



# Effects of *Lactobacillus curvatus* MG5246 on inflammatory markers in *Porphyromonas gingivalis* lipopolysaccharide-sensitized human gingival fibroblasts and periodontitis rat model

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**Abstract** This study investigated the effects of *Lactobacillus curvatus* MG5246 on periodontitis inflammation. Cell-free supernatants (CFS) prepared from *L. curvatus* MG5246 decreased prostaglandin E2 production and cyclooxygenase-2 gene expression by 60% and 78% in *Porphyromonas gingivalis*-lipopolysaccharide stimulated human gingival fibroblasts at 400 µg/mL. Gene expressions of tumor necrosis factor- $\alpha$ , interleukin-6, matrix metalloproteinases, and chemokines were significantly downregulated by CFS treatment ( $p < 0.05$ ). *L. curvatus* MG5246 ( $2 \times 10^8$  CFU/day, 8 weeks) administration significantly improved alveolar bone loss in the ligature-induced periodontitis rat model. Elevated mRNA expression of the receptor activator of nuclear factor- $\kappa$ B ligand/osteoprotegerin ratio in the gingival tissue was significantly decreased by *L. curvatus* MG5246 administration ( $p < 0.05$ ). Moreover, *L. curvatus* MG5246 showed sufficient tolerance in simulated gastrointestinal conditions (gastric tolerance: 89.48%, intestinal tolerance: 98.62%) and did not show antibiotic resistance and hemolytic activity. Therefore, *L. curvatus* MG5246 has the potential as novel oral probiotics.

**Keywords** *Lactobacillus curvatus* MG5246 · Human gingival fibroblasts · Inflammatory cytokine · Chemokine · Ligature-induced periodontitis rat

## Introduction

Periodontal disease, one of the most common and frequent chronic diseases, has become a global concern. Periodontitis is highly associated with various systemic diseases, including rheumatoid arthritis and cardiovascular diseases (Bartold et al., 2005; Beck and Offenbacher, 2005). *Porphyromonas gingivalis*, a Gram-negative anaerobic bacteria, is a major periodontal pathogen, and lipopolysaccharide (LPS) from *P. gingivalis* stimulates host inflammatory responses (Wang et al., 2000). The binding between LPS and toll-like receptors (TLR) increases the expression of proinflammatory cytokines [interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )] and promotes the destruction of periodontal tissue (Sun et al., 2010). Human gingival fibroblasts (HGF-1 cells) are dominant resident cells in gingival connective tissue and modulate periodontal homeostasis. HGF produces inflammatory cytokines upon binding of *P. gingivalis* LPS (*Pg*-LPS) to TLR4 (Wang et al., 2000).

Allaker and Stephen (2017) reported that probiotics have great potential to be used as an effective interface to recover homeostasis in oral microbial dysbiosis. *Lactobacillus curvatus* is one of the probiotics isolated from variety of fermented foods, such as vegetables, cereals and cheeses (Terán et al., 2018). *L. curvatus* SMFM2016-NK effectively decreased osteoclastic cell numbers and improved alveolar bone resorption in the periodontitis rat model (Choi et al., 2021a). In addition, fermented milk prepared with *L. curvatus* SMFM2016-NK also

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downregulated inflammatory gene expression in the oral cavity of periodontitis rats and increased the composition and diversity of the gut microbiome (Choi et al., 2021b).

This study was conducted to evaluate the efficacy of *L. curvatus* MG5246 as an oral probiotic. To achieve this goal, the cell free supernatant (CFS) of *L. curvatus* MG5246 was prepared, and the anti-inflammatory effects were analyzed using Pg-LPS-stimulated HGF-1 cells. Furthermore, the effects of *L. curvatus* MG5246 on alveolar bone loss were examined in an experimental periodontitis rat model.

## Materials and methods

### Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin were purchased from WelGENE Inc. (Daegu, Korea). Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) enzyme-linked immunosorbent assay kit was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Taqman® Universal master mix, Taqman® probes (5'-fluorescein based reporter dye; 3'-TAMRA quencher) and high capacity RNA-to-cDNA kit were purchased from Applied Biosystems (Foster City, CA, USA). *L. curvatus* MG5246 powder was kindly provided from Mediogen (Jecheon, Korea). Pepsin, pancreatin, and other reagents were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

### Characteristics of *L. curvatus* MG5246

#### *Survival in simulated gastrointestinal conditions*

To evaluate the gastrointestinal resistance of *L. curvatus* MG5246, the strain was harvested by centrifugation (3460 × g, 10 min) after culture in MRS broth at 37 °C for 24 h. The pellets were washed twice with phosphate buffered saline (PBS) adjusted to pH 7 and resuspended (10<sup>7</sup> CFU/mL) in simulated gastric fluid (SGF; 3 g/L of pepsin in PBS, pH 3 and pH 4) and simulated intestinal fluid (SIF; 1 g/L of pancreatin in PBS, pH 7 and 8). The viable cells were determined on MRS agar after incubated at 37 °C for 3 to 4 h (Maragkoudakis et al., 2006).

