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(RESEARCH ARTICLE)

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Analysis of gene expression profiles of *Enterococcus faecalis* induced by type I collagen

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Abstract

Enterococcus faecalis is an etiological agent of endodontic infections. The present study was performed to investigate the gene profiles of *E. faecalis* induced by type I collagen stimulation. *E. faecalis* ATCC 19433 was cultivated with [collagen (+)] or without type I collagen [collagen (−)], and transcriptome analysis was performed using high-throughput sequencing technology. A total of 3.6 gb of information was obtained by sequence analysis and 77 differentially expressed genes (DEGs) between the two culture conditions were identified. Among the 77 DEGs, 35 genes were upregulated in collagen (+) *E. faecalis*, whereas 42 genes were downregulated. Gene Ontology (GO) enrichment analysis was performed and 11 GO terms, including metalloendopeptidase activity (GO:0004222) and two related GO terms (GO:0031012, GO:0044421), were significantly enriched in the set of upregulated genes. We focused on an upregulated DEG belonging to the matrixin metalloprotease gene family, and matrix metalloprotease (MMP) activities of the bacterial cell were examined. The generic MMP, MMP-8, and MMP-9 activities of collagen (+) *E. faecalis* were significantly higher than those of collagen (−) *E. faecalis*. These results suggested that contact with type I collagen may alter the gene expression profile of *E. faecalis*, and upregulation of metalloprotease genes may result in enhanced MMP activities in *E. faecalis*.

Keywords: *Enterococcus faecalis*; Transcriptome analysis; Matrix metalloprotease; Gene ontology (GO) enrichment analysis

1. Introduction

Enterococcus faecalis is a facultative anaerobic gram-positive bacterium that is known to be a commensal organism in the gastrointestinal tract and oral cavity. However, this bacterium has also been shown to act as a pathogen in serious systemic infections, including bacteremia, endocarditis, urinary tract infections, surgical wound infections, and intraabdominal and intrapelvic infections, especially in immunocompromised hosts [1]. *E. faecalis* is also known to be one of the major causes of serious nosocomial infections. The spread of vancomycin-resistant enterococci (VRE) is a universal problem in hospitals and long-term care facilities [2].

In the oral cavity, *E. faecalis* has been detected in root canal infections [3] and infected dentin [4]. In endodontic infections, *E. faecalis* is one of the most frequently implicated pathogens in both primary and secondary root canal infections [5]. As *E. faecalis* has been frequently isolated from the obturated root canals of teeth with apical periodontitis, this bacterium has been suggested to have a pathogenic role in endodontic treatment failure [6]. It has been reported that *E. faecalis* shows resistance to root canal treatment, including the medicaments used [7]. It has also been reported that *E. faecalis* can survive in root canals without the support of other symbiotic bacteria [8]. Due to these characteristics, *E. faecalis* is the most widely implicated pathogen in persistent root canal infections. For the host, especially under conditions of immune compromise, root canals connected to alveolar bone could represent a route for

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oral bacteria associated with risks of morbidity and mortality. In this regard, *E. faecalis*, a persistent invader of the root canal, is one of the most high-risk pathogens.

In addition to antibiotic resistance, a series of virulence factors of *E. faecalis* have been reported. The surface protein is involved in immune evasion [9]. Cytolysin plays a role in hemolytic and bactericidal activity against other gram-positive bacteria [1]. Aggregation substances, gelatinase, collagen binding protein, etc., have also been reported to be involved in the pathogenesis of various infectious diseases [1]. Collagen-binding protein and protease have been reported to be virulence factors in endodontic infection [10]. *E. faecalis* has been reported to play critical pathogenic roles in both systemic and dentinal infections, and collagen is the common host component between these two types of infectious lesions. We hypothesized that *E. faecalis* may have common virulence factors against collagen between systemic and dentin infection. Contact with type I collagen may alter the mRNA profile of *E. faecalis* and lead to adaptation to the root canal environment and enhancement of virulence. In the present study, transcriptome analysis was performed to identify type I collagen induced genes. Further, based on the results of transcriptome analysis, the phenotypic alterations to matrix metalloprotease (MMP) activity attributable to contact with type I collagen were examined.

2. Material and methods

2.1. Bacterial strain and culture conditions

E. faecalis ATCC 19433 was cultivated in brain-heart infusion (BHI) broth (Nissui, Tokyo, Japan) at 37°C with (1 μg/mL) or without type I collagen (Nitta Gelatin, Osaka, Japan). The optical density at 660 nm (OD_{660}) of the medium was monitored using a MiniPhoto absorbance meter (Taitec, Koshigaya, Japan).

