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ORIGINAL RESEARCH PAPER

Protective effect of *Lactobacillus casei* HY2782 against particulate matter toxicity in human intestinal CCD-18Co cells and *Caenorhabditis elegans*

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Abstract
Objectives To investigate the preventive effect of *Lactobacillus casei* HY2782 on toxicity induced by particulate matter (PM, inhalable particles less than 10 μm in diameter) in human intestinal CCD-18Co cells and a model animal *Caenorhabditis elegans*.
Results *L. casei* HY2782 treatment prevented PM-induced intestinal cell death via cellular reactive oxygen species production and membrane disruption attenuation. PM significantly decreased the total number of eggs laid and the body bending activity of *C. elegans*, demonstrating PM toxicity. *L. casei* HY2782 treatment restored the reproductive toxicity and decline in locomotion activity induced by PM in *C. elegans*. Overall, *L. casei* HY2782 attenuated PM toxicity in vitro in cultured intestinal cells and in vivo in the model nematode.
Conclusion Our study provides a potential clue for developing *L. casei* HY2782 probiotics that attenuate PM-induced cellular and physiological toxicity; however, further in-depth preclinical trials using mammalian animal models and clinical trials are required.

Keywords Air pollution · *Caenorhabditis elegans* · *Lactobacillus casei* · Particulate matter · Probiotics

Introduction

Air pollution is one of the most serious social concerns worldwide because air pollution affects diverse living organisms, including humans. Air pollution chronically induces and aggravates various human diseases that result in increased social costs. Particulate matter (PM), fine particles with a diameter below 10 μm, are known to be associated with various human diseases, such as chronic respiratory, cardiovascular, and allergic diseases as well as cancers, diabetes, and premature mortality (Kim et al. 2015). The toxic effect of PM originates from mainly its toxic components, such as heavy metals (e.g., arsenic, cadmium, lead, iron, etc.) and organic substances (e.g., polycyclic aromatic

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hydrocarbons, nitro compounds, aldehydes, and bacterial endotoxins) (Forman and Finch 2018).

To prevent air pollution-associated human health problems, reduced exposure to PM of epithelial cells can be achieved through decreasing PM content in inhaled air by using an indoor air-filtering system and personal dust masks. By contrast, improvement of personal resistance to toxic chemicals via consumption of detoxifying and immune modulatory nutraceuticals can be an alternative strategy. For example, many dietary phytochemicals and bioactive food components are known to possess protective effects against PM toxicity via the induction of the intracellular antioxidant defense and detoxification enzyme systems (Jin et al. 2016; Li et al. 2013).

Lactic acid bacteria (LAB) have received much attention as functional foods for improving human health for many years. Probiotic bacteria, such as some edible *Lactobacillus* and *Bifidobacterium* bacteria, are conventionally known to have human health functions, including improvement of gut microbial composition, attenuation of gastrointestinal discomfort, alleviation of constipation, and immune modulatory effects (Sanders et al. 2014). Probiotic bacteria are generally considered alive in the host gut and exert various beneficial effects (Clark and Hodgkin 2014). In addition, a recently prodigious study regarding the gut microbiome suggested that probiotics have therapeutic effects against various human diseases, including inflammatory bowel diseases, obesity, diabetes, hypercholesterolemia, cancer, atopic dermatitis, allergy-associated disorders, and immune-mediated disorders (George Kerry et al. 2018; Sanders et al. 2014). Since, similar to detoxifying phytochemicals, probiotic bacteria can activate intracellular defense and detoxification enzymes (Mu et al. 2019; Nakagawa et al. 2016), we hypothesized here that LAB could be used for attenuating air pollution-associated health dysfunction. We especially focused on protective effects of probiotic bacteria against PM-induced toxicity because *Lactobacillus* probiotics are known to activate the Nrf2 signaling pathway in cultured human cells (Mu et al. 2019) as well as various animal models (Liu et al. 2019; Nakagawa et al. 2016; Zhao et al. 2019), and Nrf2 is a master transcription factor regulating intracellular antioxidant and detoxification enzymes (Li et al. 2013; Nakagawa et al. 2016; Zhao et al. 2019). For this purpose, we selected *Lactobacillus casei* HY2782 as a model probiotic bacterial strain for the present study because this strain has been one of the most popular bacterial strains for the commercial production of yogurt, a probiotic fermentation product of milk, for a long time in Korea.

