Journal of Oral Microbiology

ISSN: 2000-2297 (Online) Journal homepage: www.tandfonline.com/journals/zjom20

Comparative inhibition of oral pathogens, biofilm formation, and inflammation-related responses by postbiotics from *Lactobacillus kefiranofaciens* DD2

Eun-Ah Jung, Hyunsook Kim, Wallace H. Yokoyama, Nitin Nitin & Kun-Ho Seo

To cite this article: Eun-Ah Jung, Hyunsook Kim, Wallace H. Yokoyama, Nitin Nitin & Kun-Ho Seo (2026) Comparative inhibition of oral pathogens, biofilm formation, and inflammation-related responses by postbiotics from *Lactobacillus kefiranofaciens* DD2, Journal of Oral Microbiology, 18:1, 2712769, DOI: 10.1080/20002297.2026.2712769

To link to this article: https://doi.org/10.1080/20002297.2026.2712769

© 2026 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 10 Sep 2026.

Submit your article to this journal

Article views: 203

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=zjom20

JOURNAL OF ORAL MICROBIOLOGY
2026, VOL. 18, NO. 1, 2712769
https://doi.org/10.1080/20002297.2026.2712769

Taylor & Francis
by informa

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Comparative inhibition of oral pathogens, biofilm formation, and inflammation-related responses by postbiotics from *Lactobacillus kefiranofaciens* DD2

Eun-Ah Jung[a], Hyunsook Kim[b], Wallace H. Yokoyama[c], Nitin Nitin[d] and Kun-Ho Seo[a]

[a]Center for One Health, College of Veterinary Medicine, Konkuk University, Seoul, Korea; [b]Department of Food and Nutrition, Hanyang University, Seoul, Republic of Korea; [c]USDA Agricultural Research Service, Western Regional Research Center, Albany, CA, USA; [d]Department of Food Science and Technology, University of California, Davis, CA, USA

ABSTRACT

Background: Postbiotics derived from lactic acid bacteria are attracting significant interest as safe and stable alternatives to live probiotics for controlling oral biofilms and inflammation.

Objective: Building on previous research that identified *Lactobacillus kefiranofaciens* DD2 as a potent anti-cariogenic strain, we compared antimicrobial, anti-biofilm, and anti-inflammatory properties of postbiotics produced by DD2 with those from two newly isolated kefir strains (LKF4 and LK12) and two commercial probiotics, *Lactiplantibacillus plantarum* ATCC 3105 and *Lacticaseibacillus rhamnosus* ATCC 7469.

Materials and methods: Cell-free postbiotics were evaluated for their antimicrobial and anti-biofilm activities against major oral pathogens, including *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis*, and *Porphyromonas gulae*. Furthermore, their immunomodulatory effects were investigated in lipopolysaccharide-stimulated RAW 264.7 macrophages through nitric oxide assays and reverse transcription quantitative polymerase chain reaction.

Results: All tested postbiotics significantly inhibited planktonic growth and biofilm formation of the pathogens ($p < 0.05$), and DD2 consistently demonstrated the strongest suppression across all species. In macrophages, DD2-derived postbiotics markedly reduced the expression of pro-inflammatory mediators (*Cox2, Nos2, Tnf-α, and Il-1β*) to approximately 0.5-fold relative to the control, while inducing the highest levels of the anti-inflammatory cytokine *Il-10*. DD2 upregulated *Tlr4*, *Nrf2*, and *Ho-1* mRNA, indicating the preservation of pathogen sensing and the activation of cytoprotective pathways via the Nrf2/Ho-1 signaling axis. Notably, this multi-targeted approach highlighted DD2's superior efficacy compared to other kefir and commercial strains.

Conclusions: These findings support the potential of DD2-derived postbiotics as bioactive alternatives for managing oral pathogens, biofilm formation, and inflammation relevant to oral health. Further studies are needed to identify the active components and to validate these effects in oral *in vivo* models.

ARTICLE HISTORY
Received 29 March 2026
Revised 17 July 2026
Accepted 29 July 2026

KEYWORDS
Lactic acid bacteria; postbiotics; antimicrobial; anti-biofilm; anti-inflammatory

Introduction

Lactic acid bacteria (LAB) are widely recognised for their health-promoting properties and are therefore extensively incorporated into probiotic-enriched functional foods and dietary supplements [1,2]. Beyond the effects of viable cells, LAB produce a broad spectrum of bioactive metabolites during fermentation, including organic acids, exopolysaccharides (EPS), peptides, and bacteriocins [3,4]. These metabolites, collectively referred to as postbiotics, have attracted increasing attention as safe and stable functional ingredients with anti-inflammatory, antimicrobial, and other beneficial activities [5,6]. Unlike live probiotics, postbiotics do not require survival or colonisation in the host and can be formulated more robustly, which is particularly advantageous for applications targeting the oral cavity [6,7].

CONTACT Kun-Ho Seo bracstu3@konkuk.ac.kr; bracstu3@gmail.com Center for One Health, College of Veterinary Medicine, Konkuk University, 120, Neungdong-ro, Gwangjin-gu, 05029, Seoul, Korea

© 2026 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The terms on which this article has been published allow the posting of the Accepted Manuscript in a repository by the author(s) or with their consent.

Oral diseases such as dental caries and periodontitis are driven by specific bacterial pathogens. *Streptococcus mutans* and *Streptococcus sobrinus* are key aetiologic agents of dental caries, whereas *Porphyromonas gingivalis* is a principal pathogen associated with human periodontitis [2,8]. *Porphyromonas gulae* is a major periodontopathic bacterium frequently detected in dogs with periodontal disease. Similar to other oral Porphyromonas species, its pathogenicity is closely associated with biofilm formation and the induction of chronic inflammatory responses that contribute to progressive periodontal tissue destruction. In addition, certain virulent genotypes of *P. gulae* have been associated with more severe forms of periodontal disease, highlighting its clinical importance in companion animal oral health [9,10]. Since these pathogens often reside within complex biofilms on tooth surfaces, they are difficult to eradicate using conventional antimicrobials alone [2,4]. Thus, there is growing interest in LAB-derived postbiotics as multifunctional agents capable of inhibiting oral pathogens, disrupting biofilms, and modulating host inflammatory responses [4,11].

Kefir is a fermented milk product that serves as a rich reservoir of probiotic diversity. It typically contains >50 species of LAB, yeasts, and acetic acid bacteria [12–14]. Within kefir microbiota, *Lentilactobacillus kefiri* and *Lactobacillus kefiranofaciens* are among the most frequently isolated LAB and have been reported to produce EPS and other metabolites with diverse biological activities, including antimicrobial and anti-inflammatory effects [13,15]. In our previous work, we isolated *Lact. kefiranofaciens* DD2 from kefir and demonstrated its pronounced antimicrobial activity against cariogenic *Strep. mutans* and *Strep. sobrinus* as well as its ability to interfere with bacterial adhesion and biofilm formation [13].

These findings identified DD2 as a promising oral probiotic/postbiotic candidate. However, several important questions remain. First, most studies on kefir-derived LAB, including our earlier work on DD2, have focused primarily on cariogenic streptococci, with relatively limited evaluation against periodontal pathogens such as *P. gingivalis* and *P. gulae*. Second, it is not yet clear whether DD2-derived postbiotics provide distinct advantages over other kefir-derived LAB or widely used commercial probiotic strains. Without a direct comparative assessment under standardised conditions, the true value of DD2 as an oral health candidate remains uncertain. Third, although the direct antimicrobial properties of LAB-derived postbiotics are well documented, their broader impact on biofilm formation and host inflammatory pathways has not been fully elucidated in a unified experimental framework [2,16].

We aimed to determine whether DD2-derived postbiotics exhibit superior activity against oral pathogens, biofilm formation, and inflammation-related responses compared with postbiotics from other kefir-derived and commercial LAB strains. We hypothesised that DD2-derived postbiotics would show stronger multifunctional effects in oral health-relevant *in vitro* models than the comparator strains.

Materials and methods

Experimental design

We selected five LAB strains—kefir-derived *Lact. kefiranofaciens* DD2, LKF4, and LK12, together with the commercial strains *Lp. plantarum* subsp. *plantarum* ATCC 3105 and *Lc. rhamnosus* ATCC 7469—to compare the functional advantages of kefir-derived postbiotics. The study was conducted in three major phases: (i) characterisation of probiotic properties and safety profiles; (ii) assessment of antimicrobial and anti-biofilm activities against oral pathogens; and (iii) evaluation of immunomodulatory effects in LPS-stimulated RAW 264.7 macrophages, as summarised in Figure 1.

Isolation of lactic acid bacteria from kefir

Initially, we evaluated 19 lactic acid bacteria (LAB) strains, comprising 17 kefir-derived isolates and 2 commercial probiotic strains (*Lp. plantarum* subsp. *plantarum* ATCC 3105 and *Lc. rhamnosus* ATCC 7469). Among the 17 kefir-derived isolates, 3 strains, including *Lact. kefiranofaciens* DD2, had been previously isolated from kefir and maintained in the laboratory's culture collection as stock strains based on their previously identified functional properties [13,17,18]. The other 14 LAB strains, including *Lact. kefiranofaciens* LKF4 and *Lent. kefiri* LK12, were newly isolated from kefir for this study to expand the pool of candidate strains. The commercial strains were purchased from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). For the isolation of new strains, kefir was prepared by inoculating 10 g of kefir grains into

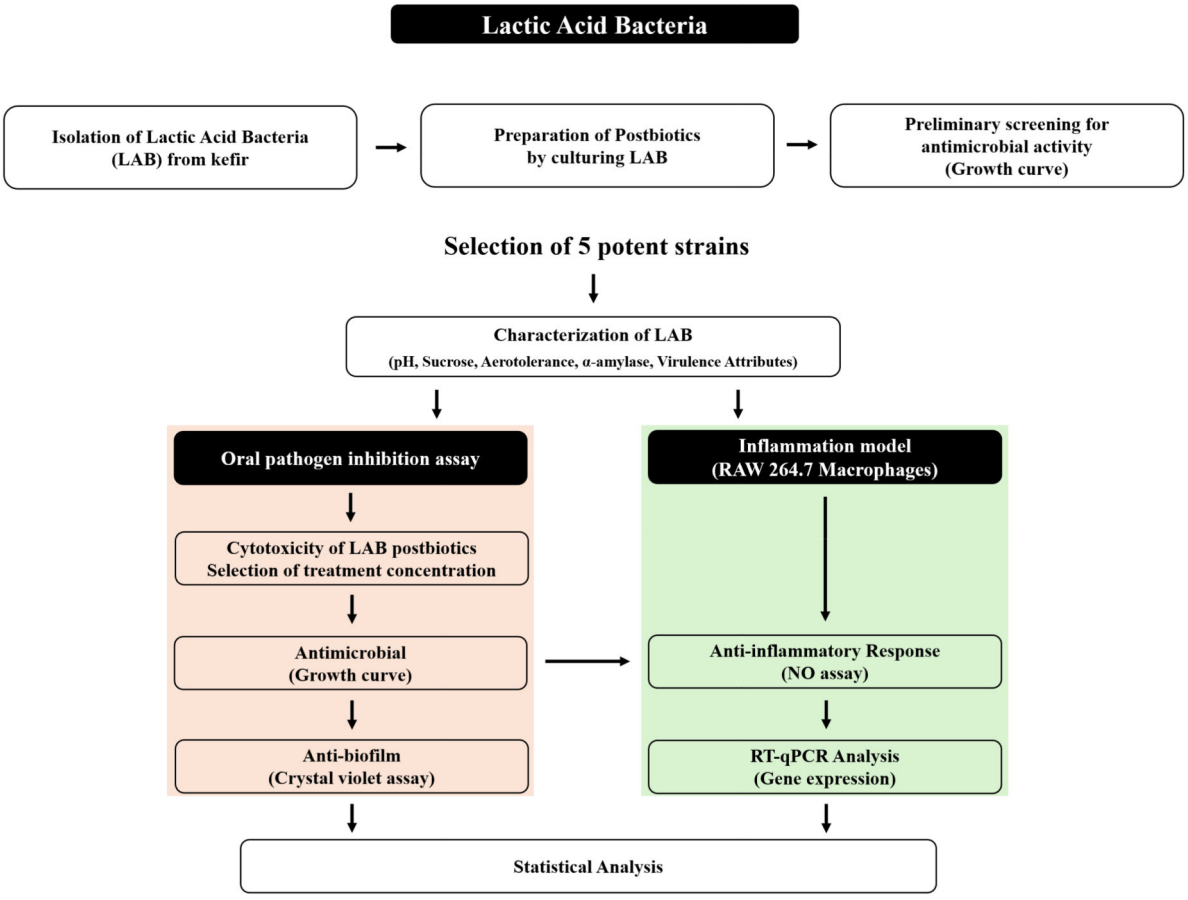

Figure 1. Schematic diagram of the experimental design and overall study workflow. LAB, lactic acid bacteria.

