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Comprehensive analysis of transcriptional profiles in oral epithelial-like cells stimulated with oral probiotic *Lactobacillus* spp.

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ABSTRACT

Objective: The mechanisms of action of probiotics can vary among species and among strains of a single species; thus, they can affect host cells in a complex manner. In the present study, *Lactobacillus* spp. were evaluated for their ability to adhere to gingival epithelial-like cells. Comprehensive analyses of transcriptional profiles of mouse gingival epithelial GE1 cells treated with *L. rhamnosus* L8020 were performed to assess the putative *in vivo* probiotic potential of this strain.

Methods: Five *Lactobacillus* spp., isolated from the oral cavity, traditional Bulgarian yoghurt, and the feces of a healthy human, were each co-cultured with GE1 cells. Adhesion assays with serial dilution plating and DNA microarray analysis were performed to identify differentially expressed genes (DEGs) in GE1 cells grown in co-culture with *L. rhamnosus* L8020.

Results: The oral isolates *L. rhamnosus* L8020, *L. casei* YU3, and *L. paracasei* YU4 demonstrated significantly greater adhesion compared with the non-oral isolates. In total, 536 genes in GE1 cells exhibited more than twofold upregulation or downregulation, compared with the 0 h timepoint, during co-culture with *L. rhamnosus* L8020. Gene ontology enrichment analysis revealed that DEGs were differentially enriched in a time-dependent manner. Early responses involved widespread changes in gene expression.

Conclusions: This study reveals changes in expression of genes involved in the epithelial physical barrier and immune response in gingival epithelial-like cells co-cultured with *L. rhamnosus* L8020. Further investigations regarding the molecular mechanisms by which *L. rhamnosus* L8020 serves as a probiotic may provide evidence to support clinical use.

1. Introduction

Probiotics are live microorganisms that can confer a health benefit to the host upon oral intake (Food & Agriculture Organization/World Health Organization, 2001; Hill et al., 2014). Various studies have investigated the use of probiotics to prevent health problems, including digestive disorders, allergic disorders, and dental and oral diseases (Bermudez-Brito, Plaza-Díaz, Muñoz-Quezada, Gómez-Llorente, & Gil, 2012; Nikawa et al., 2011).

Microbes of the genus *Lactobacillus*, which ferment saccharides to produce lactic acid, are the predominant indigenous bacteria in the human oral cavity, gastrointestinal tract, and vagina. Novel *Lactobacillus* species are regularly described, such that the genus now includes > 200 species (Salvetti, Harris, Felis, & O'Toole, 2018). Many *Lactobacillus* species are used in fermented food production (*e.g.*, as starter cultures or food preservatives) and in probiotics; accordingly, they have a long history of safe usage in foods and drug supplements (Papizadeh, Rohani, Nahrevanian, Javadi, & Pourshafie, 2017). We previously isolated 42 *Lactobacillus* spp. from healthy volunteers who had no caries or caries-treatment experience. Among the 42 isolates, five, including *L. rhamnosus* 8020, *L. casei* YU3, and *L. paracasei* YU4, showed > 95 % growth inhibition of *Porphyromonas gingivalis* and

Abbreviations: MRS, de Man Rogosa and Sharpe; CXCL, chemokine (C–X–C motif) ligand; CCL, chemokine (C–C motif) ligand; BP, biological processes; CC, cellular components; MF, molecular functions; IFIT, interferon-induced protein with tetratricopeptide repeats; PAI, plasminogen activator inhibitor

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Streptococcus sobrinus in vitro. *L. rhamnosus* 8020 also showed > 95 % growth inhibition of *Candida albicans*. On the basis of this *in vitro* study, we performed clinical studies and found that *L. rhamnosus* 8020 could reduce the oral burden of mutans streptococci and the risk of periodontal disease (Nikawa et al., 2011; Yuki et al., 2019), suggesting that daily consumption of these probiotic organisms may be useful for prevention of dental and oral diseases.