In the present study, we exploited cultured human intestinal CCD-18Co cells to evaluate PM toxicity and protective effects of *L. casei* HY2782. PM is known to induce various intestinal diseases, intestinal inflammation, and cell toxicity (Kish et al. 2013; Li et al. 2019), and probiotics can directly interact with intestinal epithelial cells and the intestinal microbiome (Cha et al. 2018; Mu et al. 2019; Sanders et al. 2014). We also used the non-mammalian *Caenorhabditis elegans* model as an animal model instead of a rodent model; *C. elegans* is a model animal used for various basic and applied biological studies, including probiotic and nutraceutical screening (Clark and Hodgkin 2014; Kim et al. 2019; Kim and Mylonakis 2012; Nakagawa et al. 2016) and risk assessment of environmental toxicants (Lee and Kang 2017; Lee et al. 2017), and is easy to handle and cost effective. Moreover, this animal system lacks animal ethics issues that are present with mammalian animal models and is favorable for developing consumer-friendly functional foods. Prolonged exposure to PM induces significant damage to the locomotion activity, reproduction, and lifespan of *C. elegans* (Chung et al. 2019; Sun et al. 2016; Zhao et al. 2014). Here, we investigated for the first time the preventive effects of *L. casei* HY2782 probiotic bacteria on PM toxicity in in vitro cultured human intestinal cells as well as an in vivo nonmammalian nematode model.

Materials and methods

Materials

PM used in this study (PM10-1, ERM-CZ100, polycyclic aromatic hydrocarbons certified; and PM10-2, ERM-CZ120, metal element certified) was purchased from Sigma. 2′,7′-Dichlorofluorescin diacetate (DCF-DA), dimethyl sulfoxide (DMSO), Hoechst 33342, and propidium iodide (PI) were purchased from Sigma. Dried powder of *L. casei* HY2782 was provided by Korea Yakurt (Yongin, Korea).

Human intestinal cell culture

Human normal intestinal CCD-18Co cells were obtained from the Korean Cell Line Bank (Seoul, Korea). CCD-18Co cells were maintained in Minimum Essential Medium (HyClone, Logan, UT, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA), 100 units/ml penicillin, and 0.1 mg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

Measurement of CCD-18Co cell viability

CCD-18Co cells were seeded in 12-well plates (5×10^4 cells per well) and incubated for 24 h. Next, the cells were incubated with serum-free medium containing PM or *L. casei* HY2782 for 72 h. After treatment, the cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS), and then, EZ-Cytox cell viability assay reagents (Daeil Lab Service, Seoul, Korea) were added and incubated for 1–4 h, as previously described (Kang et al. 2011). The media were transferred to 1.5 ml tubes and centrifuged at 15,000×*g* for 10 min to remove PM, which interferes with the measurement of optical density. Ninety microliters of the supernatant was carefully transferred to a 96-well plate, and the absorbance at 450 nm and 650 nm was measured by using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

Measurement of ROS levels in CCD-18Co cells

ROS levels in the cultured CCD-18Co cells were measured by using an ROS-sensing fluorescent dye, DCF-DA. CCD-18Co cells (6×10^4 to 1×10^5 cells per well) were seeded in 96-well black plates and incubated for 24 h. Next, the cells were washed twice with DPBS and incubated with serum-free medium for 24 h. The cells were then washed twice with DPBS and incubated with serum-free medium without (control) and with 2×10^6 CFU/ml *L. casei* HY2782 for 2 h. Then, the cells were washed twice with DPBS and incubated with serum- and phenol red-free medium containing PM (500 μg/ml) for 4 h. The cells were washed three times with DPBS and incubated with serum-free medium containing 20 μM DCF-DA. Then, the fluorescence intensity (with excitation 485 nm and emission 528 nm) of each well was measured by using a microplate reader.

Observation of the loss of cellular membrane integrity in CCD-18Co cells

CCD-18Co cells (3×10^5 cells per well) were seeded in 6-well plates and incubated for 24 h. The cells were incubated with serum-free medium supplemented with PM or *L. casei* HY2782 for 24 h. After treatment, the cells were washed twice with DPBS, and then, the cells were incubated with the medium containing Hoechst 33342 (2 ng/ml) and PI (20 μg/ml) for 15 min at 37 °C in dark conditions. After staining, the cells were washed once with DPBS and dried at room temperature for 2 h in dark conditions. Fluorescence microscopy images were obtained by using a TE2000U fluorescence microscope (Nikon, Tokyo, Japan) and a ProgRes CFcool camera system (Jenoptik, Jena, Germany). To analyze the disruption of plasma membrane integrity, three random fields were counted for each group. The percentage of PI-positive cells (portion of membrane-disrupted cells) was calculated as follows:

PI-positive cells (%) = number of Hoechst 33342 and PI double-stained cells (pink color) / total number of cells (pink or blue color).