200 mL of sterilised milk and fermented at 25 °C for 48–72 h. The fermented mixture was serially diluted and spread onto De Man, Rogosa, and Sharpe (MRS) agar (Difco, Detroit, MI, USA), then incubated under anaerobic conditions at 37 °C for 48–72 h [13,19]. Single colonies with distinct morphologies were selected and streaked onto fresh MRS agar to obtain pure isolates. The taxonomic identity of the 14 new isolates was confirmed by 16S rRNA gene sequencing, using universal primers 27F and 1492 R (Bionics, Seoul, Korea). Although all 19 strains (2 commercial, 3 existing isolates, and 14 new isolates) were subjected to preliminary characterisation, the five strains (DD2, LKF4, LK12, ATCC 3105, and ATCC 7469) were specifically selected for comparative postbiotic analysis based on their superior probiotic and safety profiles.

Preparation of postbiotics

For the preliminary screening of antimicrobial activity, postbiotics were prepared from all 19 LAB strains. Each strain was cultured on MRS agar at 37 °C for 48–72 h. After the initial incubation, single colonies were transferred into MRS broth, and the bacterial suspension was adjusted to 1×10^6 CFU/mL [4,20], followed by an additional 48 h incubation until reaching a stationary phase with a cell density of approximately 1×10^8 to 1×10^9 CFU/mL. The culture was then centrifuged at 4,000 × g for 15 min at 4 °C [11], and the supernatant was collected and filtered through a 0.22 μm pore size membrane filter (Merck Millipore, Burlington, MA, USA) to obtain cell-free postbiotics. The filtered postbiotics were stored frozen at −20 °C until further use.

Preliminary screening for antimicrobial activity against oral pathogens

To identify the most potent candidates for oral health management, the postbiotics prepared from all 19 LAB strains in Section 2.3. were subjected to preliminary antimicrobial screening against four major oral

pathogens: *S. mutans*, *S. sobrinus*, *P. gingivalis*, and *P. gulae*[8,10]. The antimicrobial activity was evaluated by confirming the bacterial growth curves in co-culture with each postbiotic following established kinetic analysis protocols [16,21]. Bacterial cultures were adjusted to a final concentration of 1×10^6 CFU/mL using brain heart infusion (BHI) broth. A volume of 100 µL of each bacterial suspension was dispensed into the wells of a 96-well plate and 100 µL of each postbiotic was added. The optical density at 600 nm (OD_{600}) was recorded every 15 min for 24 h using a Varioskan™ ALF multimode microplate reader (Thermo Fisher Scientific, USA) to verify the growth inhibition patterns. Based on these growth curve profiles, five strains that exhibited superior antimicrobial performance (DD2, LKF4, LK12, ATCC 3105, and ATCC 7469) were selected for further comparative analysis [13].

Characterisation of probiotic and safety profiles

To assess the suitability of the 19 LAB strains for oral health applications, several physiological and safety characteristics were evaluated following established protocols [13,20].

pH measurement of cultured MRS broth

A rapid reduction in pH is a key mechanism for inhibiting the growth of oral pathogens [20,22]. Therefore, to assess the acidogenic potential of the strains, we measured the pH of the cultures grown in MRS broth after incubation at 37 °C for 24 h and 48 h using a pH metre (Orion Star A211, Thermo Fisher Scientific, USA).

Sucrose utilisation

To determine the metabolic versatility of the strains and their potential interaction with dietary sugars in the oral cavity, we assessed their sucrose metabolising ability using the Vitek 2 system (bioMérieux, France) [23].

Aerotolerance test

To ensure that the strains can survive and remain active in the oxygen-rich environment of the oral cavity [13], each strain was spread onto MRS agar plates and incubated under aerobic and anaerobic conditions (37 °C, 48 h). A reduction in colony diameter to <75% of the anaerobic control indicated reduced survivability.

α-amylase tolerance

Strains were inoculated into MRS broth (pH 6.8) supplemented with α-amylase (1,000 IU/mL) and incubated at 37 °C for 4 h to ensure the stability of the strains against salivary enzymes, which is essential for maintaining probiotic functions during oral transit [13]. Survivability was calculated relative to the initial count.

Virulence attributes

To verify the biological safety of the strains, ensuring they do not exhibit pathogenic traits before clinical or functional application [20,24], haemolysis activity was assessed on blood agar plates (γ-haemolysis indicates safety), and gelatinase production was determined on gelatin agar.

Determination of non-cytotoxic concentrations of LAB postbiotics

To determine the maximum non-cytotoxic concentration of LAB-derived postbiotics for subsequent experiments, a cytotoxicity assay was performed using the WST-8 reagent (Dojindo), with slight modifications from previously published protocols [25]. Primary human gingival fibroblast (HGF) cells purchased from ATCC (Manassas, VA, USA) were used as an oral cell model to evaluate the cytocompatibility of the postbiotics. The HGF cells were seeded into 96-well plates at a density of 5×10^4 cells/well in 200 µL of Roswell Park Memorial Institute RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin. The plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24–48 h until they reached 70–80% confluency. Following incubation, the culture medium was replaced with 200 µL of fresh RPMI-1640 without phenol red (containing 10% FBS and 1% penicillin-streptomycin) supplemented with the previously prepared LAB postbiotics (1×10^8 to 1×10^9 CFU/mL equivalent) at volume-to-volume (v/v) concentrations of 50%, 25%, 10%, and 1%.

After 24 h of treatment, 10% (v/v) of WST-8 reagent was added to each well, and the plates were incubated for an additional 2 h at 37 °C in 5% CO_2. After the reaction, the plates were gently agitated to ensure uniform colour development, and absorbance was measured at 450 nm using a Varioskan™ ALF multimode microplate reader (Thermo Fisher Scientific, USA) to determine cell viability [11,25].

Cell viability (%) was calculated using the following formula:

$$(OD_{treatment}/OD_{control}) \times 100$$

Antimicrobial activity of LAB-derived postbiotics against oral pathogens

The antimicrobial activity of LAB-derived postbiotics against oral pathogens was evaluated by monitoring bacterial growth curves over time. The target bacterial strains—*Strep. mutans*, *Strep. sobrinus*, *P. gingivalis*, and *P. gulae*—were obtained from the KCTC (Daejeon, Republic of Korea). *Strep. mutans* and *Strep. sobrinus* were cultured in BHI broth at 37 °C for 24 h [20]. *P. gingivalis* and *P. gulae* were initially grown on tryptic soy agar supplemented with 5% defibrinated horse blood, 5 μg/mL hemin, and 1 μg/mL menadione at 37 °C under anaerobic conditions for 48–72 h [9]. These cultures were then subcultured in BHI broth and incubated anaerobically at 37 °C for an additional 48 h. For growth curve analysis, bacterial cultures were adjusted to a final concentration of 1×10^6 CFU/mL using BHI broth. A volume of 100 μL of each bacterial suspension was dispensed into the wells of a 96-well plate, and 100 μL of LAB-derived postbiotics at a concentration of 10% (v/v), the maximum non-cytotoxic concentration, were added. The plate was sealed with an adhesive film to prevent evaporation and incubated at 37 °C for 24 h. During incubation, the optical density at 600 nm (OD_{600}) was recorded every 15 min using a Varioskan™ ALF multimode microplate reader (Thermo Fisher Scientific, USA) [13,16].

Anti-biofilm activity of LAB-derived postbiotics against oral pathogens

The anti-biofilm activity of LAB-derived postbiotics against oral pathogens was evaluated based on a method described by [13,26], with some modifications. Each bacterial strain was adjusted to 1×10^6 CFU/mL in BHI broth, and 100 μL of the suspension was added to each well of a 96-well plate. Then, an equal volume of LAB-derived postbiotics at a concentration of 10% (v/v) was added to each well. To maintain anaerobic conditions, the plate was covered with a lid, placed in an anaerobic jar, and incubated at 37 °C for 24 h. Following incubation, the culture medium was gently removed. Each well was washed three times with 200 μL of PBS to eliminate planktonic cells. Biofilms attached to the bottom of the wells were stained with 200 μL of 0.1% crystal violet solution and incubated at room temperature for 20 min. The wells were then washed three times with PBS, and 200 μL of 99.9% ethanol was added to each well to solubilise the bound dye. After gentle pipetting to ensure complete dissolution, the absorbance was measured at 575 nm using a Varioskan™ ALF multimode microplate reader (Thermo Fisher Scientific, USA).

The percentage of biofilm formation was calculated using the following formula:

$$\text{Biofilm formation}(\%) = (OD_{treatment}/OD_{control}) \times 100$$

Anti-inflammatory response induced by LAB postbiotics in RAW 264.7 macrophages

The anti-inflammatory effects of LAB-derived postbiotics were evaluated in RAW 264.7 murine macrophages by measuring nitric oxide (NO) production using the Griess assay [27]. RAW 264.7 cells were seeded in 96-well plates at a density of 5×10^4 cells/well in phenol red-free DMEM supplemented with 10% FBS and 1% penicillin-streptomycin, followed by a 24 h incubation at 37 °C in a 5% CO_2 atmosphere for attachment. To evaluate the immunomodulatory potential of the samples, the cells were treated with the prepared postbiotics (10% v/v) under three experimental conditions—pre-treatment, co-treatment, and post-treatment—to assess both preventive and therapeutic effects under LPS stimulation [5,28]. To eliminate the confounding effect of acidity, all postbiotic treatments in cell-based assays were conducted in buffered DMEM or RPMI-1640 media, which maintained a neutral pH environment (pH 7.0–7.4) throughout the incubation period. Following treatment, 100 μL of culture supernatant was treated with 50 μL of Griess Reagent I for

10 min at room temperature, followed by the addition of 50 μL of Griess Reagent II for an additional 10 min. The absorbance was measured at 540 nm using a Varioskan™ ALF multimode microplate reader (Thermo Fisher Scientific, USA). Nitrite concentrations were determined using a sodium nitrite standard curve, and relative NO production was expressed as a percentage of the LPS-only group (100%).