Pathogenic bacteria are well known to induce altered gene expression in host cells (Handfield et al., 2005; Zhu, Lu, Wei, Cai, & Wang, 2018). Notably, multiple studies have shown that probiotics are also able to modulate host gene expression (de Andrés, Jiménez, Espinosa-Martos, Rodríguez, & García-Conesa, 2018; Plaza-Díaz et al., 2017; Taranu et al., 2018). In the oral cavity, epithelial cells act as a physical barrier against invasive and commensal microorganisms. Gingival epithelial cells may be affected by invasive pathogenic microorganisms (*e.g.*, *P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *C. albicans*) and their protective function may be modulated by probiotics.

The mechanisms of action of probiotics can vary among species and among strains of a single species; thus, they can affect host cells in a complex manner (Tsilingiri et al., 2012). Accordingly, research regarding probiotic mechanisms in the oral cavity can facilitate selection of strains for clinical and/or commercial use in treatment or prevention of particular pathological conditions. In the first part of the present work, three oral isolates of *Lactobacillus* spp. were evaluated for their ability to adhere to gingival epithelial-like cells; these oral isolates showed significantly greater adhesion compared with non-oral isolates. In the latter part of the study, one oral isolate, *L. rhamnosus* L8020, which has the widest antimicrobial spectrum among the three oral isolates used in this study, was analyzed to determine its ability to alter the transcriptional profile of gingival epithelial-like cells and contribute to host defense, indicating its putative probiotic potential.

2. Materials and methods

2.1. Bacterial strains

L. rhamnosus L8020, *L. casei* YU3, and *L. paracasei* YU4, previously isolated from healthy and caries-free volunteers (clinical isolates, Hiroshima University Hospital, Hiroshima, Japan) (Nikawa et al., 2011) were selected as the probiotics for investigation in this study. *L. gasseri* OLL2716 and *L. delbrueckii* ssp. *bulgaricus* OLL1073R-1 were used as non-oral isolates. *L. gasseri* OLL2716 was isolated from the feces of a healthy human (Kimura, 2011); *L. delbrueckii* ssp. *bulgaricus* OLL1073R-1 was originally isolated from traditional Bulgarian yoghurt (Makino et al., 2010). *Lactobacillus* spp. were preincubated in *Lactobacillus* de Man, Rogosa and Sharpe (MRS; Difco, Tokyo, Japan) broth for 24 h at 37 °C.

2.2. Cell culture

The GE1 mouse gingival epithelial cell line was obtained from RIKEN Bioresource Center Cell Bank (RCB1709; Ibaraki, Japan) (Hatakeyama et al., 2001; Mine et al., 2010). GE1 cells were previously shown to display a gingival epithelial cell phenotype (Makihira et al., 2010). Cells were cultured in antibiotic-free SFM-101 (Nissui, Tokyo, Japan) containing 1 % fetal bovine serum (HyClone, South Logan, UT, USA) and 10 ng/mL mouse epidermal growth factor (Sigma-Aldrich, St. Louis, MO, USA); cells were incubated at 33 °C in 5% CO_2/95 % air.

2.3. Adhesion of Lactobacillus spp. to GE1 cells

GE1 cells were seeded onto 24-well plates at 2×10^4 cells/well, and the culture medium was changed on day 3. After reaching confluency, there were 7×10^5 GE1 cells per well. *Lactobacillus* spp. cells were harvested during the exponential growth phase by centrifugation at 1000 × *g*, washed twice with phosphate-buffered saline (pH 6.8), and resuspended to a final concentration of 1×10^8 colony-forming units/mL. Then, 100 μL *Lactobacillus* spp. suspension were added to each well of confluent GE1 cells (multiplicity of infection 14.3:1).

To evaluate the adhesion of *Lactobacillus* spp. to GE1 cells, a serial dilution plating method was used, with a modified version of a previously reported protocol (Wang et al., 2018). After 4 h of co-culture, the wells were washed twice with phosphate-buffered saline and the GE1 cells were lysed with 1% Triton-X (Nacalai Tesque, Kyoto, Japan). Numbers of viable adherent *Lactobacillus* spp. were determined by serial plating on MRS-agar (Difco).