Bacterial survival was calculated as follows:

$$\text{Survival(\%)} = \frac{\log \text{CFU of viable cells survived}}{\log \text{CFU of initial viable cells}} \times 100$$

#### *Antibiotic resistance*

The antibiotic resistance of *L. curvatus* MG5246 was assayed using the minimum inhibitory concentration (MIC) test strip method. The *L. curvatus* MG5246 was harvested by centrifugation (3460 × g, 10 min) after culture in MRS broth at 37 °C for 18 h, washed twice with PBS (pH 7), and resuspended in PBS to obtain 0.5 McFarland turbidity. The suspended solution was incubated in LSM broth (a mixture of 90% Iso-Sensitest (OXOID, Hampshire, UK) and 10% MRS broth) with 1.5% agar using swabs (Klare et al., 2005). The plates were dried for 10 min, and MIC test strips (Liofilchem, Italy) were placed on the agar surface according to the manufacturer's instructions. Plates were incubated at 37 °C, and the results were taken after 24 h inoculation. Antibiotic susceptibility was determined according to the European Food Safety Authority (EFSA) guidelines.

#### *Hemolytic property*

To evaluate the hemolytic activity, *L. curvatus* MG5246 strain was grown in MRS broth for 18 h at 37 °C and streaked onto tryptic soy agar (Difco, Detroit, MI, USA) with 5% sheep blood (MB cell, Seoul, Korea). After incubation for 48 h (37 °C, 10% CO<sub>2</sub>), the hemolytic property of *L. curvatus* MG5246 was evaluated based on the lysis of red blood cells around the colonies (Buxton, 2005).

### Preparation of cell free supernatant (CFS)

*L. curvatus* MG5246 strain was grown in MRS broth at 37 °C for 24 h, aerobically, and CFS was obtained by centrifugation at 3460 × g for 10 min, followed by filtration using 0.2 μm filters (Advantec, Tokyo, Japan). The supernatants were lyophilized and used for the cell culture study.

### Cell culture

HGF-1 cells (American Type Culture Collection, Manassas, VA, USA) were incubated and maintained in DMEM containing 10% FBS (v/v) and 1% penicillin–streptomycin (100 units/mL) at standard conditions (37 °C, 5% CO<sub>2</sub>). The culture medium was changed every 2–3 days, and subcultures were performed when the cells were completely full. Only cells with passage numbers between 4 and 10 were used for the experiments. MTT assay was used to evaluate the cytotoxicity of the sample.

## PGE<sub>2</sub> production

HGF-1 cells were seeded in 6 cm plate at a density of  $4 \times 10^5$  cells/well and incubated until 90% confluent. Cells were treated with *Pg*-LPS (Invivogen, San Diego, CA, USA; 5 µg/mL) in fresh phenol-free DMEM medium to stimulate immune response and coincubated with various CFS concentrations (100, 200, and 400 µg/mL) of CFS for another 24 h. The production of PGE<sub>2</sub> was analyzed from the culture supernatant using the enzyme immunoassay kits (Cayman Chemical).

## Induction of experimental periodontitis

Male Sprague–Dawley rats (6 weeks old; 400 ~ 430 g; Orient Bio, Gapyeong, Korea) were maintained in a controlled temperature ( $23 \pm 3$  °C) and relative humidity ( $55 \pm 15\%$ ) with a 12 h light–dark cycle. Rats had free access to purified water and a standard chow diet (Cargill Inc., Seongnam, Korea). Animal experiments protocols were approved by the Institutional Animal Care and Use Committee (KNOTUS 21-KE-032). After 1 week adaptation period, the rats were randomly divided into three groups: (1) no ligation ( $n = 10$ ), (2) ligation control ( $n = 10$ ), and (3) ligation + *L. curvatus* MG5246 ( $2 \times 10^8$  CFU/day) ( $n = 10$ ). Based on the result of toxicity assessment that none of the *Lactobacillus* strains caused any sign of toxicity at a concentration as high as  $1 \times 10^{10}$  CFU/kg/day (Shokryazdan et al., 2016) and the suggested human dose of most probiotics is  $10^8$  to  $10^9$  CFU, a dosage of  $2 \times 10^8$  CFU/kg/day was decided. Ligatures were placed around right mandibular molar teeth as described previously (Kim et al., 2019). After 8 weeks of ligation induction and *L. curvatus* MG5246 administration, rats were sacrificed and its gingival tissue were taken for further analysis.

## Micro-computerized tomography (Micro-CT) analysis

Morphometric analysis of ligated molar tooth and the alveolar bone of rats was done using Viva CT 80 (SCANCO Medical, Switzerland) after CFS administration (8 weeks). The distance from the cemento enamel junction (CEJ) to the alveolar bone crest (ABC) was used as an index of alveolar bone loss.