RT-qPCR analysis of inflammation-related gene expression in RAW 264.7 macrophages treated with LAB postbiotics

To elucidate the multi-faceted anti-inflammatory mechanisms of postbiotics, the mRNA expression of target genes was analysed across four functions: (1) pro-inflammatory sensors and mediators (*Tlr4*, *p65*, *Nos2*, and *Cox2*); (2) pro-inflammatory cytokines (Tnf-*α* and Il-1β); (3) anti-inflammatory cytokines (*Il-10*); and (4) antioxidant signalling molecules (*Nrf2* and *Ho-1*).

Total RNA was extracted from RAW 264.7 cells using the PureLink RNA Mini Kit (Thermo Fisher Scientific, USA) following the manufacturer's instructions. Subsequently, cDNA was synthesised from the purified RNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA) on a MiniAmp Thermal Cycler (Thermo Fisher Scientific, USA). Quantitative real-time PCR (qPCR) was performed using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). The cycling conditions consisted of uracil-DNA glycosylase activation at 50 °C for 2 min and polymerase activation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55–60°C for 15 s, and extension at 72 °C for 1 min. Melt curve analysis was subsequently conducted to confirm amplification specificity. Finally, the expression levels of target genes were normalised to the housekeeping genes, *Gapdh* and *β-actin*. Relative quantification was conducted using the $2^{-\Delta\Delta CT}$ method [5,27]. The primer sequences and functional annotations of the target and reference genes used for RT-qPCR are summarised in Table 1.

Table 1. Primer sequences of inflammatory, antioxidant, and immune-related genes used for RT-qPCR analysis.

Target Gene	Function	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Reference
Tnf-α	Major pro-inflammatory cytokine, mediates inflammation and immune responses	CCC-TCA-CAC-TCA-GAT-CAT-CTT-CT	GCT-ACG-ACG-TGG-GCT-ACA-G	[29]
Il-1β	Pro-inflammatory cytokine, promotes fever and inflammatory cascades	ATG-GCA-ATC-GTT-CCT-GAA-CTC-AAC	CAG-GAC-AGG-TAT-AGA-TTC-TTT-CCT-TT	[30]
p65	Transcription factor that regulates pro-inflammatory cytokines	ACG-ACA-TTG-AGG-TTC-GGT-TC	ATC-TTG-TGA-TAG-GGC-GGT-GT	[31]
Cox2	Enzyme involved in prostaglandin synthesis that mediates inflammatory pain and fever	CAC-TCA-GTT-TGT-TGA-GTC-ATT-C	GAT-TAG-TAC-TGT-AGG-GTT-AAT-G	[32]
Il-10	Anti-inflammatory cytokine suppresses excessive immune response	GCT-CTT-ACT-GAC-TGG-CAT-GAG	CGC-AGC-TCT-AGG-AGC-ATG-TG	[29]
Nos2	Enzyme that catalysers the production of nitric oxide (NO); a key mediator of inflammatory responses.	GAC-AAG-CTG-CAT-GTG-ACA-TC	GCT-GGT-AGG-TTC-CTG-TTG-TT	[33]
Nrf2	Master transcription factor that regulates the expression of antioxidant and cytoprotective genes.	TCC-GCT-GCC-ATC-AGT-CAG-TC	ATT-GTG-CCT-TCA-GCG-TGC-TTC	[31]
Ho-1	Inducible antioxidant enzyme that degrades haem and exhibits potent anti-inflammatory activities.	TGA-AGG-AGG-CCA-CCA-AGG-AGG	AGA-GGT-CAC-CCA-GGT-AGC-GGG	[32]
Tlr4	Cell surface receptor that recognises LPS and initiates the innate immune signalling cascade.	ATG-GCA-TGG-CTT-ACA-CCA-CC	GAG-GCC-AAT-TTT-GTC-TCC-ACA	[29]
Gapdh	Housekeeping gene for normalisation of gene expression	AGT-GGC-AAA-GTG-GAG-ATT	GTG-GAG-TCA-TAC-TGG-AAC-A	[34]
β-actin	Housekeeping gene for normalisation of gene expression	GGC-TGT-ATT-CCC-CTC-CAT-CG	CCA-GTT-GGT-AAC-AAT-GCC-ATG-T	[29]

Tnf-α, tumour necrosis factor-α; ***Il***, interleukin; ***p65***, NF-κB p65 subunit; ***Cox2***, cyclooxygenase-2; ***Nos2***, nitric oxide synthase 2; ***Nrf2***, nuclear factor erythroid 2-related factor 2; ***Ho-1***, haem oxygenase-1; ***Tlr4***, Toll-like receptor 4; ***Gapdh***, glyceraldehyde 3-phosphate dehydrogenase.

Statistical analysis

All experiments were performed in at least three independent biological replicates, and the data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance followed by Tukey's post-hoc test, or Student's *t*-test where appropriate. Differences were considered statistically significant when the *p*-value was < 0.05. Data analysis was performed using SPSS software (version 28.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism (version 10; GraphPad Software, San Diego, CA, USA).

Results

Selection of potent LAB strains and their comparative probiotic characterisation

To identify the most effective candidates for oral health applications, a preliminary antimicrobial screening was conducted on the 19 initial LAB strains (including 17 kefir-derived isolates and 2 reference strains). Among these, five strains—*Lact. kefiranofaciens* DD2, *Lact. kefiranofaciens* LKF4, *Lent. kefiri* LK12, *Lp. plantarum* ATCC 3105, and *Lc. rhamnosus* ATCC 7469—were prioritised and selected for further characterisation. These five strains exhibited superior and consistent growth inhibition against major oral pathogens compared to the other 14 isolates (Table 2). The taxonomic identity and safety characteristics of the selected LAB strains are detailed in Table 2. All five isolates showed 96.0–99.9% 16S rRNA gene sequence identity to their respective reference strains, confirming their genetic classification. Phenotypically, all selected strains exhibited γ-haemolysis (no haemolytic activity) and no gelatinase activity, fulfilling the basic biological safety requirements for probiotic application. The physiological profiles indicated that all strains possessed acidogenic potential, though the rate of acidification varied significantly among the strains and across the incubation periods. There was a significant decrease in the pH of the culture media in all groups from 24 h to 48 h ($p < 0.0001$). At both time points, *Lact. kefiranofaciens* DD2 maintained a significantly higher pH (4.52 ± 0.01 at 24 h; 4.16 ± 0.02 at 48 h) compared to the other strains, particularly *Lc. rhamnosus* ATCC 7469, which exhibited the lowest pH (4.07 ± 0.01 at 24 h; 3.75 ± 0.02 at 48 h) (Table 2). Regarding metabolic versatility and environmental stability, most isolates successfully utilised sucrose and maintained high viability under aerobic conditions, which is essential for survival in the oxygen-rich oral cavity. Furthermore, the strains demonstrated robust stability in the presence of α-amylase, suggesting their ability to remain active despite exposure to salivary enzymes.

Cytotoxicity of LAB-derived postbiotics in human gingival fibroblasts

Cell viability was measured after 24 h of incubation and expressed as a percentage relative to untreated controls. At high concentrations (50% and 25%), all lactic acid bacteria postbiotics exhibited cytotoxicity. In contrast, at 10% and 1% concentrations, no cytotoxicity was observed for any strain, and viability values exceeded the safety threshold of 80% (Figure 2).

Comparative antimicrobial and anti-biofilm activities of LAB-derived postbiotics against oral pathogens

The antimicrobial activity of the postbiotics of five selected candidates against four oral pathogens was identified by measuring the optical densities of the cultures at 600 nm (OD600). The untreated controls exhibited a progressive increase in OD600 values, reaching 0.79 for *Strep. mutans*, 0.76 for *Strep. sobrinus*, 0.83 for *P. gingivalis*, and 0.50 for *P. gulae*, indicating robust planktonic growth. All tested LAB-derived postbiotics significantly suppressed the proliferation of these oral pathogens, as shown in Figure 3 for *Strep. mutans*, Figure 4 for *Strep. sobrinus*, Figure 5 for *P. gingivalis*, and Figure 6 for *P. gulae*. Notably, this inhibitory effect extended to biofilm formation, where *Lact. kefiranofaciens* DD2 demonstrated the most potent suppressive activity across all strains compared to the control group (Figure 7).

Table 2. Characteristics of lactic acid bacteria as candidate oral probiotics.

Strain		Species (16S rRNA gene sequence identity, %)	pH of cultured MRS broth		Sucrose utilisation	Growth under aerobic conditions (37℃, 48 h)		*α*-amylase tolerance (MRS, pH 6.8, 1,000 IU/ml of *α*-amylase, 37℃, 4 h)[6]	Hemo-lysis[7]	Gelatinase production[8]
			24 h[1]	48 h[2,3]		Colony size[4]	Colony count[5]			
Kefir isolates	DD2	*Lactobacillus kefiranofaciens* (99.5)	4.52 ± 0.01^{a}	$4.16 \pm 0.02^{a****}$	+	NS	+++	+++	γ	N
	LKF4	*Lactobacillus kefiranofaciens* (97.6)	4.38 ± 0.01^{c}	$3.80 \pm 0.02^{b****}$	+	NS	+++	+++	γ	N
	LK12	*Lentilactobacillus kefiri* (97.6)	4.33 ± 0.02^{b}	$3.89 \pm 0.02^{c****}$	+	NS	+++	+++	γ	N
Commercial probiotic strains	ATCC 3105	*Lactiplantibacillus plantarum* subsp. *plantarum* (99.8)	4.28 ± 0.02^{d}	$3.80 \pm 0.01^{c****}$	+	NS	+++	+++	γ	N
	ATCC 7469	*Lacticaseibacillus rhamnosus* (99.5)	4.07 ± 0.01^{e}	$3.75 \pm 0.02^{d****}$	–	NS	+++	+++	γ	N

[1,2] Values are expressed as mean ± SD and superscript letters indicate significant differences among strains within the same column ($p < 0.05$).
[3] Asterisks (****) indicate highly significant differences compared to the 24 h value for each strain ($p < 0.0001$).
[4] NS, not significant, the colony diameter in aerobic cultures reached over 75% of that measured under anaerobic conditions.
[5] +++ , >90% vs. colony count under anaerobic conditions.
[6] +++ , >90% survival vs. initial count.
[7] ***α*-haemolysis** indicates partial haemolysis with a greenish discoloration on blood agar; ***β*-haemolysis** indicates complete haemolysis with a clear zone around colonies; and ***γ*-haemolysis** indicates no haemolytic activity.
[8] "N" in the gelatinase production column indicates that no gelatinase activity was observed.

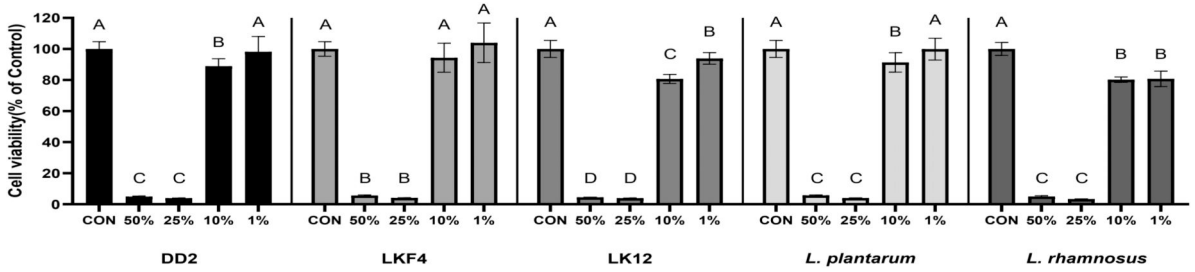

Figure 2. Cytotoxicity of LAB-derived postbiotics in HGF. Values are expressed as mean ± SD from three independent experiments. Cell viability was calculated as a percentage relative to the untreated control group (CON). A viability above 80% was considered non-cytotoxic, based on commonly accepted thresholds in ISO 10993-5 and previous literature. LAB, lactic acid bacteria.