2.4. DNA microarray analysis

Expression profiles were determined using the Mouse Clariom™ S Assay (Thermo Fisher Scientific, Waltham, MA, USA). GE1 cells were grown to confluence on 24-well plates, then co-cultured with *Lactobacillus* spp. for 1 or 4 h. Total RNA was isolated from GE1 cells using an RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was synthesized using a GeneChip WT Amplification kit (Thermo Fisher Scientific) following the manufacturer's instructions. The sense cDNA was then fragmented and biotin-labeled with terminal deoxynucleotidyl transferase using a GeneChip WT Terminal labeling kit (Thermo Fisher Scientific). Approximately 5.5 μg of labeled DNA target was hybridized to the Affymetrix GeneChip Array at 45 °C for 16 h. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner (Thermo Fisher Scientific). The data were summarized and normalized using the Signal Space Transformation-Robust Multichip Analysis method implemented in Affymetrix Power Tools (Thermo Fisher Scientific). Statistical significance of expression data was determined by using fold-change analysis. To identify differentially expressed genes, hierarchical cluster analysis was performed with MultiExperiment Viewer, version 4.9.0, using complete linkage and Euclidean distance as a measure of similarity. Gene enrichment and functional annotation analysis to identify significant terms was performed using Gene Ontology (http://geneontology.org); $p < 0.005$ was regarded as the threshold value.

2.5. Statistical analysis

One-way analysis of variance and Tukey's multiple range test were used to analyze differences among groups. Statistical analyses were performed using SPSS Statistics software, version 21.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Oral-isolate Lactobacillus spp. adhere to GE1 cells

As shown in Fig. 1, serial dilution plating revealed that *L. rhamnosus* L8020, *L. casei* YU3, and *L. paracasei* YU4 demonstrated significantly greater adhesion to mouse GE1 cells than non-oral isolates *L. gasseri* OLL2716 and *L. delbrueckii* ssp. *bulgaricus* OLL1073R-1 ($p < 0.05$, $p < 0.01$).

3.2. Identification of differentially expressed genes in GE1 cells co-cultured with L. rhamnosus L8020

To investigate the gene expression profiles underlying putative probiotic effects, DNA microarray analysis was performed in GE1 cells after 0, 1, and 4 h of co-culture with *L. rhamnosus* L8020. Transcriptional profiles observed after 1 h of co-culture were defined as early responses; profiles observed after 4 h of co-culture were defined as secondary responses. In total, 536 genes exhibited more than twofold upregulation or downregulation compared with the 0-h timepoint during co-culture (Fig. 2A); defined early responses included 476 genes with changes in expression. Defined secondary responses included 103

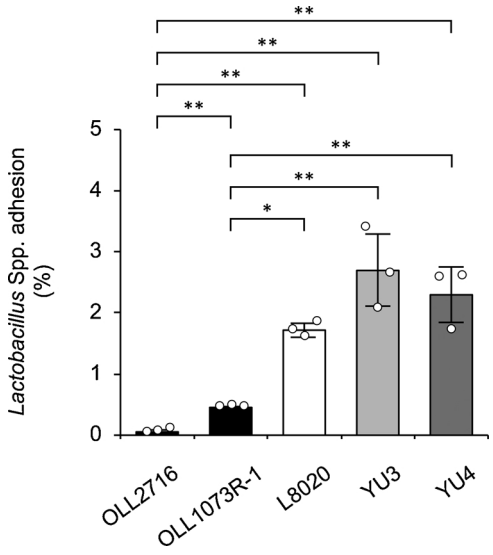

Fig. 1. Adhesion to GE1 cells, compared between *Lactobacillus rhamnosus* L8020 (L8020), *L. casei* YU3 (YU3), *L. paracasei* YU4 (YU4), *L. gasseri* OLL2716 (OLL2716), and *L. delbrueckii* ssp. *bulgaricus* OLL1073R-1 (OLL1073R-1). GE1 cells were co-cultured with *Lactobacillus* spp. for 4 h. Data in all graphs represent the means ± standard deviations of three independent cultures. Asterisks indicate statistically significant differences between *Lactobacillus* spp. (*$p < 0.05$, **$p < 0.01$).

genes with changes in expression. Forty-three genes overlapped between the 476 (early responder) and 103 (secondary responder) genes (Fig. 2A, B).