## RNA extraction and quantitative real time polymerase chain reaction (qRT-PCR)

qRT-PCR analysis was performed using HGF-1 cell lysates and rat gingival tissues as described previously (Kim et al., 2019). Target gene expressions [cyclooxygenase-2 (*COX*-

2), *TNF-α*, *IL-6*, *MMP-2*, *MMP-8*, *CXCL12*, *CXCR4*, *RANKL*, and *OPG*] were analyzed by StepOne Plus RT-PCR system (Applied Biosystems; Foster City, CA, USA). The relative changes in target gene expression levels were normalized to the value of *β-actin*.

## Statistical analysis

All cell culture experiments and analytical measurements were conducted in triplicate. Data were presented as the mean  $\pm$  SD. One-way analysis of variance and Duncan's multiple comparison test (SPSS 25; SPSS, Inc., Chicago, IL, USA) were performed to examine significant differences among treatment means ( $p < 0.05$ ).

## Results and discussion

### Simulated gastrointestinal survivability of *L. curvatus* MG5246

The viability of probiotics in the gastrointestinal tract is one of the key functional requirements to provide health benefits to the body (Tokatlı et al., 2015). The viability of *L. curvatus* MG5246 in simulated gastrointestinal conditions is shown in Table 1. Under the simulated gastric conditions adjusted pH 3, *L. curvatus* MG5246 had an 82% survival rate compared to the initial. In addition, *L. curvatus* MG5246 exhibited a viable cell count of  $> 6$  log CFU/mL, and the survival rate was  $> 96\%$  after exposure to pH 4–8. Generally, the viability of probiotic strains was in the range of 70% ~ 103% in SGF (Kim et al., 2020). Thus, *L. curvatus* MG5246 has acceptable viability to be used as a probiotic strain.

### Antibiotic resistance and hemolytic activity of *L. curvatus* MG5246

The possible antibiotic resistance gene transfer to the pathogen is one of the safety issues in probiotic strains. In addition, evaluation of hemolytic activity of probiotics is strongly recommended to use in food products, even if they have GRAS (Generally Recognized as Safe) or QPS (Quality Presumption of Safety) status (FAO/WHO, 2002). Thus, the MICs of eight antibiotics and hemolytic activity were determined to ensure the safety of *L. curvatus* MG5246.

In Table 2, the MICs of all eight antibiotics against *L. curvatus* MG5246 showed lower values than the cut-off MIC values of the European Food Safety Authority guidelines. Also, MG5246 showed neither alpha nor beta hemolytic activity on the blood agar plates (data not shown). Based on these results, MG5246 does not pose

**Table 1** Survival ability of *L. curvatus* MG5246 under simulated gastrointestinal conditions

Strain	Initial counts <sup>a</sup>	Simulated gastric fluid <sup>b</sup> (Survival rate %)		Simulated intestinal fluid <sup>c</sup> (Survival rate %)	
		pH 3	pH 4	pH 7	pH 8
<i>Lactobacillus curvatus</i> MG5246	6.89 ± 0.27	5.65 ± 0.05 (82.00%)	6.68 ± 0.05 (96.95%)	6.80 ± 0.00 (98.69%)	6.79 ± 0.01 (98.55%)

The results are expressed as means ± SD. All values are shown as viable counts (log CFU/mL)

<sup>a</sup>Initial counts evaluated at 0 h. <sup>b</sup>Simulated gastric tolerance results were obtained at pH 3 and pH 4 for 37 °C after 3 h. <sup>c</sup> Simulated intestinal tolerance results were obtained at pH 3 and pH 4 for 37 °C after 4 h

**Table 2** Minimum inhibitory concentrations of antibiotics for *L. curvatus* MG5246

Antibiotics	Microbiological cut-off values (mg/L)	
	EFSA	<i>L. curvatus</i> MG5246
Ampicillin	4	1
Chloramphenicol	4	3
Clindamycin	4	< 0.016
Erythromycin	1	0.04
Gentamycin	16	0.5
Kanamycin	64	2
Streptomycin	64	6
Tetracycline	8	1.5

MIC values were recorded as the lowest concentration of antibiotic that prevents visible bacterial growth. Antibiotic susceptibilities were evaluated according to the guidelines by European Food Safety Authority (EFSA)

safety concerns regarding antibiotic resistance and hemolytic activity.

