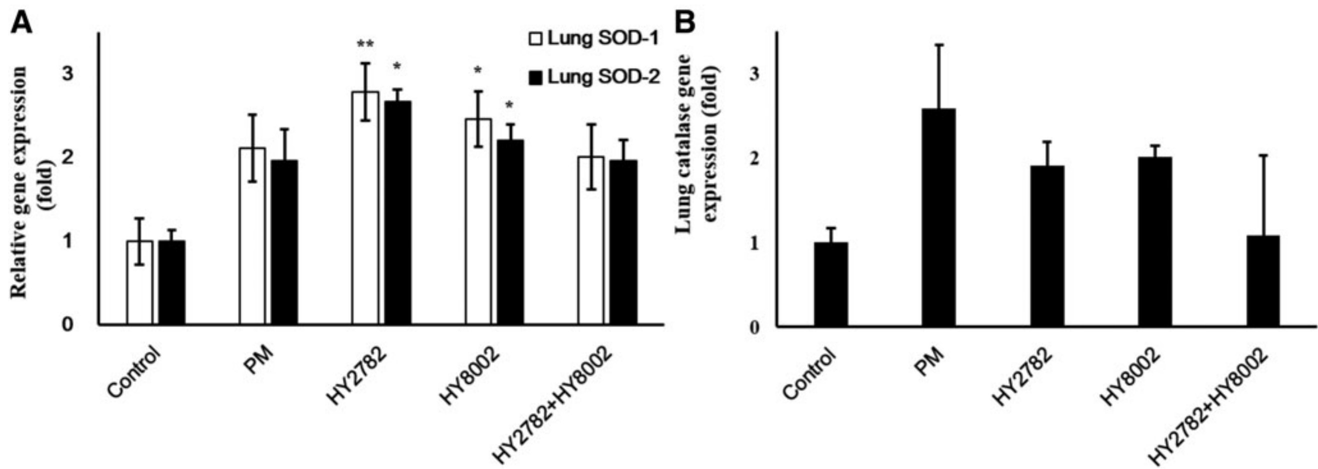

FIG. 8. Antioxidant gene expression in organs. Changes in the expression level of **(A)** SOD-1/2 and **(B)** CAT in the lung. Data are shown as the mean ± SD. $*P<.05$ and $**P<.01$ when compared with the normal control group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice. CAT, catalase; SOD-1/2, superoxide dismutase 1/2.

feeding of probiotics to the mice relieved lung tissue inflammation. In addition, measurement of the lung mucosa thickness showed that the value in normal mice was $73.5 \pm 8.93\,\mu$m, whereas that in mice exposed to PM2.5 increased to $158.98 \pm 54.09\,\mu$m. Intake of probiotics decreased mucosa thickness to $60.10 \pm 9.62\,\mu$m (HY2782), $58.66 \pm 12.76\,\mu$m (HY8002), and $49.35 \pm 7.75\,\mu$m (HY2782 + HY8002) (Fig. 10).

Taken together, ROS induced by PM2.5 increased airway inflammatory factors such as MCP-1, MIP-2, LTC4, IL-4, and IL-5 and induced lung inflammation along with the recruitment of immune cells. Ingestion of probiotics increases antioxidant factors such as NRF2 to regulate ROS *in vivo*. Increased NRF2 appears to reduce lung inflammation by increasing antioxidant-related factors such as GPX and SOD, inhibiting intracellular ROS production, and decreasing chemokine and cytokine secretion, thereby inhibiting immune cell infiltration.

DISCUSSION

The World Health Organization estimates that 3 million deaths annually can be attributed to air pollution, Considering the number of reported deaths, and the lack of certainty for many suspected pollution-related deaths, the actual death toll may be as high as 6 million. These figures are used worldwide that's 5% of deaths per year (55 million). In particular, Korea has a relatively higher concentration of fine dust than most other countries.[13,14] Among the air pollutants, fine dust, sulfur dioxide, nitrogen dioxide, ozone, and carbon monoxide are important in the development and exacerbation of asthma and lung disease progression.[15] These substances affect allergen sensitization by acting on asthma patients' airways and cause airway epithelial damage, as well as facilitate the infiltration of allergens through damaged airways, increasing the immune response and airway inflammation.