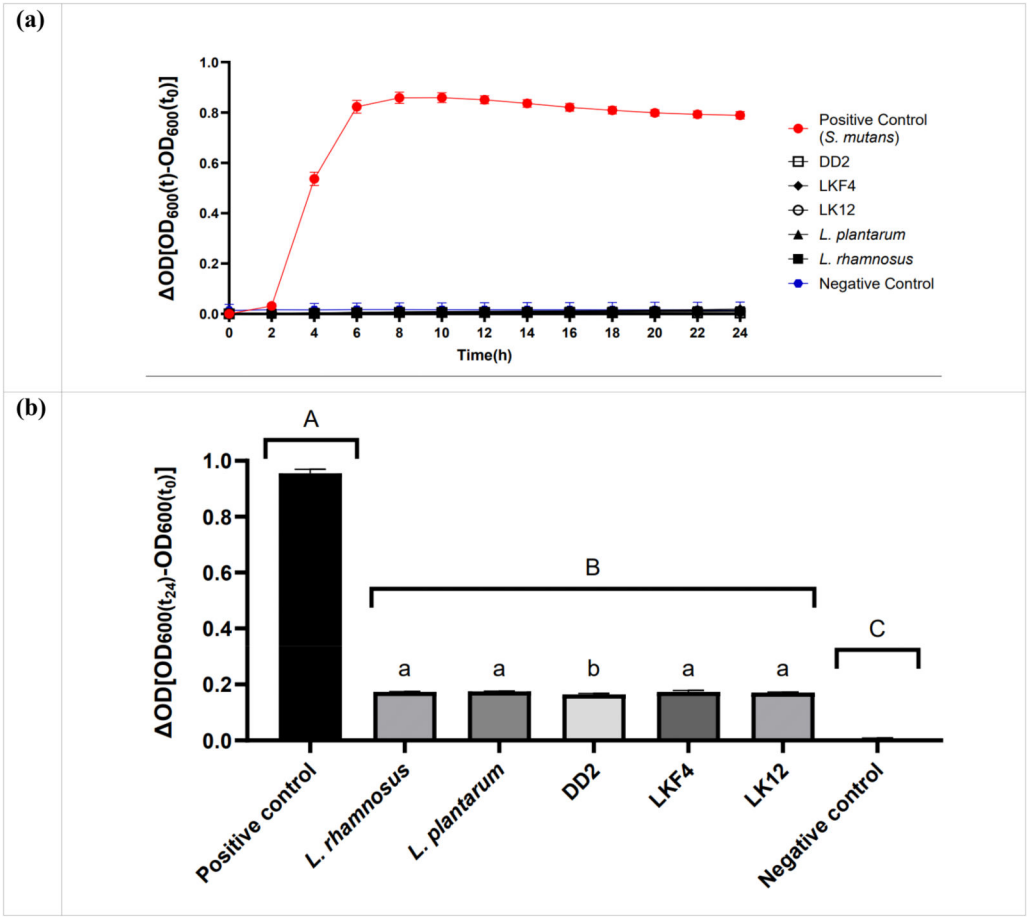

Figure 3. Antimicrobial effects of LAB-derived postbiotics against Strep. mutans. Positive control represents oral pathogens cultured without any treatment. The negative control consists of sterile BHI broth. Statistical analysis was performed based on ΔOD_{600} values at 24 h and is shown in panel (b). Different uppercase letters (A–C) indicate significant differences among the positive control, LAB-treated, and negative control groups, while lowercase letters (a–b) indicate differences among LAB-treated groups at 24 h (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$). LAB, lactic acid bacteria; ANOVA, analysis of variance; OD, optical density; OD600, optical densities of the cultures at 600 nm.

Strain-dependent effects of LAB-derived postbiotics on nitric oxide production in LPS-stimulated macrophages

The cytotoxicity of LAB-derived postbiotics was assessed in RAW 264.7 cells via WST assay. Treatment with all postbiotic candidate samples for 24 h did not significantly reduce cell viability compared to the NC group, confirming that subsequent NO inhibition was not attributable to cytotoxicity (Figure 8a). NO

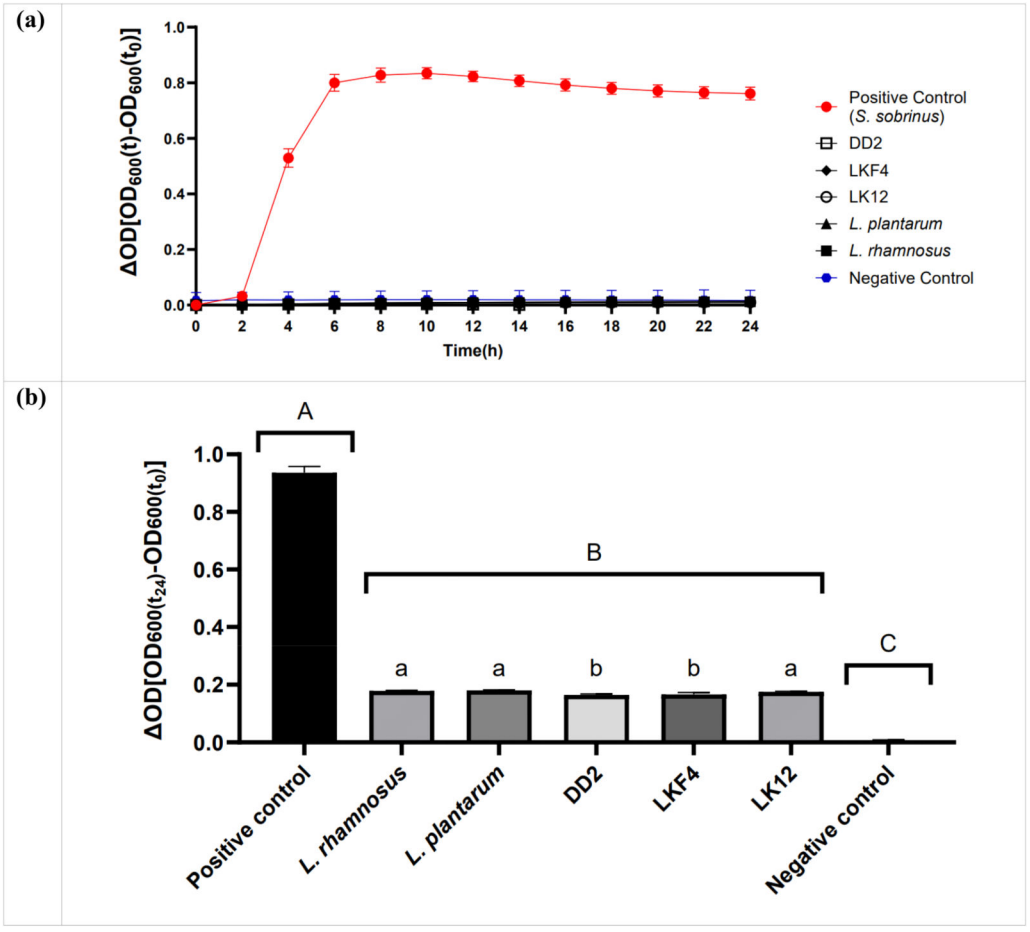

Figure 4. Antimicrobial effects of LAB-derived postbiotics against *Strep. sobrinus*. Positive control represents oral pathogens cultured without any treatment. The negative control consists of sterile BHI broth. Statistical analysis was performed based on ΔOD_{600} values at 24 h and is shown in panel (b). Different uppercase letters (A–C) indicate significant differences among the positive control, LAB-treated, and negative control groups, while lowercase letters (a–b) indicate differences among LAB-treated groups at 24 h (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$). LAB, lactic acid bacteria; ANOVA, analysis of variance; OD, optical density; OD600, optical densities of the cultures at 600 nm.

production was significantly suppressed across all treatment strategies, in the following order: pre-treatment > co-treatment > post-treatment, with dexamethasone (positive control) showing the highest inhibitory activity. Among the tested postbiotic samples, DD2 showed the most prominent inhibitory effect in the co-treatment condition (Figure 8b). Pre-treatment resulted in the strongest suppression, where DD2 and LK12 reduced NO levels to amounts comparable to or lower than those of the Blank group (Figure 8c). Although post-treatment exhibited weaker efficacy than pre-treatment, significant inhibitory activity was still observed (Figure 8 d). Overall, DD2 consistently demonstrated the highest anti-inflammatory activity among the candidates across all tested conditions.

Distinct immunomodulatory gene expression profiles induced by DD2-derived postbiotics

Significant differences in the mRNA expression levels of inflammatory, anti-inflammatory, and antioxidant-related genes were observed in LPS-stimulated RAW 264.7 macrophages following treatment with LAB-derived postbiotics (Figure 9). The mRNA expression levels of pro-inflammatory mediators, including *Cox2*, *Nos2*, and *Tnf-α*, were markedly reduced in the DD2-treated group compared with the LPS-stimulated control. Specifically, DD2 treatment suppressed the expression of these three genes to < 0.5-fold relative to the control, showing the most pronounced inhibitory effect among the tested postbiotics ($p < 0.05$). Although the magnitude of reduction varied across strains, the expression of *Il-1β* was also significantly decreased by DD2 treatment, reaching an approximate relative expression level of 0.5 ($p < 0.05$). The transcriptional regulator