### Effect of CFS on PGE<sub>2</sub> production and inflammatory gene expression in *Pg*-LPS-stimulated HGF-1 cells

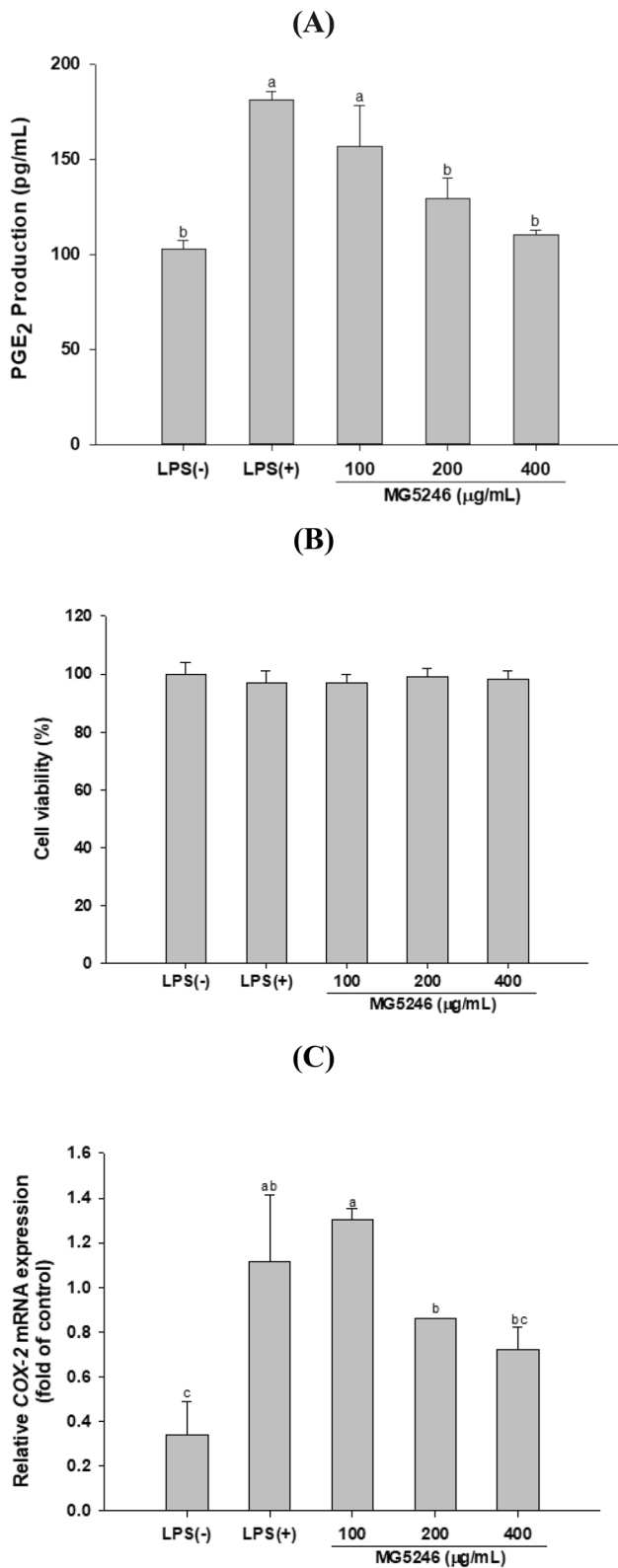
The effects of CFS on PGE<sub>2</sub> production and inflammatory gene expression were analyzed in *Pg*-LPS-stimulated HGF-1 cells. As shown in Fig. 1a, PGE<sub>2</sub> production was significantly decreased by CFS treatment at 200 and 400 µg/mL. No cytotoxicity was observed at all tested concentration ranges (Fig. 1b). *COX-2* gene expression was significantly downregulated at 200 and 400 µg/mL of CFS ( $p < 0.05$ ) (Fig. 1c), whereas *TNF-α* was substantially decreased by 60% with 200 µg/mL CFS ( $p < 0.05$ ) (Fig. 1d). The expression of *IL-6*, one of the major proinflammatory cytokines, was significantly suppressed at 200 and 400 µg/mL CFS ( $p < 0.05$ ) (Fig. 1e). Gene expression of both *MMP-2* and *MMP-8* was also effectively

downregulated even with 100 µg/mL CFS ( $p < 0.05$ ; Fig. 1f and g).

PGE<sub>2</sub> plays an important role in the progression of inflammatory response. Proinflammatory immune responses such as cytokine secretion, extracellular matrix degradation, and alveolar bone loss are caused by the action of PGE<sub>2</sub> (Offenbacher et al., 1993). *TNF-α* is a primary inflammatory mediator and promotes induction of secondary mediators such as chemokines and *COX-2*. The *COX-2* expression and PGE<sub>2</sub> production are significantly upregulated in inflamed gingival tissue, and *IL-1β* and periodontal bacterial components accounted for the elevated PGE<sub>2</sub> production (Morton and Dongari-Bagtzoglou, 2001).

There have been several reports that orally administered probiotics resulted in beneficial effects in periodontitis. *Lactobacillus gasseri* SBT2055 positively prevented *Pg*-mediated periodontal disease in mice by decreasing levels of *IL-1β* and *TNF-α* levels. These reports also demonstrated that the antiperiodontitis effect caused by LG2055 administration was related to  $\beta$ -defensin expression in the oral cavity (Kobayashi et al., 2017). The application of probiotic oral tablet containing *Lactobacillus reuteri* significantly suppressed inflammatory cytokines in chronic periodontitis patients and principal clinical parameters, including bleeding index, periodontal probing depth, and clinical attachment level (Szkardkiewicz et al., 2014).