C. elegans maintenance and *L. casei* HY2782 culture for *C. elegans* feeding

Wild-type *C. elegans* N2 (var. Bristol) and the bacterial strain *Escherichia coli* OP50 were obtained from the Caenorhabditis Genetics Center (CGC, University of Minnesota, MN, USA). *C. elegans* was maintained on NGM agar plates at 20 °C using *E. coli* OP50 as a food source. For *C. elegans* feeding experiments, *L. casei* HY2782 was cultured for 14 h at 37 °C in 500 ml of MRS broth (Difco, Detroit, MI, USA). *L. casei* HY2782 was harvested by centrifugation at 3220×*g* and 4 °C for 30 min and resuspended in 100 ml of MRS broth. The *L. casei* culture was stored 4 °C for up to 2 weeks. The bacterial foods, *E. coli* OP50 and *L. casei* HY2782, were mixed with PM and dried overnight before *C. elegans* toxicity testing.

Measurement of *C. elegans* growth

Effects of PM and *L. casei* HY2782 feeding on the growth of *C. elegans* were evaluated as described previously (Lee and Kang 2017; Lee et al. 2017). Age-synchronized nematode eggs were incubated on NGM plates coated with bacterial foods (*E. coli* OP50 or *L. casei* HY2782) and PM for 72 h at 20 °C. Then, the body size of worms was measured by using stereomicroscopy and ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Evaluation of reproductive toxicity in *C. elegans*

Reproductive toxicity was evaluated in the model animal *C. elegans* as described previously (Lee and Kang 2017; Lee et al. 2017). Age-synchronized nematode eggs were incubated on NGM plates coated with bacterial foods (*E. coli* OP50 or *L. casei* HY2782) and PM for 64 h at 20 °C. Then, 10 adult worms were transferred to new NGM plates supplemented with 50 µg/ml ampicillin and 50 µg/ml nystatin to minimize bacterial and fungal contamination, and the worms were allowed to lay eggs for 24 h. These egg-laying plates contained heat-inactivated *E. coli* OP50 as a food source and no PM. Worms were transferred to new egg-laying plates, and the egg number was counted every day for 6 days. This experiment was performed in triplicate. The total numbers of egg laid per worms were calculated as previously described (Lee and Kang 2017).

Evaluation of *C. elegans* locomotion activity

Age-synchronized nematode eggs were incubated on NGM plates coated with bacterial foods (*E. coli* OP50 or *L. casei* HY2782) and PM for 72 h at 20 °C. Then, 15–20 adult worms were transferred to slide glass with 10 µl of S-buffer, and video was immediately recorded. The numbers of body bending events for 20 s were counted. Fifteen worms were monitored from each group.

Statistical analysis

The data are expressed as the mean ± standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism 7.0 software (La Jolla, CA, USA). p values < 0.05 were considered statistically significant.

Results

Effect of *L. casei* HY2782 on the cellular toxicity induced by PM10 in CCD-18Co cells

First, we evaluated the toxic effects of PM in human intestinal epithelial CCD-18Co cells because PM and its chemical components, such as heavy metals and polycyclic aromatic hydrocarbons, are reported to induce intestinal cell toxicity and are associated with various intestinal diseases (Harris et al. 2013; Li et al. 2019; Mutlu et al. 2011; Yu et al. 2016). Treatment with PM (500 µg/ml, 1 mg/ml) significantly decreased the viability of CCD-18Co cells compared to that of the vehicle control cells ($p < 0.001$, Fig. 1). Cotreatment with *L. casei* HY2782 significantly