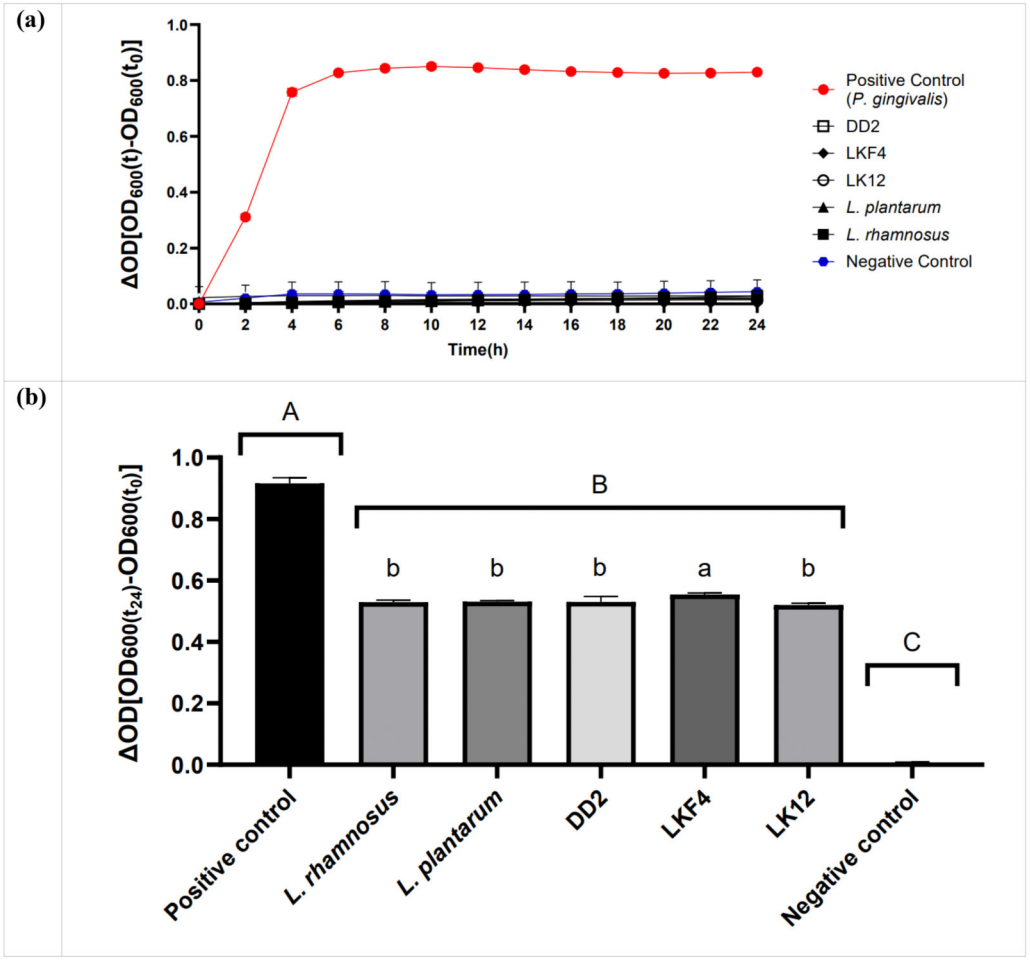

Figure 5. Antimicrobial effects of LAB-derived postbiotics against *P. gingivalis*. Positive control represents oral pathogens cultured without any treatment. The negative control consists of sterile BHI broth supplemented with hemin (5 μg/mL) and menadione (1 μg/mL). Statistical analysis was performed based on ΔOD_{600} values at 24 h and is shown in panel (b). Different uppercase letters (A–C) indicate significant differences among the positive control, LAB-treated, and negative control groups, while lowercase letters (a–b) indicate differences among LAB-treated groups at 24 h (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$). LAB, lactic acid bacteria; ANOVA, analysis of variance; OD, optical density; OD600, optical densities of the cultures at 600 nm.

p65 was downregulated in all postbiotic-treated groups compared with the control ($p < 0.05$). Interestingly, the expression of *Tlr4* significantly increased to nearly 4-fold in all postbiotic groups except the *L. rhamnosus*-treated group. In contrast, the anti-inflammatory cytokine *Il-10* showed an increasing trend across all treated groups, with the DD2 group exhibiting the highest induction level ($p < 0.05$) (Figure 9g). Regarding antioxidant signalling, both *Ho-1* and *Nrf2* were upregulated in all postbiotic-treated groups. *L. rhamnosus* and LKF4 induced the highest expression of *Ho-1* and *Nrf2*, respectively. Although the expression of these antioxidant genes in the DD2-treated group was significantly higher than in the control, it remained lower than that observed in several other postbiotic-treated groups.

Discussion

In this study, we demonstrated that postbiotics derived from kefir-associated LAB, particularly *Lactobacillus kefiranofaciens* DD2, exhibited multifunctional biological activities relevant to oral health. These postbiotics showed antimicrobial and anti-biofilm effects against major oral pathogens, together with immunomodulatory activity in an *in vitro* macrophage model, supporting their potential as adjunctive bioactive agents for oral health applications [2,6].

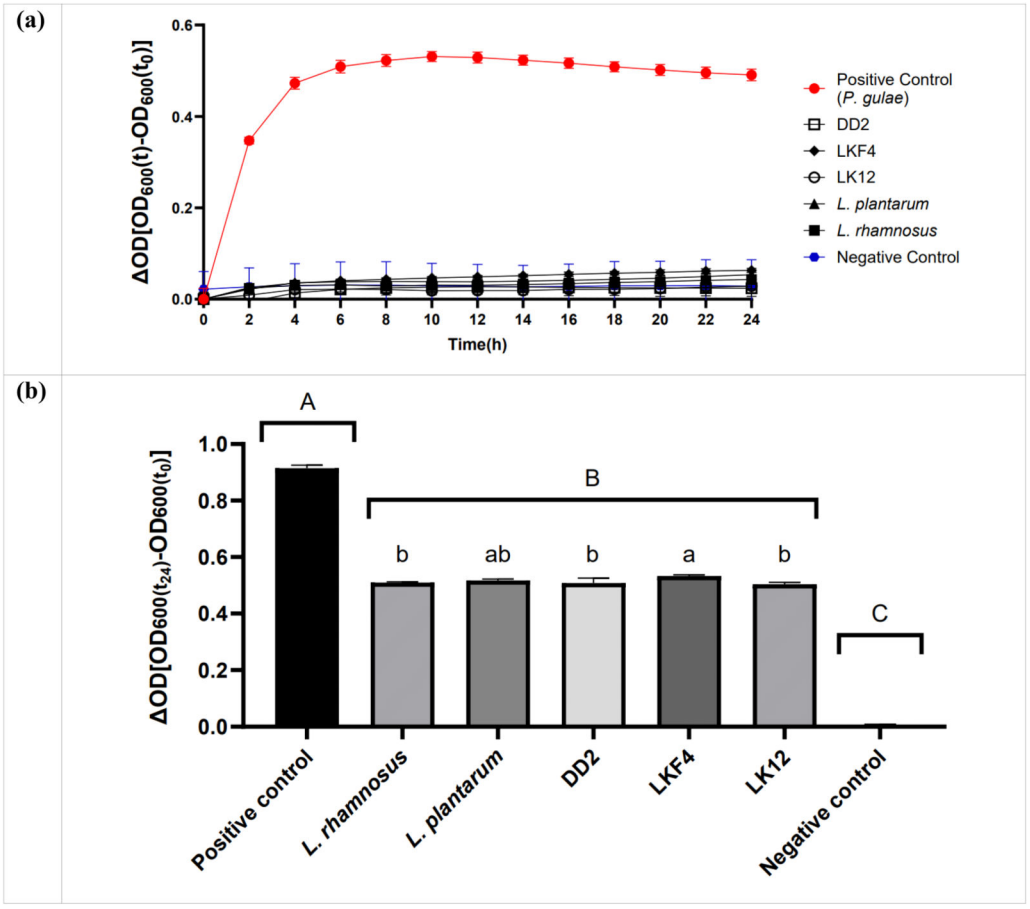

Figure 6. Antimicrobial effects of LAB-derived postbiotics against *P. gulae*. Positive control represents oral pathogens cultured without any treatment. The negative control consists of sterile BHI broth supplemented with hemin (5 μg/mL) and menadione (1 μg/mL). Statistical analysis was performed based on ΔOD_{600} values at 24 h and is shown in panel (b). Different uppercase letters (A–C) indicate significant differences among the positive control, LAB-treated, and negative control groups, while lowercase letters (a–b) indicate differences among LAB-treated groups at 24 h (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$). LAB, lactic acid bacteria; ANOVA, analysis of variance; OD, optical density; OD600, optical densities of the cultures at 600 nm; BHI, brain heart infusion.

The antimicrobial activity of LAB-derived postbiotics against a broad spectrum of pathogens, including *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis*, and *Porphyromonas gulae,* was evident [8,9,20]. Although *P. gulae* is primarily associated with periodontal disease in companion animals, its inclusion in this study broadened the comparative assessment of *Porphyromonas* susceptibility, whereas *P. gingivalis*, *S. mutans*, and *S. sobrinus* represent the main human oral pathogens of direct relevance to this work [8]. Unlike live probiotics, postbiotics consist of diverse bioactive metabolites, including organic acids, bacteriocin-like peptides, and other soluble microbial products, which can directly affect pathogenic bacteria across species [4,35,36]. Organic acids such as lactic acid may reduce local pH and impair bacterial metabolic activity, whereas bacteriocin-like substances are known to disrupt cell membrane integrity or inhibit essential cellular processes [37,38]. These combined effects likely contributed to the growth inhibition observed in this study [4,13].

In addition to suppressing planktonic bacterial growth, LAB-derived postbiotics significantly inhibited biofilm formation by the tested pathogens [3,39]. Biofilms are a universal challenge in oral health; early colonisers like *S. mutans* initiate plaque development, whereas *Porphyromonas* species drive the inflammatory destruction of periodontal tissues within mature biofilms [9,40,41]. The observed reduction in biofilm biomass suggests that postbiotics may interfere with bacterial adhesion or early biofilm establishment, regardless of the host species [20]. Although the precise molecular mechanisms were not investigated, previous studies have suggested that postbiotic components can affect biofilm development through physicochemical