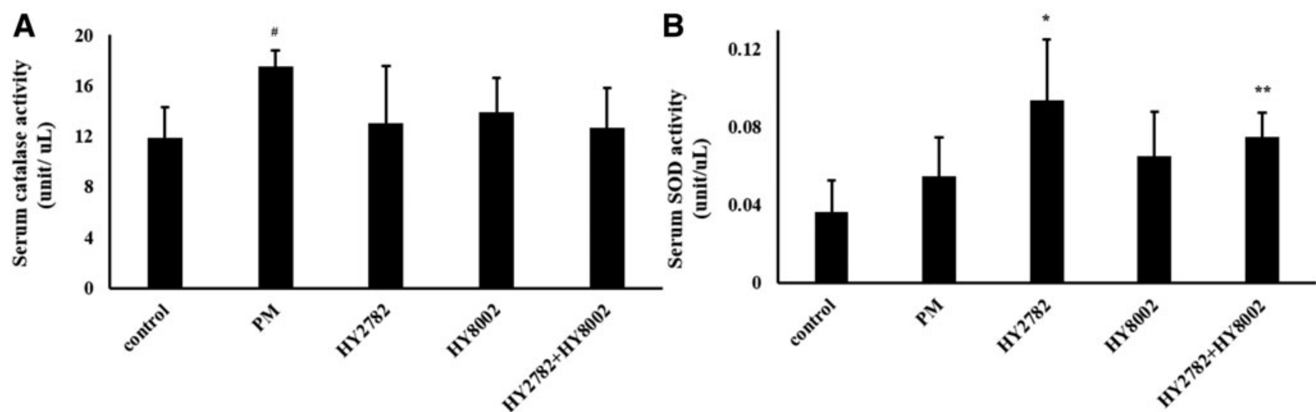

FIG. 9. Antioxidant enzyme activity in the serum. **(A)** Serum CAT activity. **(B)** Serum SOD activity. Data are shown as the mean ± SD. $^{\#}P<.05$ when compared with the normal group, $*P<.05$, and $**P<.01$ when compared with the PM2.5-exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice.

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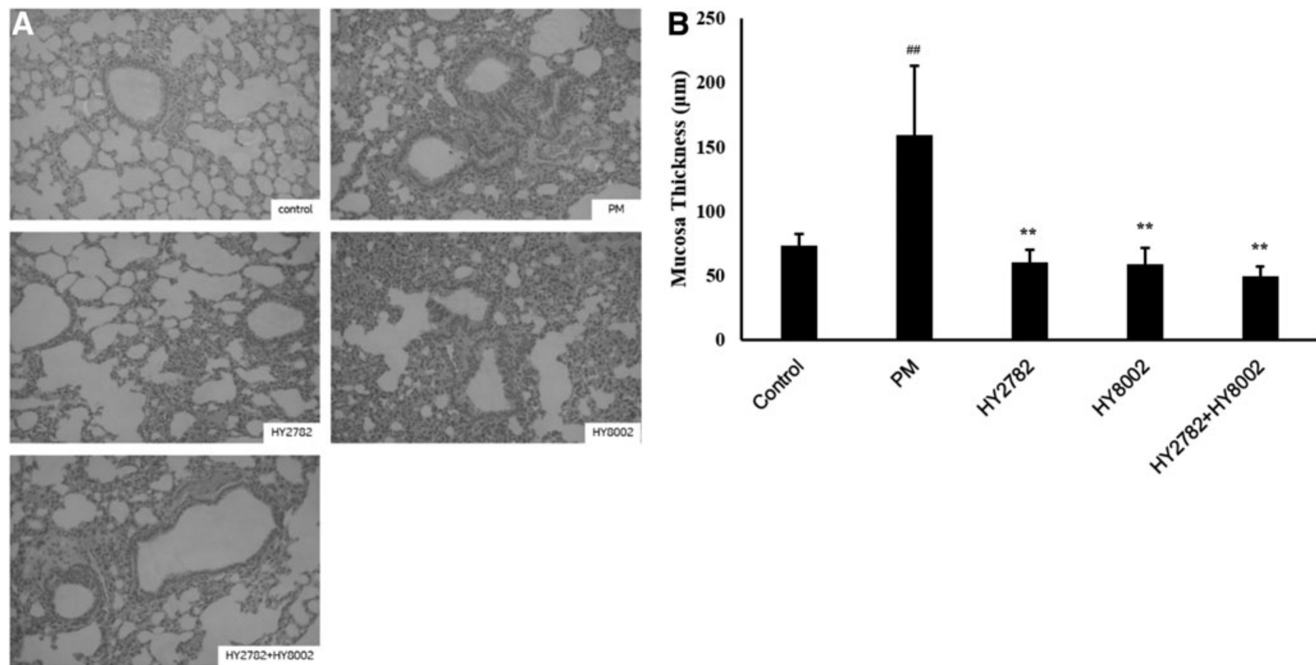