Progression of periodontitis leads to increased collagen degradation via MMP secreted from gingival fibroblasts. Fibroblasts express multiple MMPs and increased MMP-2 level was observed in gingival tissue of chronic adult periodontitis (Makela et al., 1994). *MMP-8* is an important periodontitis-associated biomarker, as it is a major collagen degrading protease and is closely related to the severity of periodontal inflammation (Sorsa et al., 2011). Based on these reports, inhibition of *MMP-2* and *MMP-8* is an important target to delay the progression or severity of the periodontal disease (Fig. 2).



**Fig. 1** Effects of CFS on **a** PGE<sub>2</sub> production, **b** cell viability, **c** COX-2, **d** TNF- $\alpha$ , **e** IL-6, **f** MMP-2, and **g** MMP-8 mRNA expression in *Pg*-LPS-stimulated human gingival fibroblasts. Cells were treated for 24 h in the presence of *Pg*-LPS (5  $\mu$ g/mL) and samples. **a** PGE<sub>2</sub> production was determined by enzyme immunoassay kit. **b** Cell viability was measured by MTT assay. The level of target gene expressions was analyzed after normalization to that of  $\beta$ -actin using qPCR. Data are expressed as the mean  $\pm$  SD. Bars with different letters (a-c) indicate significant differences at  $p < 0.05$ . *Pg*-LPS: *Porphyromonas gingivalis* lipopolysaccharide; PGE<sub>2</sub>: prostaglandin E<sub>2</sub>; COX-2: cyclooxygenase-2, TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ; IL-6: interleukin-6; MMP: matrix metalloproteinases

**Effects of CFS on chemokine gene expression in *Pg*-LPS stimulated HGF-1 cells**

Chemokine is a type of cytokine that promotes the migration of immune cells through specific receptors. Based on the result of qRT-PCR, *L. curvatus* MG5246 treatment resulted in significant differences in chemokine gene expression of *Pg* LPS-stimulated HGF-1 cells. The gene expression of *CXCL12* and its receptor *CXCR4* was decreased by 42% and 70%, respectively, with 400  $\mu$ g/mL of *L. curvatus* MG4265 (Fig. 3a and b). The gene expression of *ICAM-1* was downregulated in a dose-dependent manner by the addition of *L. curvatus* MG5246 (Fig. 3c).

Grassi et al., (2004) reported that *CXCL12* expression significantly increased in synovial and bone tissue of rheumatoid arthritis and osteoarthritis patients. Elevated *CXCL12* was closely linked with osteoclast-induced bone resorption. *Pg*-LPS-stimulated the expression of *CXCL12* and its receptor *CXCR4* in HGF-1 cells and amplified NF- $\kappa$ B signaling. LPS-mediated elevated *CXCL12* expression was counteracted by the phosphatidylinositol 3-kinase inhibitor, LY2940002 (Sun et al., 2017). Nagashima et al. (2017) demonstrated that administration of a *CXCR4* neutralizing antibody significantly reduced alveolar bone loss in the periodontitis mice model. This suggested that suppression of *CXCR4* signaling can be a potential target for periodontitis treatment.

The expression of *ICAM-1* was augmented by typical proinflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$  in HGF, but suppressed by NF- $\kappa$ B inhibitor (MG-132) (Hosokawa et al., 2006). Chu et al. (2010) reported that *Lactobacillus plantarum* treatment for 4 weeks improved chronic inflammation by lowering proinflammatory cytokine and *ICAM-1* expressions in the IL-10 knockout clots mice model. Based on this speculation, suppression of *CXCL12*, *CXCR4*, and *ICAM-1* expression in *Pg*-LPS-stimulated HGF might play an important role in alleviating periodontal inflammation.

Although the exact active components in the CFS of *L. curvatus* MG5246 are unclear, culture supernatants may