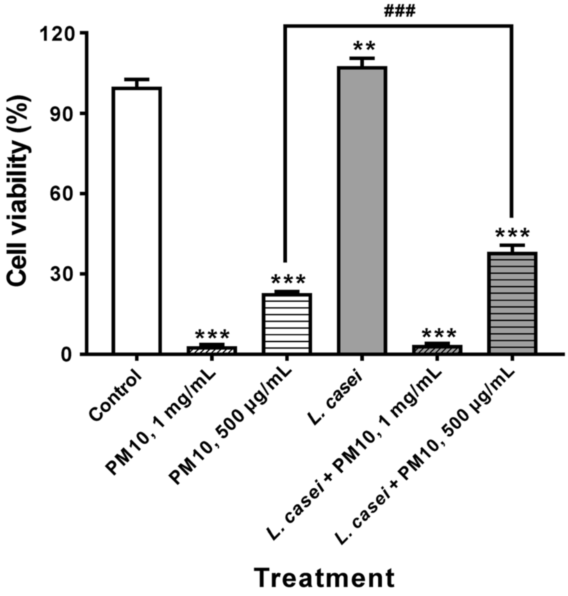

Fig. 1 Effect of *L. casei* HY2782 on the cellular toxicity induced by PM (PM10-1) in human normal intestinal CCD-18Co cells. CCD-18Co cells were treated with PM (0, 0.5, and 1 mg/ml) or *L. casei* HY2782 (2×10^6 CFU/ml) for 72 h. Then, cell viability was determined by using a cell viability assay. The values shown are the means ± SDs from triplicate experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$, compared to the vehicle control. $^{\#\#\#}p < 0.001$, compared between the PM10 (500 µg/ml) single treatment group and the *L. casei* HY2782 + PM10 (500 µg/ml) cotreatment group. The graphs shown are representative of at least three independent experiments

rescued the decreased cell viability of CCD-18Co cells induced by the 500 μg/ml PM treatment ($p < 0.001$). Cotreatment with *L. casei* HY2782 did not reverse the complete cell death of CCD-18Co cells induced by high-concentration PM (1 mg/ml) treatment (Fig. 1).

Effect of *L. casei* HY2782 on the intracellular ROS accumulation induced by PM10 in CCD-18Co cells

Next, we investigated the effect of *L. casei* HY2782 on ROS production in CCD-18Co cells treated with PM (500 μg/ml) because PM increased ROS generation in the cultured intestinal cells in a previous study (Mutlu et al. 2011). In accordance with the previous report, PM treatment significantly increased the cellular ROS levels in CCD-18Co cells ($p < 0.001$). Pretreatment with *L. casei* HY2782 significantly decreased ROS generation in CCD-18Co cells compared to that in cells treated with only PM ($p < 0.001$, Fig. 2).

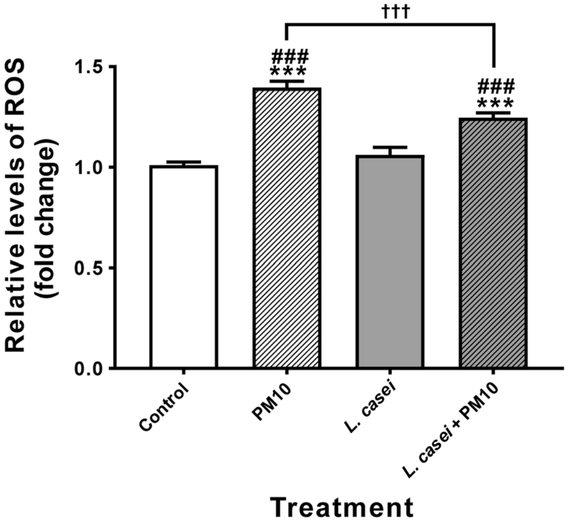

Fig. 2 Effect of *L. casei* HY2782 on the cellular ROS levels induced by PM (PM10-1) in human intestinal CCD-18Co cells. CCD-18Co cells were treated with *L. casei* HY2782 (0 or 2×10^6 CFU/ml) for 2 h. Next, the cells were treated with PM (PM10-1; 0 or 500 μg/ml) for 4 h. Then, the cellular ROS levels were determined by using the DCF-DA assay. Values shown are the means ± SDs ($n = 5$). $^{***}p < 0.001$, compared to the vehicle control. $^{\#\#\#}p < 0.001$, compared to the *L. casei* HY2782 single treatment group. $^{\dagger\dagger\dagger}p < 0.001$, compared between the PM10 only-treated group and the *L. casei* HY2782 + PM10 cotreated group. The graphs shown are representative of three independent experiments

Effect of *L. casei* HY2782 on the loss of plasma membrane integrity induced by PM10 in CCD-18Co cells.