2.2. Transcriptome analysis

E. faecalis was harvested at mid-exponential phase, and total RNA was extracted using a RiboPure™ RNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA). Transcriptome analysis was performed by Rhelixa (Tokyo, Japan). Briefly, 16S and 23S rRNA were removed from the total RNA using a Ribo-Zero Plus rRNA Depletion Kit (Illumina, San Diego, CA, USA), and a strand-specific library was constructed by cDNA synthesis and adaptor ligation using NEBNext an Ultra RNA Library Prep Kit for Illumina (Illumina). Sequencing was performed using an Illumina NovaSeq 6000 (Illumina). Quality check and trimming bad quality reads and adaptor sequence were performed using FastQC software version 11.7 (Babraham Instituite: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and Trimmomatic version 0.38 (Illumina), respectively. The trimmed sequence reads were mapped to the reference genome sequence of *E. faecalis* ATCC 19433 on the website of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genome/browse#!/prokaryotes/enterococcus) using HISAT2 version 2.1.0 (11).

The expression level of each gene was evaluated as transcripts per million (TPM) calculated with featureCounts version 1.6.3 (SOURCEFORGE SUBREAD: https://sourceforge.net/projects/subread/files/subread-1.5.2/). Differentially expressed genes (DEGs) between the samples were detected using edgeR software version 3.26.8 (Bioconductor: https://bioconductor.org/packages/release/bioc/html/edgeR.html). Transcripts showing a 2-fold increase or reduction to 1/2 or less in expression with $P < 0.05$ were considered as DEGs, and a volcano plot was made using plotly version 4.9.2.1. (Plotly Python Open-Source Graphing Library: https://plotly.com/python/).

Gene Ontology (GO) enrichment analysis of a subset of DEGs and creation of an oriented graph were conducted with TopGO[3] version 2.36.0 (Bioconductor: https://bioconductor.org/packages/release/bioc/html/topGO.html). GO terms (MF, molecular function; BP, biological process; CC, cellular component) with $P < 0.05$ (Fisher's exact test) were distinguished in this study.

2.3. MMP activity assay

The MMP activities of the whole-cell lysate of *E. faecalis* were assessed using a SensoLyte® 520 (Generic MMP, MMP-1, 2, 8, 9) Activity Kit *Fluorimetric* (AnaSpec, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, 50 μL of the sonicated bacterial cell suspension in assay buffer (3 mg/mL) was mixed with 50 μL of MMP substrate solution provided with the kit. The enzyme reaction was carried out on 96-well plates (Brand GmbH + Co. KG, Wertheim, Germany) and the fluorescence intensity was measured at 490 nm/520 nm and recorded every 5 min for 8 h using a SpectraMax M5 Multi-Mode Microplate Reader (Molecular Devices Co., San Jose, CA, USA). The fluorescence intensity was normalized relative to assay buffer with the substrate and shown in relative fluorescence units (RFU). Recombinant human MMP-1, -2, -8, and -9 (0.5 units/mL; Life Laboratory Co., Sendai, Japan) were used as controls at final concentrations of 0.25 unit/mL. Triplicate measurements were performed twice. Mean RFU at the measurement time

of 210 min were compared between the samples by the Mann–Whitney U test. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. Sequencing

Totals of 20173020 and 16078348 reads were obtained from *E. faecalis* cultivated with type I collagen [collagen(+)] and without collagen [collagen(−)], respectively. The reads were mapped to the *E. faecalis* ATCC 19433 genome sequence (NCBI Reference Sequence: NZ_ASDA00000000.1), and 99.10% [collagen(+)] and 99.11% [collagen(−)] of the reads were matched to the annotated coding sequences.

3.2. DEG and GO enrichment analysis

A total of 77 genes were found to be differentially regulated in collagen(+) versus collagen(−) *E. faecalis* (Fig. 1). Thirty-five genes were upregulated and 42 were downregulated in collagen(+) *E. faecalis* in comparison to collagen(−) *E. faecalis* (Tables 1 and 2, respectively). Genes for ABC transporter permease and dipeptide epimerase showed the highest log2FC (fold change) of 5.97 ($P = 0.001$). The upregulated DEGs included multiple genes for sugar uptake, peptide and amino acid metabolism, and membrane structural proteins. Three virulence-associated genes encoding a matrixin family metalloprotease, WxL domain-containing protein, and LPXTG cell wall anchor domain-containing protein were also included in the upregulated DEGs. The downregulated DEGs included genes associated with membrane structure (LysM peptidoglycan-binding domain-containing protein) and phage.