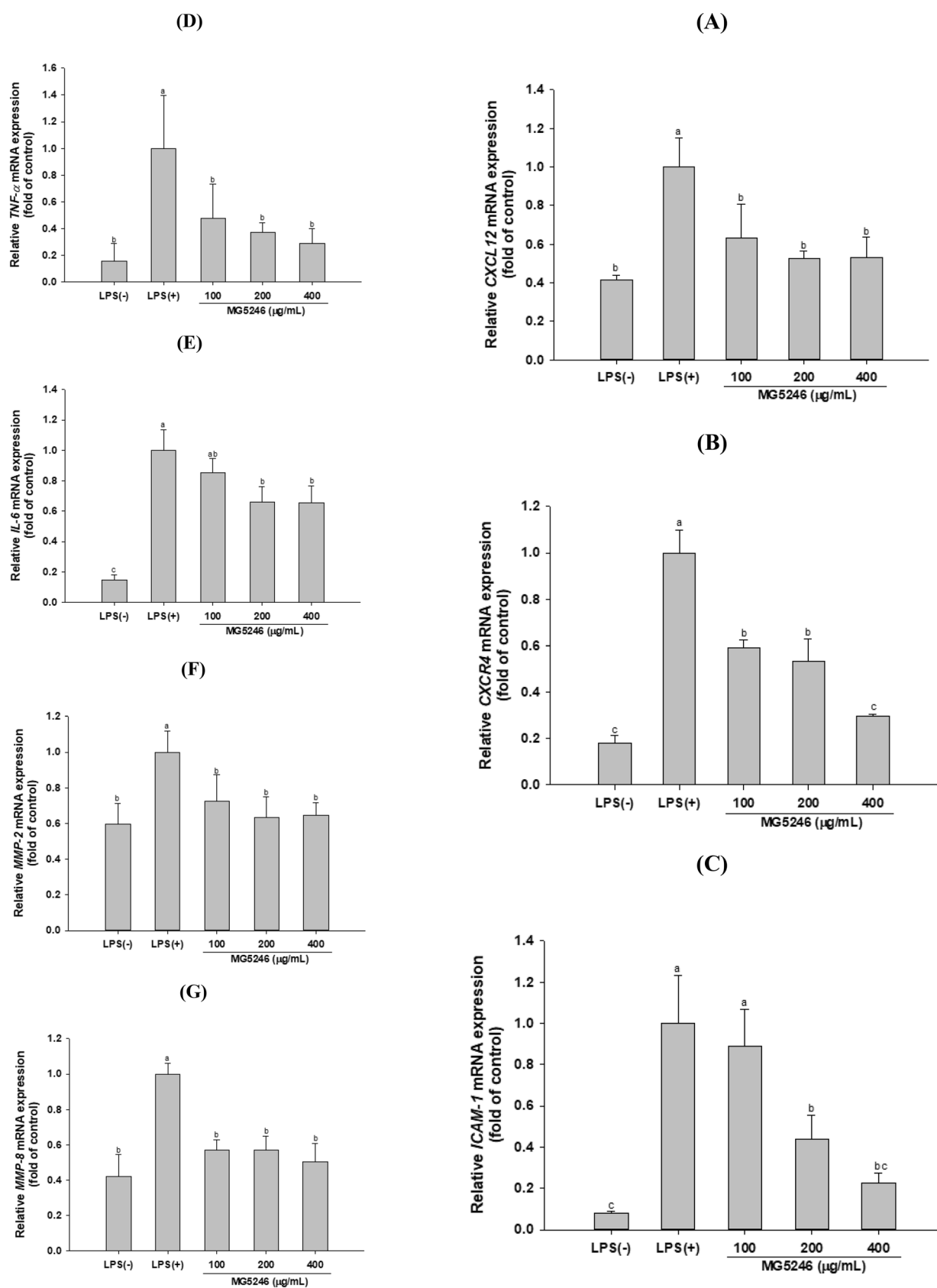


Fig. 1 continued



**Fig. 2** Effects of CFS on **a** CXCL12, **b** CXCR4 and **c** ICAM-1 mRNA expression in *Pg*-LPS stimulated human gingival fibroblasts. Cells were treated for 24 h in the presence of *Pg*-LPS (5 µg/mL) and samples. The level of target gene expressions was analyzed after normalization to that of β-actin using qPCR. Data are expressed as the mean ± SD. Bars with different letters (a-c) indicate significant differences at  $p < 0.05$ . *Pg*-LPS: *Porphyromonas gingivalis* lipopolysaccharide; ICAM-1: intercellular adhesion molecule-1

contain various compounds that originated from microbial action, such as bacterial metabolites and fragments (Aguilar-Toalá et al., 2018). Postbiotics derived from *Lactobacillus* species include various surface proteins, bacteriocins, neurotransmitters, and short-chain fatty acids. They were actively involved in immune modulation and antimicrobial activity (Teame et al., 2020).