We also investigated the loss of membrane integrity to elucidate the cellular mechanism underlying the preventive effects of *L. casei* HY2782 against the intestinal cell death induced by PM. PM treatment significantly increased the percentage of PI-positive cells compared to that of the vehicle control cells ($p < 0.001$), which indicates that PM induced the loss of membrane integrity in CCD-18Co cells. Cotreatment with *L. casei* HY2782 significantly rescued the loss of plasma membrane integrity of CCD-18Co cells compared with that of PM only-treated cells ($p < 0.001$, Fig. 3). Based on these data, we concluded that *L. casei* HY2782 could attenuate the intestinal cell death induced by PM through the attenuation of intracellular ROS production and loss of plasma membrane integrity.

Effect of *L. casei HY2782* on the reproductive toxicity induced by PM10 in *C. elegans*

Next, we evaluated the in vivo protective effect of *L. casei* HY2782 against PM toxicity in the model animal *C. elegans*. The toxic effects of PM, such as growth retardation, reproductive toxicity, locomotion activity depression and lifespan reduction, were previously reported in a *C. elegans* model (Sun et al. 2016; Zhao et al. 2014). In our study, both PMs (PM10-1 and PM10-2) significantly decreased the total number of eggs laid per worm compared with that for the vehicle control (worms fed *E. coli* OP50 in the absence of PM; $p < 0.05$ and $p < 0.01$ for PM10-1 and PM10-2, respectively), demonstrating strong reproductive toxicity in *C. elegans*. Cotreatment with *L. casei* HY2782 instead of *E. coli* OP50 significantly rescued the reproductive toxicity in *C. elegans* ($p < 0.05$). The single treatment of *L. casei* HY2782 alone did not result in a significant change in the total number of eggs laid (Fig. 4).

We also tested the effect of *L. casei* HY2782 on the growth of *C. elegans* exposed to PM (Supplementary Fig. 1). PM treatment significantly decreased the body size of worms fed *E. coli* OP50 ($p < 0.01$), and these data are in accordance with data in a previous study (Zhao et al. 2014). Cotreatment with *L. casei* HY2782

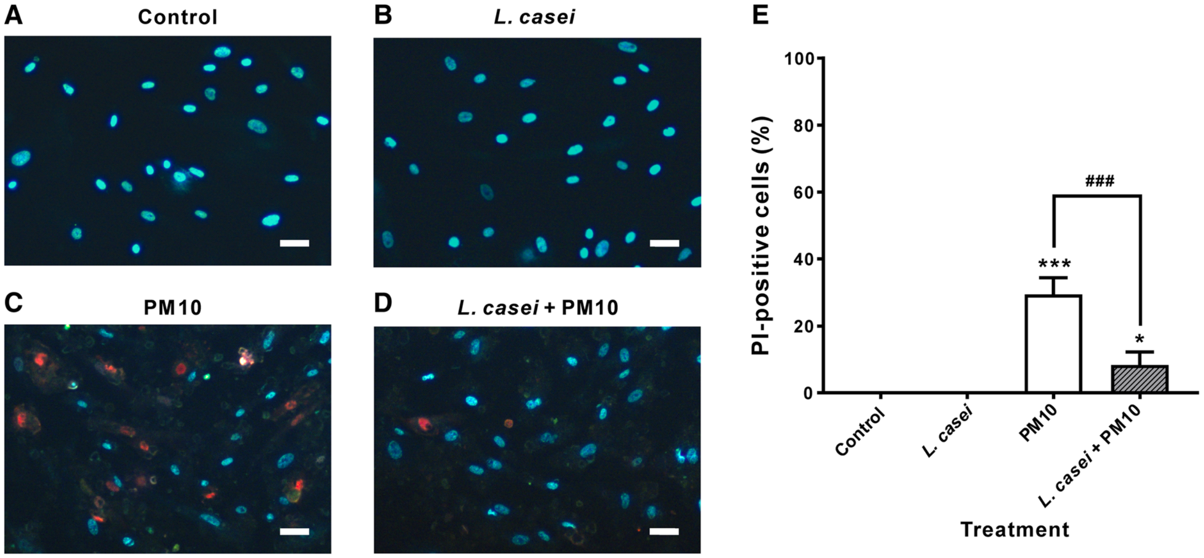

Fig. 3 Effect of *L. casei* HY2782 on the loss of membrane integrity in CCD-18Co cells, which is induced by particulate matter (PM10-1). **a–d** Fluorescence microscopy image of CCD-18Co cells stained with Hoechst 33342 and PI (bar = 50 µm). CCD-18Co cells were treated with PM (500 µg/ml) and *L. casei* HY2782 (2×10^6 CFU/ml) for 24 h. Then, the cells were stained with Hoechst 33342 and PI. **a** Vehicle control cells. **b** *L. casei* HY2782 only-treated cells. **c** PM only-treated cells. **d** Cells treated with both *L. casei* HY2782 and PM. **e** The percentage of PI-positive cells was calculated as described in the Materials and Methods section. The values shown are the means ± SDs from triplicate experiments. $^{*}p < 0.05$ and $^{***}p < 0.001$, compared to the vehicle control. $^{\#\#\#}p < 0.001$, compared between the PM10 single treatment group and the *L. casei* HY2782 + PM10 cotreatment group. The graphs shown are representative of two independent experiments

slightly rescued the growth retardation; however, its effects were not statistically significant.