Early responses included upregulation of genes such as encoding Claudin-1, Desmocollin-2, and Desmocollin-3, all of which are involved in epithelial barrier function (Table 1). Downregulated genes identified in early responses are shown in Table 2. Upregulated genes identified in secondary responses included chemokine (C–X–C motif) ligand (CXCL) 1, CXCL10, and chemokine (C–C motif) ligand (CCL) 2; all of these are known immunomodulatory chemokines (Table 3). Downregulated genes identified in secondary responses are shown in Table 4.

3.3. Gene ontology enrichment analysis of GE1 cells co-cultured with L. rhamnosus L8020

Gene ontology analysis identified 712 terms that were significantly enriched at the 1 h co-culture timepoint, including biological processes (BP), cellular components (CC), and molecular functions (MF). The top 10 Gene Ontology terms in each sub-ontology (*i.e.* BP, CC, and MF) are shown in Fig. 3A. Moreover, 383 terms were significantly enriched at the 4 h co-culture timepoint; the top 10 Gene Ontology terms in each sub-ontology are shown in Fig. 3B. Co-culture with *L. rhamnosus* L8020 for 1 h had potential impacts on numerous BP, including system development, anatomical structure morphogenesis, anatomical structure development, animal organ development, and multicellular organism development. Furthermore, co-culture with *L. rhamnosus* L8020 for 4 h had potential impacts on response to cytokines, defense response, response to biotic stimulus, response to another organism, and response to external biotic stimulus.

4. Discussion

Microbes in the oral cavity coexist with the host in a mostly harmonious symbiotic relationship. However, biofilm accumulation can act as a triggering event, which results in complex immune interactions that induce gingivitis and lead to periodontitis (Kilian et al., 2016). The oral epithelial barrier is composed of two main systems: a physical barrier with an intact epithelial multilayer and intracellular junctions,

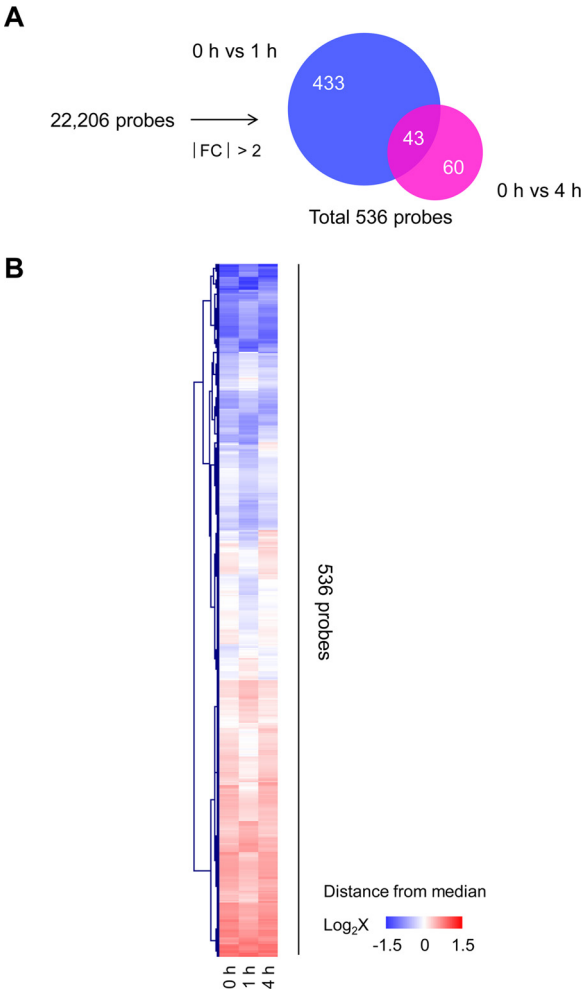

Fig. 2. Comprehensive DNA microarray analysis of mouse GE1 cells co-cultured with *L. rhamnosus* L8020. To identify *L. rhamnosus* L8020-responsive genes, GE1 cells were co-cultured with *L. rhamnosus* L8020 for 1 or 4 h. (A) Venn diagram illustrating subsets of altered genes among 536 genes with significant change in expression (fold change > 2) compared with the 0-h timepoint. (B) Heatmap of microarray data.