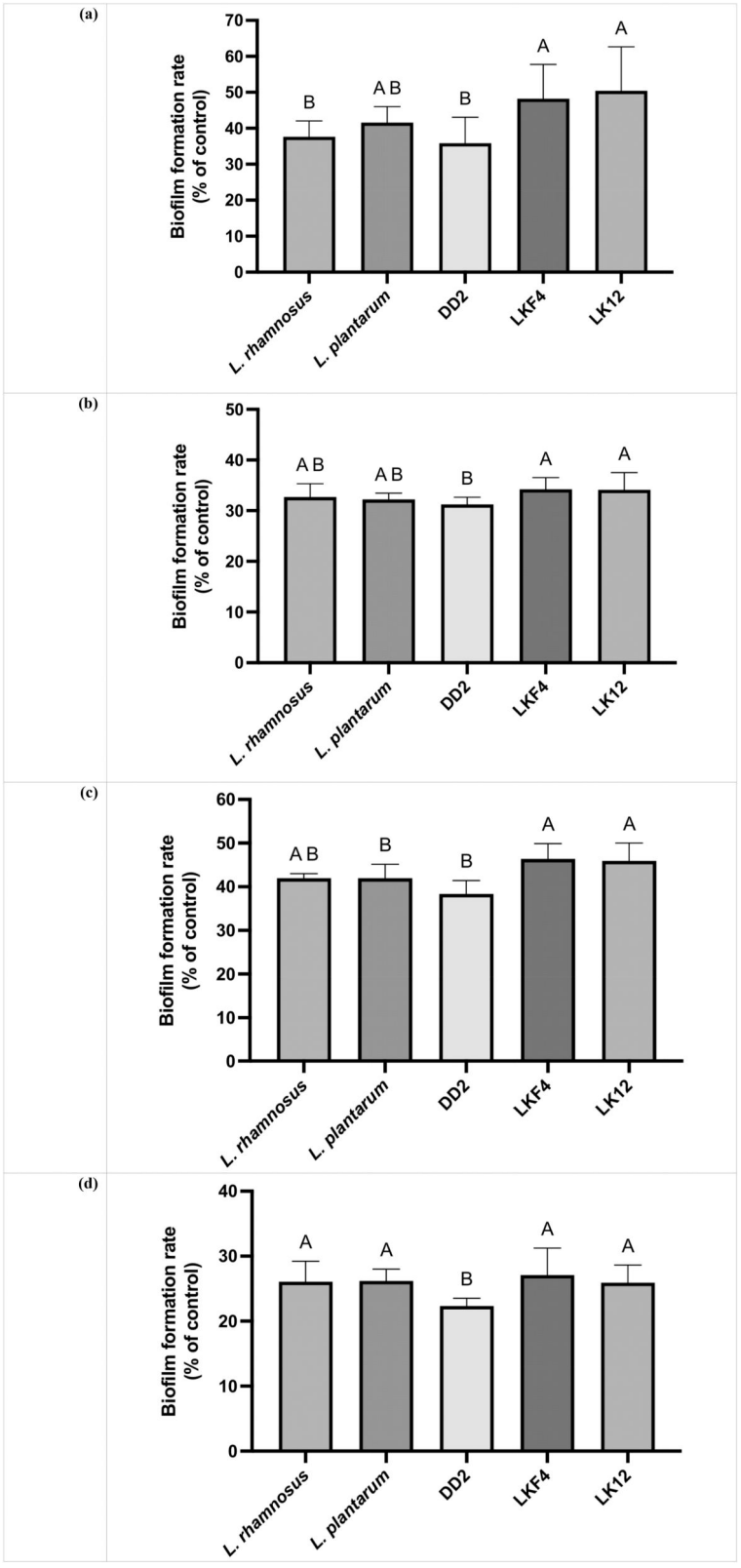

Figure 7. Inhibition of biofilm formation by postbiotics derived from five LAB strains against four oral pathogens. (a) *Strep. mutans*, (b) *Strep. sobrinus*, (c) *P. gingivalis,* (d) *P. gulae*. Biofilm formation rates were expressed as a percentage of the untreated control group (set at 100%). Data represent mean ± SD ($n = 3$). Statistical differences were evaluated by one-way ANOVA followed by Tukey's post hoc test. Different uppercase letters indicate significant differences ($p < 0.05$). LAB, lactic acid bacteria; ANOVA, analysis of variance; OD, optical density; OD600, optical densities of the cultures at 600 nm; SD, standard deviation.

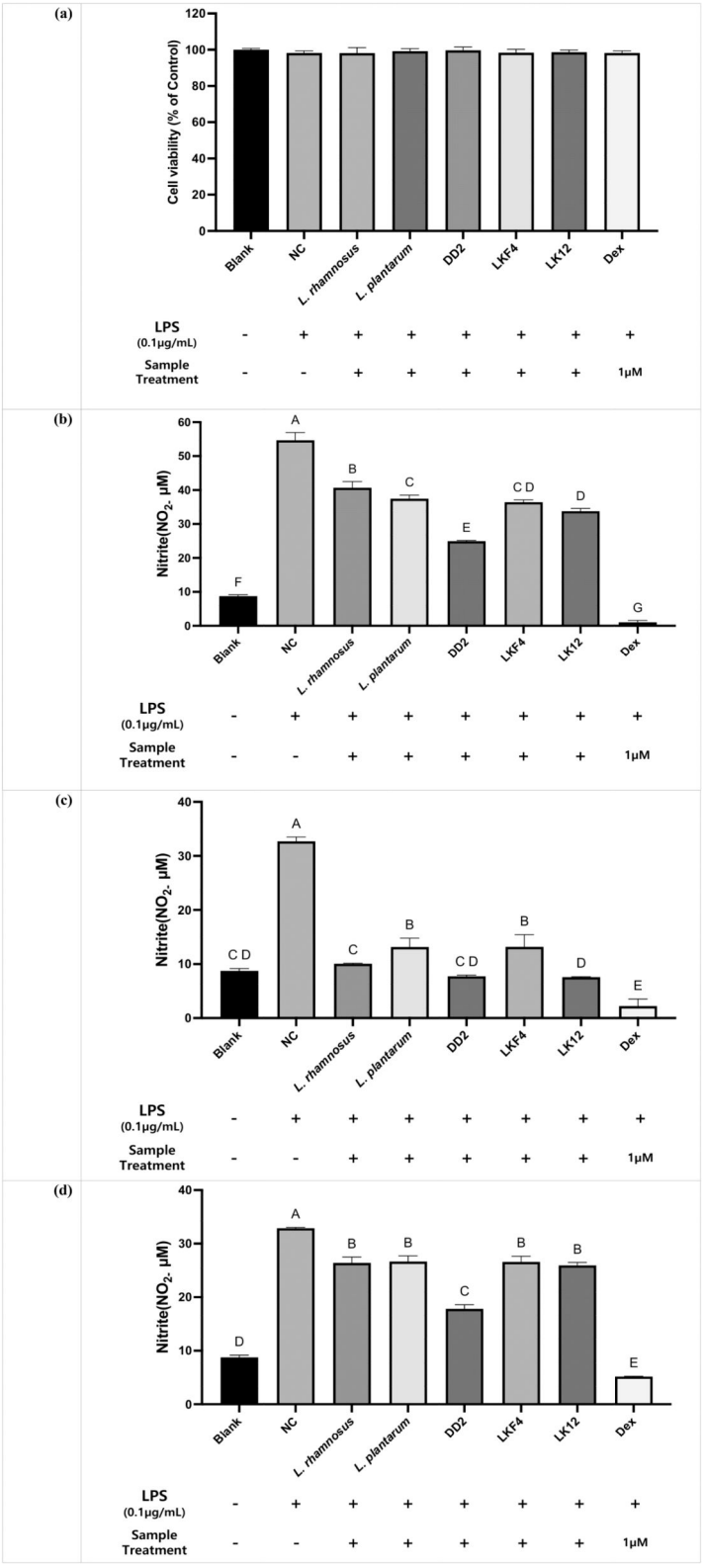

Figure 8. Cytotoxicity and anti-inflammatory effects of LAB in LPS-stimulated RAW 264.7 macrophages. (a) Cytotoxicity of LAB-derived postbiotics evaluated by WST assay after 24 h co-treatment with LPS (0.1 μg/mL). (b-d) Nitric oxide (NO) production measured by Griess assay: (b) 24 h co-treatment with LPS; (c) 2 h pre-treatment with postbiotics followed by 24 h LPS stimulation; (d) 2 h pre-treatment with LPS followed by 24 h postbiotic treatment. Cell viability is expressed as % of control. **Blank:** untreated cells (no LPS, no sample); **NC**: negative control (LPS only); **Dex**: positive control (1 μM dexamethasone). Data are shown as mean ± SD ($n = 3$). Bars with different uppercase letters indicate statistically significant

(Caption on next page)

differences ($p < 0.05$, one-way ANOVA, Tukey's post hoc test). LAB, lactic acid bacteria; ANOVA, analysis of variance; LPS, lipopolysaccharide; Dex, dexamethasone; SD, standard deviation.

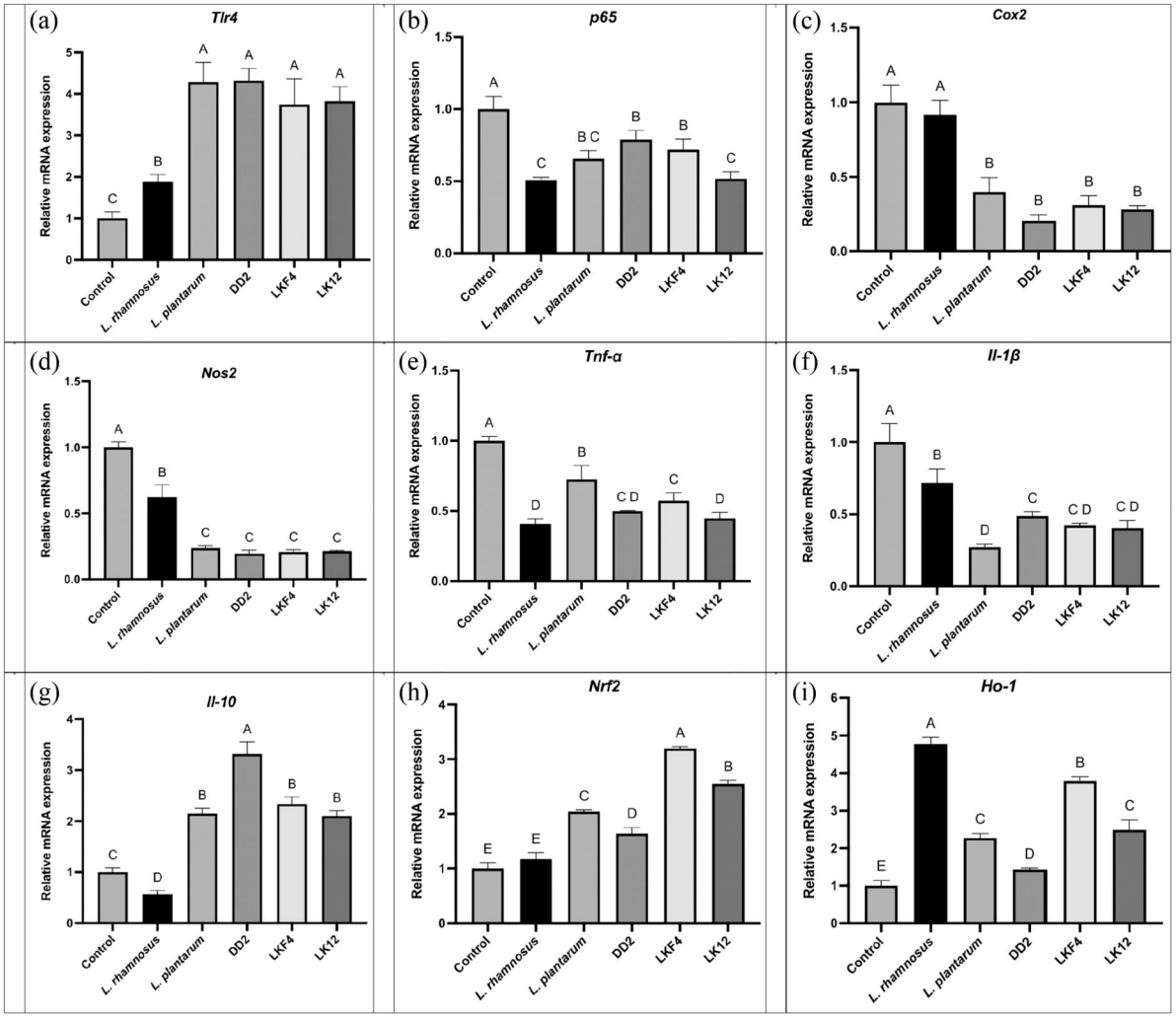

Figure 9. Effects of LAB-derived postbiotics on pro-inflammatory, anti-inflammatory, and antioxidant gene expression in LPS-stimulated RAW 264.7 macrophages. (a) *Tlr4,* (b) *p65*, (c) *Cox2*, (d) *Nos2*, (e) *Tnf-α*, (f) *Il-1β*, (g) *Il-10*, (h) *Nrf2*, and (i) *Ho-1*. RAW 264.7 macrophages were stimulated with LPS and treated with postbiotics derived from the five LAB strains (DD2, LKF4, LK12, *Lactiplantibacillus plantarum* ATCC 3105, and *Lacticaseibacillus rhamnosus* ATCC 7469). Data are expressed as fold changes in mRNA expression relative to the LPS-stimulated control group, which was set to 1.0. Fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method with Gapdh and *β*-actin as reference genes. Values are presented as mean ± SD ($n = 3$). Bars with different uppercase letters differ significantly among groups (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$). LAB, lactic acid bacteria; ANOVA, analysis of variance; OD, optical density; OD600, optical densities of the cultures at 600 nm; SD, standard deviation.

alterations of the local microenvironment or interference with bacterial cell–cell interactions [3,6]. Therefore, the anti-biofilm effects observed here are likely a multifaceted strategy for maintaining oral microbial balance [16,39]. However, growth-curve analysis and crystal violet staining have limitations in distinguishing viable from non-viable cells and in characterising the three-dimensional structure of biofilms. Future studies should incorporate colony-forming unit enumeration, confocal laser scanning microscopy, and live/dead fluorescence staining to more precisely evaluate the effects of DD2-derived postbiotics on biofilm viability and architecture. In addition, although DD2-derived postbiotics inhibited biofilm formation by all tested oral pathogens in single-species biofilm models, oral diseases are driven by complex interactions among multiple microbial species within the oral microbiome. Future studies using dual-species and multi-species biofilm

models would better reflect oral microecology and improve the translational relevance of these findings. Finally, differences in pH among postbiotic preparations may have contributed to the observed antimicrobial effects. Because pH-normalised postbiotic samples and acid-only controls were not included in the present study, the relative contribution of specific bioactive metabolites versus general acidification remains unclear. Future studies should incorporate pH-adjusted postbiotics and acid-matched controls to distinguish metabolite-specific antimicrobial activity from acidity-mediated inhibition.