Effect of *L. casei* HY2782 on the decline in locomotion activity induced by PM10 in *C. elegans*.

Then, we evaluated the preventive effect of *L. casei* HY2782 feeding on the decline in locomotion activity of worms induced by exposure to PM. Both particular matters (PM10-1 and PM10-2) significantly decreased the number of body bending events of *C. elegans* fed *E. coli* OP50 ($p < 0.001$, Fig. 5). Cotreatment with *L. casei* HY2782 and PM significantly increased the number of body bending events compared with those of *C. elegans* treated with PM and *E. coli* OP50 ($p < 0.01$ and $p < 0.001$ for PM10-1 and PM10-2, respectively). *L. casei* HY2782 feeding apparently rescued the locomotion activity to the level of the vehicle control worms (*C. elegans* fed *E. coli* OP50 in the absence of PM) (Fig. 5). Based on these data, we concluded that *L. casei* HY2782 feeding significantly rescued the toxicity induced by PM not only in in vitro cultured intestinal cells but also in an in vivo model animal, *C. elegans*.

Discussion

Here, we found that *L. casei* HY2782 attenuates the toxicity generated by PM in both in vitro cultured human intestinal cells and an in vivo *C. elegans* animal model. This study is the first report of the preventive effect of probiotic bacteria against PM toxicity in cultured human cells and the nematode model. We hope that our study may present a starting point for use of *L. casei* HY2782 probiotic bacteria as a functional food material to alleviate environmental pollutant toxicity. We did not use a mammalian animal model in this study; therefore, our technique using cultured intestinal cells and the *C. elegans* model might be useful for testing various health functions of probiotic bacteria, including *Lactobacillus*, without animal ethics issues that are present with conventional mammalian animal models. Our experimental strategy using cultured intestinal cells and *C. elegans* may be efficiently used for screening new probiotic bacteria

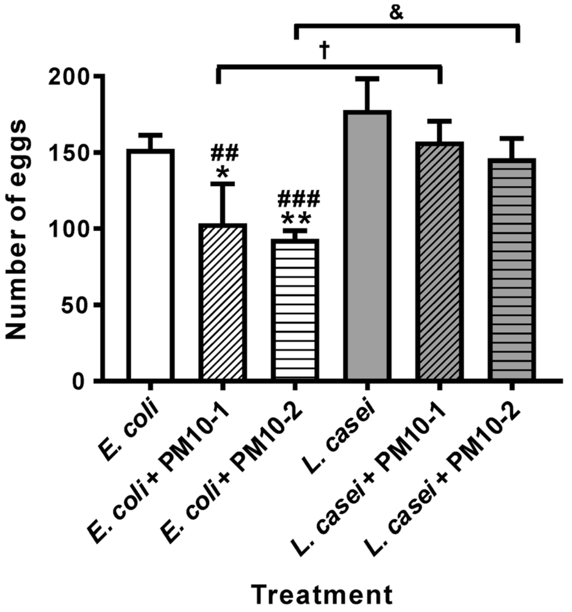

Fig. 4 Effect of *L. casei* HY2782 feeding on the reproductive toxicity induced by PM in *C. elegans*. Age-synchronized N2 eggs were transferred to NGM plates supplemented with PM (PM10-1 or PM10-2, 1 mg/ml) and live bacterial foods (*E. coli* OP50 or *L. casei* HY2782) and incubated for 64 h. The total number of eggs was counted every day for 6 days, and the total number of eggs per worm was calculated. The values shown are the means ± SDs from triplicate experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared to the *E. coli* OP50 without PM group. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$, compared to the *L. casei* HY2782 without PM group. $^{\dagger}p < 0.05$, compared between the *E. coli* OP50 + PM10-1 group and the *L. casei* HY2782 + PM10-1 group. $^{\&}p < 0.05$, compared between the *E. coli* OP50 + PM10-2 group and the *L. casei* HY2782 + PM10-2 group. The graphs shown are representative of at least three independent experiments

and their physiological functions, while further in-depth preclinical rodent studies and human clinical trials certainly should be required.