Table 1
Top 20 upregulated genes at 1 h.

Gene symbol	Description	Fold change
Ntrk2	neurotrophic tyrosine kinase, receptor, type 2	13.698443
Cldn1	claudin 1	7.434419
Txnip	thioredoxin interacting protein	7.419490
Arrdc3	arrestin domain containing 3	6.339988
Fam83b	family with sequence similarity 83, member B	5.561022
Kitl	kit ligand	5.463896
Dusp6	dual specificity phosphatase 6	4.746256
Spp1	secreted phosphoprotein 1	4.729278
Dbp	D site albumin promoter binding protein	4.064140
Spry1	sprouty homolog 1 (Drosophila)	3.813564
Gm17651	predicted gene, 17651 [Source:MGI Symbol;Acc:MGI:4937285]	3.645104
Dsc2	desmocollin 2	3.614184
Mlip	muscular LMNA-interacting protein	3.589219
Fzd4	frizzled homolog 4 (Drosophila)	3.487033
Dsc3	desmocollin 3	3.485752
Egr1	early growth response 1	3.435000
Dock8	dedicator of cytokinesis 8	3.284947
Etv5	ets variant 5	3.215092
Bhlhe41	basic helix-loop-helix family, member e41	3.211706
Trps1	trichorhinophalangeal syndrome I (human)	3.163628

Table 2
Top 20 downregulated genes at 1 h.

Gene symbol	Description	Fold change
Ctgf	connective tissue growth factor	−14.995939
Il1rn	interleukin 1 receptor antagonist	−11.316924
Abi3bp	ABI gene family, member 3 (NESH) binding protein	−10.077808
Piezo2	piezo-type mechanosensitive ion channel component 2	−8.759448
Tgtp2	T cell specific GTPase 2	−7.712775
Pmepa1	prostate transmembrane protein, androgen induced 1	−7.656049
Sfn	stratifin	−7.640780
Gm12185	predicted gene 12185	−6.480746
Tns1	tensin 1	−6.113167
Serpine1	serine (or cysteine) peptidase inhibitor, clade E, member 1	−5.926786
Tagln	transgelin	−5.811498
Wisp1	WNT1 inducible signaling pathway protein 1	−5.743141
Cyp1b1	cytochrome P450, family 1, subfamily b, polypeptide 1	−5.595744
Ccl5	chemokine (C-C motif) ligand 5	−5.506135
Fam101a	family with sequence similarity 101, member A	−5.372822
Loxl2	lysyl oxidase-like 2	−5.352897
Gm11096	predicted gene 11096 [Source:MGI Symbol;Acc:MGI:3779332]	−5.293053
Impdh1	inosine 5-phosphate dehydrogenase 1	−5.244263
Fbln5	fibulin 5	−5.176439
Ifit1	interferon-induced protein with tetratricopeptide repeats 1	−5.066291

Table 3
Top 20 upregulated genes at 4 h.

Gene symbol	Description	Fold change
Cxcl1	chemokine (C-X-C motif) ligand 1	19.687527
Cxcl5	chemokine (C-X-C motif) ligand 5	12.676196
Ifit1	interferon-induced protein with tetratricopeptide repeats 1	9.113214
Ccl2	chemokine (C-C motif) ligand 2	8.344930
Nfkbiz	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta	7.305181
Cxcl10	chemokine (C-X-C motif) ligand 10	6.735243
Zc3h12a	zinc finger CCCH type containing 12A	6.432190
Gbp2b	guanylate binding protein 2b	6.385059
Ccl7	chemokine (C-C motif) ligand 7	6.291439
Ifit3b	interferon-induced protein with tetratricopeptide repeats 3B	5.855003
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	5.395101
Irgm2	immunity-related GTPase family M member 2	4.609371
Usp18	ubiquitin specific peptidase 18	4.560810
Oasl2	2-5 oligoadenylate synthetase-like 2	4.545125
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	4.307109
Nos2	nitric oxide synthase 2, inducible	4.224147
Vcam1	vascular cell adhesion molecule 1	3.998974
Nfkbie	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, epsilon	3.947447
Nfkbia	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha	3.852540
Gm4951	predicted gene 4951	3.605052