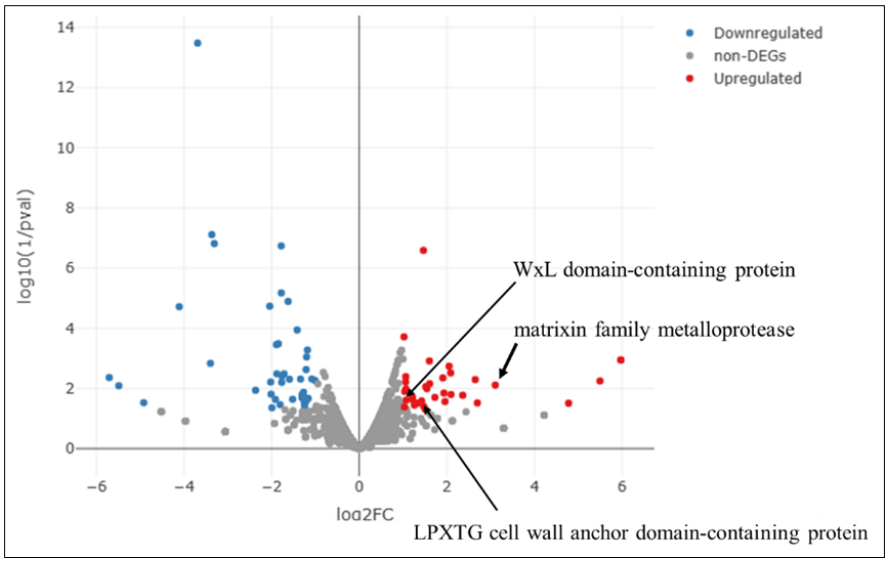

Figure 1 Volcano plot of gene distribution in collagen(+) *E. faecalis* compared with collagen(−) *E. faecalis* samples. The locations of the three upregulated differentially expressed genes (DEGs) associated with the virulence are indicated by arrows

Table 1 DEGs (upregulated)

Gene ID	Description	logFC	*P*-value
WMC_RS14520	beta-glucoside-specific PTS transporter subunit IIABC	1.46	2.57E-07
WMC_RS14735	glycerate kinase	1.02	0.00019
WMC_RS08950	ABC transporter permease	5.97	0.00113
WMC_RS10065	dipeptide epimerase	5.97	0.00113
WMC_RS03480	SDR family oxidoreductase	1.60	0.00121
WMC_RS05280	bifunctional 4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxy-phosphogluconate aldolase	2.05	0.00182

WMC_RS03470	transcriptional regulator GutM	2.09	0.00304
WMC_RS15835	hypothetical protein	1.06	0.00403
WMC_RS16230	PTS sugar transporter subunit IIC	1.91	0.00445
WMC_RS07700	matrixin family metalloprotease	2.65	0.00508
WMC_RS14035	helix-hairpin-helix domain-containing protein	5.50	0.00563
WMC_RS17445	putative holin-like toxin	1.06	0.00604
WMC_RS08470	transporter substrate-binding domain-containing protein	1.61	0.00694
WMC_RS09810	NAD(P)H-dependent oxidoreductase subunit E	3.10	0.00773
WMC_RS10680	ethanolamine ammonia-lyase subunit EutB	1.52	0.00866
WMC_RS09815	NADH-quinone oxidoreductase subunit F	1.54	0.01015
WMC_RS13675	PTS sugar transporter subunit IIB	1.07	0.01030
WMC_RS11820	DUF87 domain-containing protein	1.04	0.01260
WMC_RS13940	purine permease	1.93	0.01425
WMC_RS09300	hypothetical protein	2.09	0.01591
WMC_RS08515	PTS sugar transporter subunit IIB	2.36	0.01681
WMC_RS17350	PTS system mannose/fructose/sorbose family transporter subunit IID	1.21	0.01915
WMC_RS06620	LPXTG cell wall anchor domain-containing protein	1.72	0.01980
WMC_RS10100	PTS fructose transporter subunit IIB	1.21	0.02284
WMC_RS17220	WxL domain-containing protein	1.07	0.02450
WMC_RS03725	pyridoxal phosphate-dependent aminotransferase	1.42	0.02597
WMC_RS16235	PTS sugar transporter subunit IIB	2.00	0.02723
WMC_RS13605	IS6-like element IS1216 family transposase	1.26	0.02924
WMC_RS10640	TIGR02536 family ethanolamine utilization protein	2.70	0.03019
WMC_RS17550	cytosine permease	1.33	0.03083
WMC_RS11745	bifunctional lysozyme/C40 family peptidase	4.78	0.03096
WMC_RS08475	amino acid ABC transporter ATP-binding protein	1.26	0.03576
WMC_RS18065	IS6-like element IS1216 family transposase	1.47	0.03933
WMC_RS03465	PTS glucitol/sorbitol transporter subunit IIC	1.03	0.04103
WMC_RS10625	ethanolamine utilization protein EutQ	1.51	0.04906

DEGs, differentially expressed genes; logFC, log2(fold change).