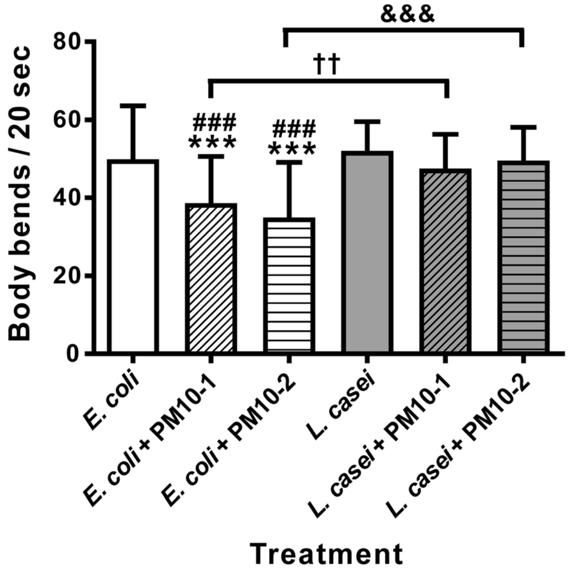

Fig. 5 Effects of *L. casei* HY2782 feeding on the locomotion activity depression induced by PM in *C. elegans*. Age-synchronized N2 eggs were transferred to NGM plates supplemented with PM (PM10-1 or PM10-2, 1 mg/ml) and live bacterial foods (*E. coli* OP50 or *L. casei* HY2782) and incubated for 72 h. Then, the number of body bending events for 20 s was measured. The values shown are the means ± SDs from three independent experiments ($n = 45$). $^{***}p < 0.001$, compared to the *E. coli* OP50 without PM group. $^{\#\#\#}p < 0.001$, compared to the *L. casei* HY2782 without PM group. $^{\dagger\dagger}p < 0.01$, compared between the *E. coli* OP50 + PM10-1 group and the *L. casei* HY2782 + PM10-1 group. $^{\&\&\&}p < 0.001$, compared between the *E. coli* OP50 + PM10-2 group and the *L. casei* HY2782 + PM10-2 group

Based on the present study and our knowledge, we speculate that probiotic consumption, including that of *L. casei* HY2782, may be helpful in preventing PM toxicity in mammalian models because of the following evidence. First, classical probiotics have been considered to improve immune function and have antiinflammatory and antiallergenic effects (George Kerry et al. 2018; Kim and Mylonakis 2012; Sanders et al. 2014). *L. paracasei* L9 consumption attenuated the PM-induced allergic response in a mouse model of asthma (Wang et al. 2017b). Therefore, the antiallergenic and immune modulatory function of probiotics may contribute to the protective effect of *L. casei* against PM-induced toxicity. Second, probiotics including *Lactobacillus* bacteria are known to absorb various heavy metals, decrease the absorption of heavy metals across the intestinal barrier in cultured human intestinal Caco-2 cells (Daisley et al. 2019), and facilitate the excretion of heavy metals in a mouse model (Zhai et al. 2019). Because heavy metals such as lead, cadmium, and mercury are harmful components of PM, biosorption and facilitated excretion of heavy metals by *Lactobacillus* probiotics may contribute to their ameliorative effect on PM toxicity. Third, *Lactobacillus* probiotics are known to activate the Nrf2 signaling pathway and induce various antioxidant and detoxification enzymes in cultured human intestinal Caco-2 cells (Mu et al. 2019), *C. elegans* (Nakagawa et al. 2016), zebrafish (Liu et al. 2019), and mice (Zhao et al. 2019). Polycyclic aromatic hydrocarbons and heavy metals are the main toxicants of PMs (Forman and Finch 2018), and oxidative stress from ROS production is the main cause of PM toxicity (Sun et al. 2016); therefore, activation of cellular antioxidant and detoxification enzymes by probiotic consumption significantly contributes to the protective effect of *Lactobacillus* probiotics against PM-induced toxicity. Fourth, exposure to PM is known to alter intestinal microbial composition and correlate with metabolic dysfunction and intestinal inflammatory responses (Kish et al. 2013; Li et al. 2019). Improvement of the gut microbial composition is one of the most reliable and classic health claims of probiotics (Cha et al. 2018; Sanders et al. 2014). *L. casei* consumption dramatically improved the gut microbial composition in a three-stage continuous microbiome culture system (Cha et al. 2018) and mouse (Wang et al. 2017a), and human trials (Sanders et al. 2014). Therefore, gut microbial dysfunction induced by PM exposure may be improved by the consumption of *Lactobacillus casei* probiotics, which may contribute to ameliorating various health problems induced by PM.