and an immune barrier that maintains immune responses or immune tolerance to microbial antigens (Wang et al., 2019). Thus, a clear understanding of interactions between the gingival epithelium and probiotics is important for the maintenance of oral health.

The ability to adhere to the gingival epithelium is a critical probiotic function, which varies among bacteria and host tissues. Notably, the probiotic *L. rhamnosus* GG (ATCC 53103; isolated from human feces) and most yoghurt starter lactobacilli cannot adhere to saliva-coated hydroxyapatite and microtiter plates, which are used to simulate the oral environment (Stamatova, Kari, Vladimirov, & Meurman, 2009).

Table 4
Top 20 downregulated genes at 4 h.

Gene symbol	Description	Fold change
Gjb3	gap junction protein, beta 3	−2.497042
Ctgf	connective tissue growth factor	−2.366672
Rnf17	ring finger protein 17	−2.326998
Serpine1	serine (or cysteine) peptidase inhibitor, clade E, member 1	−2.315173
Myrfl	myelin regulatory factor-like	−2.306300
Olfr23	olfactory receptor 23	−2.299818
Tns1	tensin 1	−2.289623
Ube2d2b	ubiquitin-conjugating enzyme E2D 2B	−2.225979
Olfr978	olfactory receptor 978	−2.219293
Mok	MOK protein kinase	−2.213716
Gm11084	predicted gene 11084 [Source:MGI Symbol;Acc:MGI:3779315]	−2.175188
Osgin1	oxidative stress induced growth inhibitor 1	−2.155944
Grhl3	grainyhead-like 3 (Drosophila)	−2.114007
Runx1	runt related transcription factor 1	−2.091677
6030408B16Rik	RIKEN cDNA 6030408B16 gene	−2.083083
Fam19a1	family with sequence similarity 19, member A1	−2.074293
Pmepa1	prostate transmembrane protein, androgen induced 1	−2.048517
Hs6st3	heparan sulfate 6-O-sulfotransferase 3	−2.041472
Cga	glycoprotein hormones, alpha subunit	−2.034945
Gm17482	predicted gene, 17482 [Source:MGI Symbol;Acc:MGI:4937116]	−2.017740

Moreover, a clinical study implied that *L. rhamnosus* SD11 (derived from the human oral cavity) may exhibit better adhesion to the gingival epithelium than non-oral strains of human origin (Rungsri et al., 2017). Consistent with these prior results, we confirmed that *L. rhamnosus* L8020, *L. casei* YU3, and *L. paracasei* YU4 exhibited better adhesion to GE1 cells than did non-oral strains of human origin (*i.e.*, *L. gasseri* OLL2716 and *L. delbrueckii* ssp. *bulgaricus* OLL1073R-1).

The mechanisms underlying the ability of *L. rhamnosus* L8020 to reduce the oral burden of mutans streptococci and the risk of periodontal disease have not been fully elucidated. Our comprehensive transcriptomic analysis revealed that defined early responses to *L. rhamnosus* L8020 involved widespread changes in gene expression, which supported reinforcement of the physical barrier in healthy gingival epithelial-like cells. Claudins, a multigene family with at least 27 members in humans and mice, are the main components of tight junctions. Claudin-1, a major component of tight junctions in the oral epithelium (Belibasakis, Kast, Thurnheer, Akdis, & Bostanci, 2015; Fujita et al., 2012), was significantly upregulated as an early response as defined in the present study. Notably, Claudin-1 has been identified as a crucial component for physical barrier function; experimental periodontal disease causes reduced expression of Claudin-1, suggesting involvement in the initiation of periodontal disease (Fujita et al., 2012). Desmocollins constitute desmosomes in combination with desmogleins; notably, desmocollins (Desmocollin-2 and Desmocollin-3) were significantly upregulated as early responses. Desmocollins play crucial roles in cell–cell junctions; furthermore, DNA microarray and/or immunostaining analysis demonstrated low desmocollin expression levels in human periodontitis tissues compared with healthy tissues (Gürsoy et al., 2016; Kim, Ramoni, Nevins, & Fiorellini, 2006).