### Effects of *L. curvatus* MG5246 on alveolar bone loss in experimental periodontitis in rats

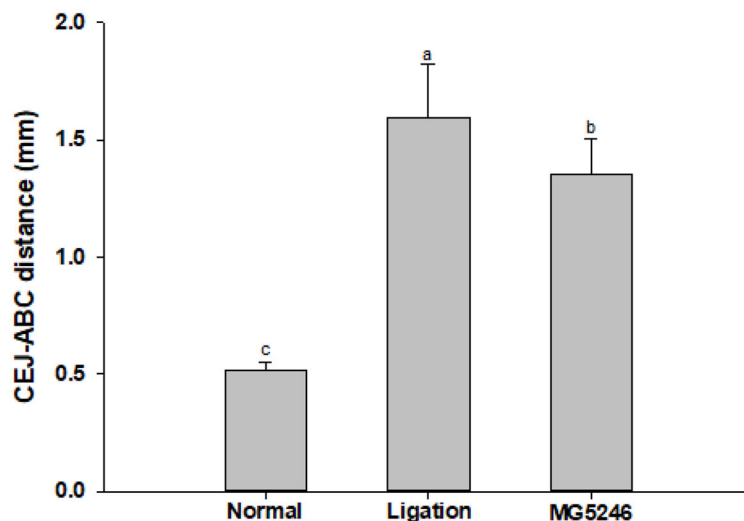
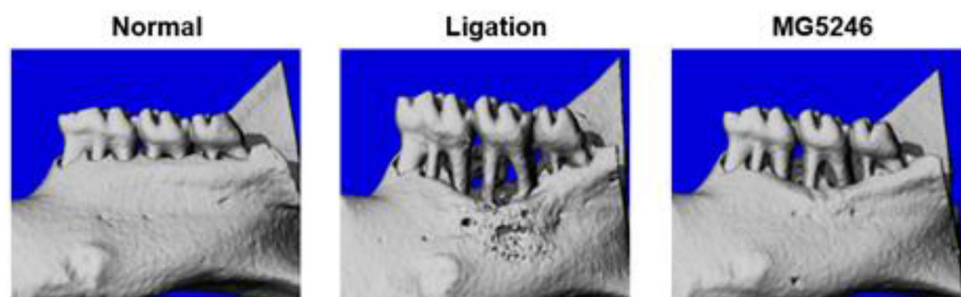
As periodontitis progresses, alveolar bone loss increases. The effects of *L. curvatus* MG5246 on alveolar bone loss

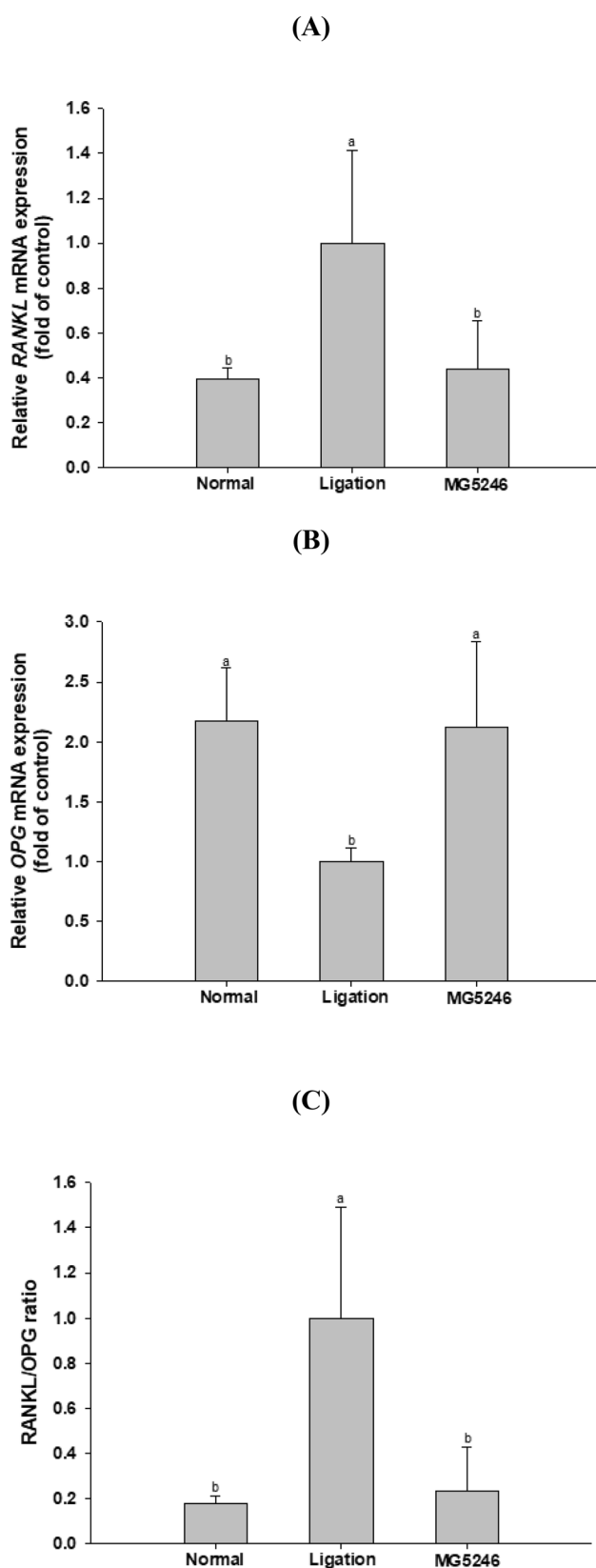
were evaluated after 8 weeks of the feeding trial, and the changes in periodontal bone height were calculated by the distance between cemento-enamel junction (CEJ) and crest of the alveolar bone (ABC). The distance of CEJ-ABC increased in the ligation control group, and *L. curvatus* MG5246 administration significantly decreased the distance of CEJ-ABC distance (Fig. 4).