However, the present study has some limitations, such as undefined molecular and biochemical mechanisms underlying the preventive effects of *L. casei* HY2782 probiotics. In a previous study, *L. gasseri* SBT2055 feeding enhanced longevity and increased oxidative stress resistance of *C. elegans* in a Nrf2/SKN-1 and p38 MAPK/PMK-1-dependent manner. *L. gasseri* SBT2055 feeding increased SOD and glutathione synthesis and, as a result, decreased ROS levels in *C. elegans* (Nakagawa et al. 2016). *Lactobacillus* probiotics also increased cellular antioxidant activity through Nrf2 signaling activation and protected against oxidative stresses in cultured mammalian cells and human intestinal Caco-2 cells (Kobatake et al. 2017; Mu et al. 2019). PM contains various toxic heavy metals and toxic organic chemicals, such as polycyclic aromatic hydrocarbons, and induced ROS production and toxic effects in *C. elegans* (Sun et al. 2016; Zhao et al. 2014). *L. casei* strains and *L. acidophilus* NCFM enhanced immune function in *C. elegans* via the Toll-like receptor homolog TOL-1 and p38 MAPK/PMK-1 signaling pathway (Kamaladevi and Balamurugan 2016; Kim and Mylonakis 2012). The activation of Nrf2 and p38 MAPK signaling and the subsequent upregulation of various detoxification and antioxidant enzymes might be responsible for the protective effects of *L. casei* HY2782 against PM toxicity. The immune modulatory effects via Toll-like receptor and p38 MAPK signaling also contribute to the preventive effects of *L. casei* HY2782 against PM-induced inflammatory and immune dysfunction. Therefore, the reevaluation of preventive activity against PM toxicity in mutant *C. elegans* strains lacking *skn-1*, *tol-1*, or *pmk-1* genes is worthwhile to elucidate the molecular mechanism underlying the *L. casei* HY2782 protective effects against PM toxicity. Currently, we are investigating gene expression profiling in both the cultured human intestinal cells and *C. elegans* treated with PM and *L. casei* HY2782 to elucidate the molecular mechanism underlying the preventive effects. We also tested the preventive effects of various other LAB against PM toxicity to elucidate whether the protective effects are strain specific.

In addition, further in-depth preclinical studies using mammalian animal models and clinical trials are required to develop *L. casei* HY2782 as a functional food material. In particular, intranasal treatment with PM in a rodent model should be required in the next step. Chronic exposure to PM or acute exposure to PM with immune stimulants should be a possible PM toxicity model in the rodent model. PM exposure by using an aerosol chamber may be more precise for testing PM inhalation toxicity in various animal models. The efficacy, side effects, and minimum dose of *L. casei* HY2782 in preclinical animal models as well as its detailed immunological mechanism in rodent models should be fully elucidated.

Here, we report for the first time that *L. casei* HY2782 ameliorated PM toxicity in cultured human intestinal CCD-18Co cells and the model animal *C. elegans*. *L. casei* HY2782 attenuated CCD-18Co cell toxicity induced by PM through the prevention of intracellular ROS production and of the loss of membrane integrity. PM significantly aggravated the reproduction and locomotion activity of *C. elegans*, and *L. casei* HY2782 feeding rescued these PM toxicity effects. Our data using in vitro cultured intestinal cells and an in vivo nematode model may reveal the potential of *L. casei* HY2782 as a functional food material for alleviating PM toxicity; however, further in-depth studies of the molecular mechanism underlying the *L. casei* HY2782 effects as well as preclinical tests using mammalian animals and clinical trials should be required.

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Supporting information Supplementary Fig. 1 Effect of *L. casei* HY2782 feeding on the growth retardation induced by PM in *C. elegans*.

Compliance with ethical standards

Conflict of interest A portion of the technology described in this article (Korea patent, 10–2017-0177415) has been transferred from KIST to Korea Yakurt Co., Ltd. The funding sponsors had no roles in the design of the study; in the collection, analyses, or interpretation of data; or in the writing of the manuscript.

Ethical approval This study does not contain any experiments involving human or mammalian subjects.

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