Regarding defined secondary responses, co-culture with *L. rhamnosus* L8020 treatment served to reinforce the immune barrier in the present study. The immunomodulatory properties of probiotics have been clearly elucidated in gut tissue through *in vitro*, *in vivo*, and clinical studies (van Baarlen et al., 2011). Some previous investigations showed that exposure to probiotic *Lactobacillus* spp. led to upregulation of chemokines (*e.g.*, CXCL1, CXCL10, CCL2, and interleukin-8) in human intestinal epithelial cells and *in vivo* healthy adult mucosa (O'Callaghan, Buttó, MacSharry, Nally, & O'Toole, 2012; O'Flaherty & Klaenhammer, 2012; van Baarlen et al., 2009, 2011). Despite tissue-related and cell-related differences, the results from our *in vitro* model and other

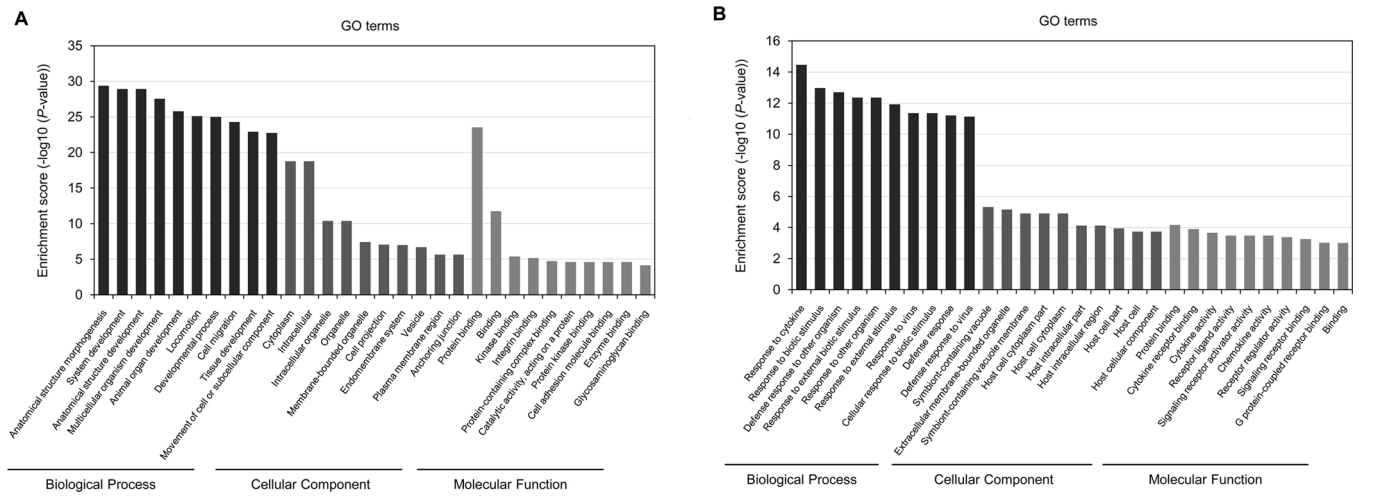

Fig. 3. Gene ontology analysis revealed enriched terms based on differentially expressed genes in GE1 cells co-cultured with *L. rhamnosus* L8020 for (A) 1 h or (B) 4 h. The top 10 Gene Ontology terms in each sub-ontology are shown.