Similar to human periodontitis, the ligation of rat molar teeth leads to periodontal tissue destruction and alveolar bone loss. Plaque accumulation was the main factor for bone resorption and the bone loss was decreased by antibiotic treatment (Abe and Hajishengallis, 2013). The reduction in CEJ-ABC distance indicated that *L. curvatus* MG5246 administration improved alveolar bone loss associated with severe periodontitis.

*Lactobacillus rhamnosus* GG (LGG) Administration reduced gingival inflammation and alveolar bone loss in *Pg* inoculation-induced periodontitis mice. LGG treatment resulted in positive effects on periodontitis regardless of the way of administration (oral gavage vs. oral inoculation) (Gatej et al., 2018). Topical administration of *Bifidobacterium animalis subsp. lactis* inhibited bone resorption and

**Fig. 3** Effects of *L. curvatus* MG5246 administration on CEJ-ABC distance in ligation-induced periodontitis rats. The micro-CT images were used to measure the distance between the cemento-enamel junction (CEJ) and the crest of the alveolar bone (ABC). Data are expressed as the mean ± SD. Bars with different letters indicate significant differences at  $p < 0.05$





**Fig. 4** Effects of *L. curvatus* MG5246 administration on **a** RANKL, **b** OPG mRNA expression, and **c** RANKL/OPG ratio in gingival tissue of ligature-induced periodontitis rats. The level of target gene expressions was analyzed after normalization to that of  $\beta$ -actin using qPCR. Data are expressed as the mean  $\pm$  SD. Bars with different letters indicate significant differences at  $p < 0.05$ . RANKL: receptor activator of nuclear factor kappa-B ligand; OPG: osteoprotegerin

detachment of connective tissue in ligature-induced periodontitis rats. The probiotic treatment group also showed a greater proportion of healthy oral commensal bacteria than the control group (Oliveira et al., 2017).

#### Effects of *L. curvatus* MG5246 on alveolar bone loss-related gene expression in gingival tissue

The effects of *L. curvatus* MG5246 administration on the bone resorption and tissue damage-related gene expression in the gingival tissue were analyzed using qRT-PCR. The *L. curvatus* MG5246 group significantly decreased RANKL expression, whereas OPG expression was increased by 2.2-fold compared to the ligation control group (Fig. 4a and b). Thus, the significantly elevated RANKL/OPG ratio by ligation was effectively lowered by *L. curvatus* MG5246 administration (Fig. 4c).

RANKL is a cell membrane-bound ligand and stimulates the differentiation and maturation of osteoclasts, followed by bone resorption. The binding of RANKL to its receptor RANK is modulated by OPG, a decoy receptor of RANKL (Belibasakis and Bostanci, 2012). Decreased RANKL but increased OPG expression inhibited bone loss by reducing the number of osteoclasts in periodontitis (Teng et al., 2000). Thus, RANKL/RANK/OPG modulation is a therapeutic target for periodontitis associated bone lysis (Belibasakis and Bostanci, 2012). Bostanci et al. (2007) reported that the relative mRNA expression of RANKL/OPG was significantly different between healthy and periodontitis groups. In addition, they found that OPG expression was reduced in chronic periodontitis, whereas acute periodontitis displayed increased RANKL expression. These results indicated that the decrease in alveolar bone resorption by *L. curvatus* MG5246 administration is closely related to the lowering relative expression of mRNA expression of RANK/OPG in gingival tissue of periodontitis rats.

The mode of action of probiotics in oral inflammation is not fully elucidated. Some indirect anti-inflammatory effects through the modulation of the gut immune system have been proposed, in addition to direct local inhibition of pathogenic bacteria in the oral cavity (Kitamoto et al., 2020). Oral administration of *L. gasseri* SBT2055 improved the disintegration of the periodontal ligament and



alveolar bone loss by modulation of the gastrointestinal immune system. In gingival tissue, the level of proinflammatory cytokines, such as TNF- $\alpha$  and IL-6, was also significantly decreased in *P. gingivalis*-infected mice (Kobayashi et al., 2017).

In this study, *L. curvatus* MG5246 CFS showed anti-inflammatory effects on Pg-LPS-stimulated HGF-1 cells. Expressions of inflammatory mediators (PGE<sub>2</sub>), proinflammatory enzymes (COX-2 and MMPs), cytokines (TNF- $\alpha$  and IL-6), and chemokines (CXCL12 and CXCR4) and adhesion molecule (ICAM-1) were significantly downregulated. Administration of *L. curvatus* MG5246 effectively improved alveolar bone loss in an experimental periodontitis rat model, and the decreased bone resorption was associated with the suppression of mRNA expression RANKL/OPG in gingival tissue. Therefore, *L. curvatus* MG5246 has great potential to be developed as novel oral probiotic.

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#### Declarations

**Conflicts of interest** YongGyeong Kim and Chang-Ho Kang are employees of Mediogen. The authors declare that they had no role in the design of the study; in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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