FIG. 10. Effect of probiotics on histology of lung tissue in PM2.5-induced mice. C57BL/6 mice were challenged with PM intranasal administration. **(A)** Lung was stained with H&E (×200 magnification). **(B)** Mucosa thickness was measured by ImageJ software. $^{\#\#}P<.01$ when compared with the normal group and $^{**}P<.01$ when compared with the PM2.5 exposed group. Control, normal mice; PM, PM2.5-exposed mice; HY2782, *Lactobacillus* HY2782-fed mice; HY8002, *Bifidobacterium* HY8002-fed mice; HY2782+HY8002, HY2782+HY8002-fed mice.

Most allergy disorders disrupt the Th1/Th2 balance, resulting in a more active Th2 immune response and high levels of Th2 type cytokines, such as IL-4, IL-5, and IL-13, which increases the production of immunoglobulin E. The immune control function of probiotics, which is strain-specific, increases the release of IL-12 and TNFα in resin cells, whereas *Lactobacillus* suppresses the inflammatory cytokines IL-12 and TNFα. In contrast, most probiotics commonly increase the secretion of IL-10 or transforming growth factor-*β*, a cytokine with immune control functions. Therefore, the Th2 immune response is suppressed and eventually the balance changes toward the Th1 immune response, alleviating the allergic symptoms.

When PM2.5 enters the respiratory tract, a large number of inflammatory cells moves to the airways or bronchi, induce excessive immune response activity, increase airway hyperresponsiveness and mucus secretion, and leads to asthma symptoms such as cough, wheezing, and dyspnea.[16] According to our data, *L. casei* HY2782 and *B. animalis* sp. lactis exhibited antiallergic effects against allergy symptoms caused by external environmental factors by reducing MCP-1 and MIP-2 activity that had been rapidly increased in mice which inhaled PM2.5 and reduced the activity of IL-4 and IL-5 induced by oxidative stress; these effects reduce the incidence of asthma. MCP-1 recruits monocytes, memory T cells, and dendritic cells to inflammation sites produced by either tissue injury or infection,[17] but does not attract neutrophils or eosinophils.[18] MIP-2 is a chemokine that functions as a chemotactic for neutrophils. Asthma is mainly caused by the action of eosinophils and neutrophils; neutrophils are particularly important in severe asthma. Neutrophils in peripheral blood infiltrate into the lung bronchus through cell migration, and migrated neutrophils accumulate without being removed by normal cell death, resulting in various inflammatory reactions and secretion of inflammatory substances; these events eventually cause tissue damage. Probiotics can help prevent asthma symptom caused by PM2.5

In addition to its proinflammatory properties, ultrafine PM has been suggested to be a strong prooxidant. Using diesel exhausted particle (DEP) as a model of air PM, studies have shown that DEP induces oxidative stress and inflammatory response in different cell lines and animal models.[19] DEPs have also been shown to induce superoxide anion (O_2^-) production in macrophages, bronchial epithelial cells, and microsomes.[20,21] A previous study demonstrated induction of ROS and oxidative damage in human lung epithelial cell line exposed to PM10 as evidence in decreased glutathione levels and activity of antioxidant enzymes such as SOD, CAT, glutathione reductase, and glutathione-*S*-transferase.[22] According to our data, ROS induced by PM increased the expression of antioxidant-related enzymes in the body by increasing the expression of the antioxidant enzyme regulatory gene Nrf2, which was induced by HY2782 and HY8002. In addition, SOD and CAT activities were very high in the blood of PM-inhaled mice. In conclusion, these finding show that HY2782 and HY8002 can scavenge PM-induced ROS and attenuate ROS-mediated pulmonary inflammation induced by PM.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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