investigations suggest that probiotics can regulate the expression of immunomodulatory genes; moreover, the induction of transient epithelial cell activation may aid in maintenance of mucosal homeostasis. The interferon-induced protein with tetratricopeptide repeats (IFIT) family includes antiviral proteins that can inhibit viral replication by means of an interferon-dependent innate immune response. Notably, expression levels of IFIT1, IFIT3, and IFIT3b were significantly upregulated as secondary responses as defined in the present study. Although the cell types are different from gingival epithelial cells, *Lactobacillus* spp. can induce IFIT-1 activation in mouse cells and human macrophages (Gutierrez-Merino, Isla, Combes, Martinez-Estrada, & Maluquer De Motes, 2020). Some studies have shown interactions between external stimuli and gingival epithelial cells that result in downregulation of IFIT family expression levels. Co-culture of a human buccal epithelial carcinoma cell line with *C. albicans* led to downregulation of IFIT1, IFIT3, and IFIT5 (Moyes et al., 2014). Moreover, exposure to cigarette smoke led to downregulation of the expression levels of IFIT1, IFIT2, IFIT3, and IFIT5 in human oral keratinocytes (Rajagopalan et al., 2018).

CTGF/CCN2 was a noteworthy gene that was downregulated in both defined early and secondary responses in the present study; this gene encodes a secretory protein, which was originally isolated from conditioned medium of human vascular endothelial cells. In previous studies, the expression level of CTGF/CCN2 was upregulated in gingival cells of patients with periodontitis, in a manner similar to the expression of TGF-β (Mize, Sundararaj, Leite, & Huang, 2015). Plasminogen activator inhibitor (PAI)-1, encoded by Serpine1, was also downregulated at both the 1- and 4-h timepoints. Multiple studies have revealed elevated PAI-1 protein levels in plasma of patients with periodontitis, in a manner associated with disease progression (Akman, Fentoğlu, Yılmaz, & Arpak, 2012; Bizzarro et al., 2007). However, analysis of gingival tissues obtained from periodontal surgery revealed that expression levels of PAI-1 were comparable between healthy and inflamed epithelial tissues (Xiao, Bunn, & Bartold, 1998).

In vivo rodent models have been used to clarify novel mechanisms and interactions between probiotic bacteria and oral diseases such as oral candidiasis (Ishijima et al., 2012), periodontal disease (Maekawa & Hajishengallis, 2014), and caries (Hillman, McDonell, Cramm, Hillman, & Zahradnik, 2009). We believe that applying the transcriptional profiles generated in this study to rodent models and further analysis will be useful for clarifying the molecular mechanisms of *L. rhamnosus* L8020 as a probiotic.

5. Conclusions

The probiotic potential of *L. rhamnosus* L8020 was investigated by means of transcriptional profiling analyses in mouse gingival epithelial-like GE1 cells. Early and secondary responses in the present study revealed changes in expression of genes involved in the epithelial physical barrier and immune response. Notably, early responses involved widespread changes in gene expression. Moreover, *L. rhamnosus* L8020 was able to regulate the expression of immunomodulatory genes; the induction of transient epithelial cell activation may also aid in maintenance of mucosal homeostasis. Several of the affected genes may be promising targets for maintenance and promotion of oral health using probiotics. The present study does not elucidate the molecular mechanism of *L. rhamnosus* L8020 efficacy in humans. However, the results provide important suggestions for further studies to clarify the detailed mechanism. It should be emphasized that we have deliberately used gingival epithelial-like mouse cells rather than human cell lines to apply the generated transcriptional profiles to rodent models. In keeping with the present study, we will perform comparative *in vitro* and *in vivo* experiments to elucidate the molecular mechanisms of action by which *L. rhamnosus* L8020 serves as a probiotic, which may provide evidence to support clinical use.

CRediT authorship contribution statement

Kimika Endo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Yuichi Mine:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Takahiro Shuto:** Validation, Writing - review & editing. **Tsuyoshi Taji:** Investigation, Validation, Writing - review & editing. **Takeshi Murayama:** Supervision, Writing - review & editing. **Hiroki Nikawa:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

H.N. has patents for *L. rhamnosus* L8020, *L. casei* YU3, and *L. paracasei* YU4. All other authors declare no conflicts of interest.

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