LAB-derived postbiotics also modulated host inflammatory responses, a critical factor in managing chronic oral diseases. In LPS-stimulated RAW 264.7 macrophages, postbiotic treatment led to significant transcriptional changes in inflammation-related genes. Notably, the immunomodulatory effects were evaluated under neutral pH conditions (pH 7.0–7.4) maintained by the culture media. However, because these experiments were conducted *in vitro* using RAW 264.7 macrophages and HGF cells, the findings should be interpreted as mechanistic support rather than direct evidence of clinical efficacy in the oral cavity. The significant anti-inflammatory activity observed in these models—specifically the marked suppression of *Nos2*, *Cox2*, and *Tnf-α* by DD2—suggests that factors other than acidity may also contribute to the functional properties of DD2-derived postbiotics, potentially involving specific bioactive metabolites such as bacteriocin-like substances or exopolysaccharides. The expression of *Il-1β* was also significantly decreased following DD2 treatment [5,27]. These findings indicate that postbiotics can attenuate inflammatory signalling at the transcriptional level, offering a broader protective effect against inflammation-induced tissue damage.

Interestingly, the expression of *Tlr4* was significantly increased in most postbiotic-treated groups, suggesting that postbiotics may enhance or maintain pathogen recognition capacity while limiting excessive downstream inflammatory signalling [5,6,42]. This pattern is consistent with a balanced immune-modulatory effect rather than global immune suppression, which is essential for maintaining oral mucosal homoeostasis [27,42]. Despite the upregulation of *Tlr4* mRNA, this increase did not translate into amplified inflammatory signalling, as evidenced by the concurrent suppression of NF-κB–associated pro-inflammatory genes and the induction of *Il-10* [5,42]. This pattern suggests that DD2-derived postbiotics preserve pathogen recognition capacity while promoting immune regulatory balance rather than inflammatory amplification.

In parallel, the anti-inflammatory cytokine *Il-10* showed an increasing trend in all postbiotic-treated groups, with the DD2-derived postbiotic exhibiting the highest level of induction [22]. The coordinated suppression of pro-inflammatory mediators and enhancement of *Il-10* expression indicate a shift toward an anti-inflammatory immune profile. Additionally, the upregulation of *Ho-1* and *Nrf2* observed in postbiotic-treated groups suggests a possible association with cytoprotective or stress-responsive signalling pathways [27]. Although antioxidant function was not directly assessed in this study, these observations suggest that DD2 may support cellular resilience.

Taken together, the results of this study suggest that kefir-derived LAB postbiotics, particularly those produced by *L. kefiranofaciens* DD2, exert combined antimicrobial, anti-biofilm, and immunomodulatory effects that are beneficial for managing oral health. By simultaneously targeting pathogens and host inflammatory responses, postbiotics may offer a multifaceted approach to managing oral inflammatory diseases [7,13]. Although this study utilised human and murine cell lines to establish the initial functional efficacy of DD2-derived postbiotics, these models provide fundamental insights into their potential oral bioactivity. Further investigations using canine-specific gingival cells and *in vivo* trials are warranted to fully confirm their clinical effectiveness in companion animals. Although DD2-derived postbiotics demonstrated superior antimicrobial and anti-biofilm activities compared with postbiotics derived from other LAB strains, direct comparisons with clinically used oral antimicrobial agents, such as chlorhexidine, were beyond the scope of the present study. Therefore, future studies should evaluate DD2-derived postbiotics alongside established antimicrobial agents to better determine their translational potential and clinical relevance for oral health applications. Future studies should also identify the key bioactive components responsible for these effects and validate their efficacy, oral retention, persistence, and sustained antibacterial activity using appropriate *in vivo* models and clinical settings. The overall dual antimicrobial and immunomodulatory actions of DD2-derived postbiotics are summarised in Figure 10.

In conclusion, postbiotics derived from kefir-isolated lactic acid bacteria, particularly *Lactobacillus kefiranofaciens* DD2, exhibited multifunctional *in vitro* activities relevant to oral health, including inhibition of oral pathogens and biofilm formation together with modulation of inflammation-related signalling. These findings support DD2-derived postbiotics as promising candidates for further investigation as oral bioactive

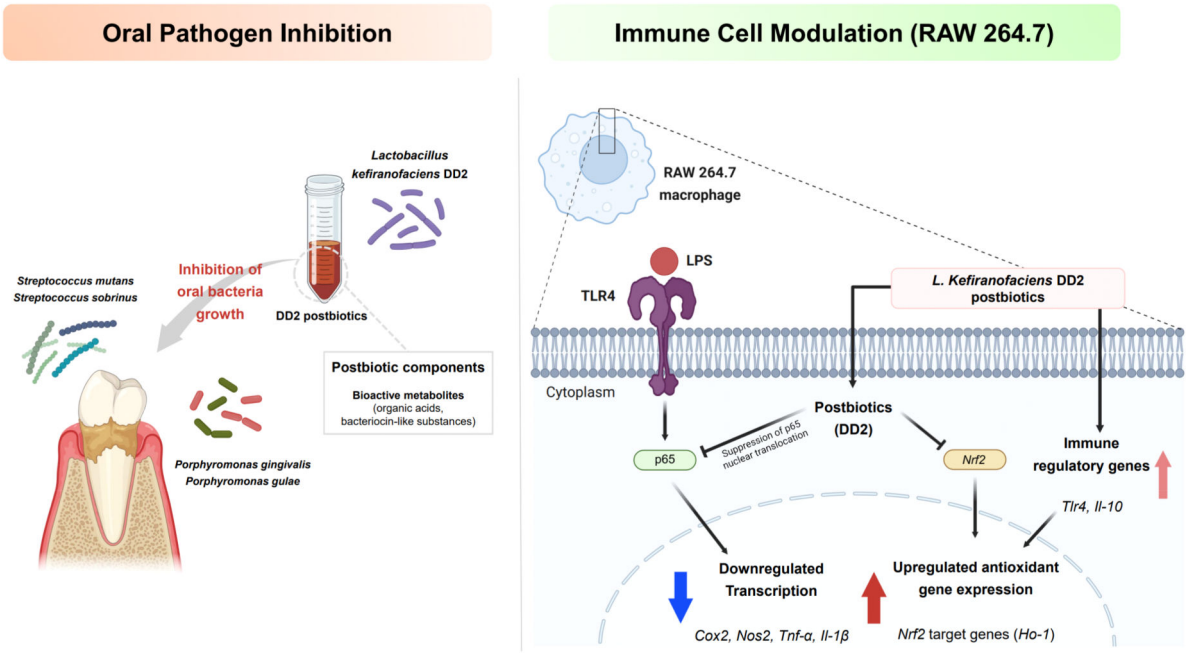

Figure 10. Graphical summary of dual antimicrobial and immunomodulatory effects of *Lactobacillus kefiranofaciens* DD2-derived postbiotics on oral health. DD2-derived postbiotics exert direct antimicrobial effects against major oral pathogens, including *Streptococcus mutans*, *Streptococcus sobrinus*, *Porphyromonas gingivalis*, and *Porphyromonas gulae*, primarily through bioactive postbiotic components such as organic acids and bacteriocin-like substances (left panel). In parallel, DD2-derived postbiotics modulate host immune responses in LPS-stimulated RAW 264.7 macrophages by suppressing NF-κB (p65) nuclear translocation and downregulating pro-inflammatory gene expression (*Cox2, Nos2, Tnf-α, and Il-1β*), while enhancing immune regulatory and antioxidant responses via the induction of *Il-10* and *Nrf2*-dependent target genes (e.g. *Ho-1*) (right panel). Together, these findings illustrate the dual functional roles of DD2-derived postbiotics in inhibiting oral pathogens and maintaining immune regulatory balance.

agents. Although DD2-derived postbiotics exhibited potent antimicrobial, anti-biofilm, and anti-inflammatory activities, the specific bioactive components responsible for these effects remain unidentified. Since postbiotics are complex mixtures of metabolites and other biologically active molecules, future studies should employ advanced analytical approaches, including mass spectrometry-based metabolomic profiling, to characterise their composition and identify the key compounds responsible for the observed biological activities.

Acknowledgements

This work was supported by the National Research Foundation of Korea grant funded by the Korea government [Grant No. RS-2024-00341262] and Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry [Grant No. RS-2024-00401232].

Author contributions

CRediT: **Eun-Ah Jung:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft; **Hyunsook Kim:** Methodology, Resources, Validation, Writing – review & editing; **Wallace H. Yokoyama:** Methodology, Resources, Validation, Writing – review & editing; **Nitin Nitin:** Methodology, Resources, Validation, Writing – review & editing; **Kun-Ho Seo:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Research Foundation of Korea (Grant No. RS-2024-00341262) and the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (Grant No. RS-2024-00401232).

ORCID

Eun-Ah Jung https://orcid.org/0009-0002-5756-8070
Hyunsook Kim https://orcid.org/0000-0001-7345-4167
Wallace H. Yokoyama https://orcid.org/0000-0002-1118-551X
Kun-Ho Seo https://orcid.org/0000-0001-5720-0538

Ethics statement

No human participants or animals were directly involved in this study. Commercially obtained primary human gingival fibroblast cells were used *in vitro*.

Data availability statement

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

References

[1] Ahuja V, Ahuja A. Efficacy of probiotic lactobacillus reuteri on chronic generalised periodontitis: a systematic review of randomized controlled clinical trials in humans. The Journal of Dental Panacea. 2021;3(3):106–117. doi: 10.18231/j.jdp.2021.024
[2] Kamianowski C, Kamianowski A, Nikitiuk J, et al. The effect of probiotic lactobacillus strains on porphyromonas gingivalis, the etiological factor of periodontal Diseases—A systematic review. Probiotics and Antimicrobial Proteins. 2025;18:3055–3093. doi: 10.1007/s12602-025-10607-1
[3] Ahn KB, Baik JE, Park O-J, et al. Lactobacillus plantarum lipoteichoic acid inhibits biofilm formation of streptococcus mutans. PLoS One. 2018;13(2):e0192694. doi: 10.1371/journal.pone.0192694
[4] Wasfi R, Abd El-Rahman OA, Zafer MM, et al. Probiotic lactobacillus sp. Inhibit growth, biofilm formation and gene expression of caries-inducing streptococcus mutans. J Cell Mol Med. 2018;22(3):1972–1983. doi: 10.1111/jcmm.13496
[5] Kang C-H, Kim J-S, Kim H, et al. Heat-killed lactic acid bacteria inhibit nitric oxide production via inducible nitric oxide synthase and Cyclooxygenase-2 in RAW 264.7 cells. Probiotics and Antimicrobial Proteins. 2021;13(6):1530–1538. doi: 10.1007/s12602-021-09781-9
[6] Vale GC, Mayer MPA. Effect of probiotic lactobacillus rhamnosus by-products on gingival epithelial cells challenged with porphyromonas gingivalis. Arch Oral Biol. 2021;128:105174. doi: 10.1016/j.archoralbio.2021.105174
[7] Geraldo BMC, Batalha MN, Milhan NVM, et al. Heat-killed lactobacillus reuteri and cell-free culture supernatant have similar effects to viable probiotics during interaction with porphyromonas gingivalis. J Periodont Res. 2020;55(2):215–220. doi: 10.1111/jre.12704
[8] Bakri HH, Syed Abdul Rahman SN, Abd Halim AA, et al. *In vitro* study of probiotic lactobacillus helveticus: antibacterial effects on porphyromonas gingivalis. PLoS One. 2025;20(8):e0329497. doi: 10.1371/journal.pone.0329497
[9] Nomura R, Inaba H, Yasuda H, et al. Inhibition of porphyromonas gulae and periodontal disease in dogs by a combination of clindamycin and interferon alpha. Sci Rep. 2020;10(1):3113. doi: 10.1038/s41598-020-59730-9
[10] Park S, Shin J, Jun W, et al. Lipoteichoic acid from a canine probiotic strain lacticaseibacillus rhamnosus possesses anti-biofilm capacity against clinically isolated canine periodontopathic porphyromonas species. Microb Pathog. 2025;205:107660. doi: 10.1016/j.micpath.2025.107660
[11] Yang KM, Kim J-S, Kim H-S, et al. Lactobacillus reuteri AN417 cell-free culture supernatant as a novel antibacterial agent targeting oral pathogenic bacteria. Sci Rep. 2021;11(1):1631. doi: 10.1038/s41598-020-80921-x
[12] Deogade SC, Gupta P, Ariga P. Probiotics: contributions to oral and dental health. Journal of International Society of Preventive & Community Dentistry. 2015;5(1):1–7. doi: 10.4103/2231-0762.151977
[13] Jeong D, Kim D-H, Song K-Y, et al. Antimicrobial and anti-biofilm activities of lactobacillus kefiranofaciens DD2 against oral pathogens. J Oral Microbiol. 2018;10(1):1472985. doi: 10.1080/20002297.2018.1472985
[14] Kim D-H, Jeong D, Kim H, et al. Modern perspectives on the health benefits of kefir in next generation sequencing era: improvement of the host gut microbiota. Crit Rev Food Sci Nutr. 2019;59(11):1782–1793. doi: 10.1080/10408398.2018.1428168
[15] Wu S, Fang J-Y, Ng C-C, et al. Anti-inflammatory activity of lactobacillus-fermented adlay-soymilk in LPS-induced macrophages through suppression of NF-κB pathways. Food Res Int. 2013;52(1):262–268. doi: 10.1016/j.foodres.2013.02.053

[16] Zanetta P, Squarzanti DF, Di Coste A, et al. Growth conditions influence lactobacillus cell-free supernatant impact on viability, biofilm formation, and co-aggregation of the oral periodontopathogens fusobacterium nucleatum and porphyromonas gingivalis. Biomedicines. 2023;11(3):859. doi: 10.3390/biomedicines11030859
[17] Bae D, Kim D-H, Chon J-W, et al. Synergistic effects of the early administration of lactobacillus kefiranofaciens DN1 and kluyveromyces marxianus KU140723-05 on the inhibition of salmonella enteritidis colonization in young chickens. Poult Sci. 2020;99(11):5999–6006. doi: 10.1016/j.psj.2020.07.032
[18] Kim D, Jeong D, Kang I, et al. Dual function of lactobacillus kefiri DH5 in preventing high-fat-diet-induced obesity: direct reduction of cholesterol and upregulation of PPAR-α in adipose tissue. Mol Nutr Food Res. 2017; 61(11):1700252. doi: 10.1002/mnfr.201700252
[19] Gündoğdu AK, Karahan AG, Çakmakç ML. Production of nitric oxide (NO) by lactic acid bacteria isolated from fermented products. Eur Food Res Technol. 2006;223(1):35–38. doi: 10.1007/s00217-005-0097-8
[20] Lim S-M, Lee N-K, Paik H-D. Antibacterial and anticavity activity of probiotic lactobacillus plantarum 200661 isolated from fermented foods against streptococcus mutans. LWT. 2020;118:108840. doi: 10.1016/j.lwt.2019.108840
[21] Meurman JH, Antila H, Korhonen A, et al. Effect of lactobacillus rhamnosus strain GG (ATCC 53103) on the growth of streptococcus sobrinus *in vitro*. Eur J Oral Sci. 1995;103(4):253–258. doi: 10.1111/j.1600-0722.1995.tb00169.x
[22] Fang F, Xu J, Li Q, et al. Characterization of a lactobacillus brevis strain with potential oral probiotic properties. BMC Microbiol. 2018;18(1):221. doi: 10.1186/s12866-018-1369-3
[23] Okada A, Imai S, Kikuchi T, et al. Evaluation of the cariogenic potential of a probiotic candidate strain lactobacillus gasseri YIT 12321. Arch Oral Biol. 2022;136:105364. doi: 10.1016/j.archoralbio.2022.105364
[24] Mann S, Park MS, Johnston TV, et al. Isolation, characterization and biosafety evaluation of lactobacillus fermentum OK with potential oral probiotic properties. Probiotics and Antimicrobial Proteins. 2021;13(5):1363–1386. doi: 10.1007/s12602-021-09761-z
[25] Zhao J, Jiang L, Zhu Y, et al. Effect of lactobacillus acidophilus and porphyromonas gingivalis on proliferation and apoptosis of gingival epithelial cells. Adv Med Sci. 2019;64(1):54–57. doi: 10.1016/j.advms.2018.04.008
[26] Kim H-J, Youn H-Y, Moon J-S, et al. Comparative anti-microbial and anti-biofilm activities of postbiotics derived from kefir and normal raw milk lactic acid bacteria against bovine mastitis pathogens. LWT. 2024;191:115699. doi: 10.1016/j.lwt.2023.115699
[27] Han KJ, Lee J-E, Lee N-K, et al. Antioxidant and anti-inflammatory effect of probiotic lactobacillus plantarum KU15149 derived from Korean homemade diced-radish kimchi. J Microbiol Biotechnol. 2020;30(4):591–598. doi: 10.4014/jmb.2002.02052
[28] Tejada-Simon MV, Pestka JJ. Proinflammatory cytokine and nitric oxide induction in murine macrophages by cell wall and cytoplasmic extracts of lactic acid bacteria. J Food Prot. 1999;62(12):1435–1444. doi: 10.4315/0362-028X-62.12.1435
[29] Puspitaningrum I, Ikawati M, Fakhrudin N, et al. Immunomodulatory effect of dioscorea esculenta L. On NF-κB, TLR-4, TNF-α, and IL-10 expressions in LPS-stimulated RAW 264.7 mouse macrophages. The Indonesian Biomedical Journal. 2025;17(3):307–316. doi: 10.18585/inabj.v17i3.3630
[30] An SJ, Pae HO, Oh GS, et al. Inhibition of TNF-alpha, IL-1beta, and IL-6 productions and NF-kappa B activation in lipopolysaccharide-activated RAW 264.7 macrophages by catalposide, an iridoid glycoside isolated from catalpa ovata G. Don (Bignoniaceae). Int Immunopharmacol. 2002;2(8):1173–1181. doi: 10.1016/S1567-5769(02)00085-1
[31] Chen Y, Xu S, Lu Y, et al. Asperuloside suppressing oxidative stress and inflammation in DSS-induced chronic colitis and RAW 264.7 macrophages via Nrf2/HO-1 and NF-κB pathways. Chem Biol Interact. 2021;344:109512. doi: 10.1016/j.cbi.2021.109512
[32] Oh Y-C, Cho W-K, Oh JH, et al. Fermentation by lactobacillus enhances anti-inflammatory effect of oyaksungisan on LPS-stimulated RAW 264.7 mouse macrophage cells. BMC Complementary Altern Med. 2012;12(1):17. doi: 10.1186/1472-6882-12-17
[33] Lee YS, Yang JH, Bae MJ, et al. Anti-oxidant and anti-hypercholesterolemic activities of wasabia japonica. Evidence-Based Complementary and Alternative Medicine. 2010;7(4):517871. doi: 10.1093/ecam/nen038
[34] Wang Z, Jiang W, Zhang Z, et al. Nitidine chloride inhibits LPS-induced inflammatory cytokines production via MAPK and NF-kappaB pathway in RAW 264.7 cells. J Ethnopharmacol. 2012;144(1):145–150. doi: 10.1016/j.jep.2012.08.041
[35] Loyola-Rodriguez JP, Morisaki I, Kitamura K, et al. Purification and properties of extracellular mutacin, a bacteriocin from streptococcus sobrinus. J Gen Microbiol. 1992;138(2):269–274. doi: 10.1099/00221287-138-2-269
[36] Silva M, Jacobus NV, Deneke C, et al. Antimicrobial substance from a human lactobacillus strain. Antimicrob Agents Chemother. 1987;31(8):1231–1233. doi: 10.1128/AAC.31.8.1231
[37] Khalaf H, Nakka SS, Sandén C, et al. Antibacterial effects of lactobacillus and bacteriocin PLNC8 αβ on the periodontal pathogen porphyromonas gingivalis. BMC Microbiol. 2016;16(1):188. doi: 10.1186/s12866-016-0810-8

[38] Pangsomboon K, Kaewnopparat S, Pitakpornpreecha T, et al. Antibacterial activity of a bacteriocin from lactobacillus paracasei HL32 against porphyromonas gingivalis. Arch Oral Biol. 2006;51(9):784–793. doi: 10.1016/j.archoralbio.2006.03.008

[39] Rossoni RD, Velloso MDS, De Barros PP, et al. Inhibitory effect of probiotic lactobacillus supernatants from the oral cavity on streptococcus mutans biofilms. Microb Pathog. 2018;123:361–367. doi: 10.1016/j.micpath.2018.07.032

[40] Nikawa H, Tomiyama Y, Hiramatsu M, et al. Bovine milk fermented with lactobacillus rhamnosus L8020 decreases the oral carriage of mutans streptococci and the burden of periodontal pathogens. Journal of Investigative and Clinical Dentistry. 2011;2(3):187–196. doi: 10.1111/j.2041-1626.2011.00056.x

[41] Park JY, Yoon HJ, Lee N-K, et al. Antioxidant and immunostimulatory effects of lactobacillus strains in RAW 264.7 macrophages via NF-κB and MAPK signaling pathways. Probiotics and Antimicrobial Proteins. 2025;18:353–367. doi: 10.1007/s12602-025-10526-1

[42] Jia L, Wu R, Han N, et al. Porphyromonas gingivalis and lactobacillus rhamnosus GG regulate the Th17/Treg balance in colitis via TLR4 and TLR2. Clinical & Translational Immunology. 2020;9(11):e1213. doi: 10.1002/cti2.1213