Table 2 DEGs (downregulated)

Gene ID	**Description**	**logFC**	***P*-value**
WMC_RS16775	putative holin-like toxin	-3.68	3.30E-14
WMC_RS12355	HK97 gp10 family phage protein	-3.36	7.67E-08
WMC_RS12365	hypothetical protein	-3.31	1.53E-07
WMC_RS12335	tape measure protein	-1.78	1.83E-07
WMC_RS12400	phage portal protein	-1.78	6.66E-06
WMC_RS12345	Ig-like domain-containing protein	-1.62	1.28E-05
WMC_RS12405	PBSX family phage terminase large subunit	-2.04	1.84E-05
WMC_RS13175	ParB N-terminal domain-containing protein	-4.11	1.92E-05
WMC_RS12375	DUF5309 domain-containing protein	-1.42	0.00011
WMC_RS12340	hypothetical protein	-1.85	0.00032
WMC_RS13245	hypothetical protein	-1.89	0.00035
WMC_RS12325	phage tail protein	-1.18	0.00052

WMC_RS12280	LysM peptidoglycan-binding domain-containing protein	-1.20	0.00089
WMC_RS06650	hypothetical protein	-3.40	0.00146
WMC_RS12380	DUF4355 domain-containing protein	-1.21	0.00234
WMC_RS12385	hypothetical protein	-1.88	0.00324
WMC_RS17480	NusG domain II-containing protein	-1.72	0.00327
WMC_RS12350	hypothetical protein	-1.76	0.00384
WMC_RS12285	phage holin	-5.71	0.00434
WMC_RS11580	GHKL domain-containing protein	-1.08	0.00483
WMC_RS13715	hypothetical protein	-1.34	0.00486
WMC_RS05935	YibE/F family protein	-1.59	0.00492
WMC_RS11700	response regulator transcription factor	-1.01	0.00544
WMC_RS04645	16S ribosomal RNA	-2.02	0.00611
WMC_RS09135	hypothetical protein	-1.77	0.00627
WMC_RS16180	hypothetical protein	-5.49	0.00811
WMC_RS13645	transposase	-2.37	0.01147
WMC_RS11765	hypothetical protein	-1.27	0.01340
WMC_RS12505	YegP family protein	-2.01	0.01551
WMC_RS12475	YqaJ viral recombinase family protein	-1.31	0.01619
WMC_RS17960	DDE-type integrase/transposase/recombinase	-1.31	0.02036
WMC_RS14685	hypothetical protein	-1.19	0.02132
WMC_RS12410	small subunit of terminase	-1.16	0.02144
WMC_RS05030	DUF4822 domain-containing protein	-1.52	0.02295
WMC_RS11740	conjugal transfer protein	-1.91	0.02320
WMC_RS16000	hypothetical protein	-1.26	0.02638
WMC_RS11975	5-bromo-4-chloroindolyl phosphate hydrolysis family protein	-1.22	0.02665
WMC_RS08075	23S ribosomal RNA	-4.92	0.02973
WMC_RS13725	zinc ribbon domain-containing protein	-1.25	0.03393
WMC_RS12305	hypothetical protein	-1.81	0.03461
WMC_RS04265	ABC transporter ATP-binding protein	-1.99	0.04378
WMC_RS06395	DUF3290 domain-containing protein	-1.24	0.04431

DEGs, differentially expressed genes; logFC, log2(fold change).

Table 3 GO enrichment analysis and functional annotation of DEGs (upregulated)

Category	**GO.ID**	**Term (DEGs/annotated gene numbers)**		***P*-value**
BP	GO:0032196	Transposition (2/8)		0.0052
BP	GO:0015074	DNA integration (2/18)		0.026
	Gene ID	Description	logFC	*P*-value
DEGs	WMC_RS13605	IS6-like element IS1216 family transposase	1.26	0.029
	WMC_RS18065	IS6-like element IS1216 family transposase	1.47	0.039
Category	**GO.ID**	**Term (DEGs/annotated gene numbers)**		***P*-value**
CC	GO:0031012	extracellular matrix (1/1)		0.020
CC	GO:0044421	extracellular region part (1/1)		0.020
MF	GO:0004222	metalloendopeptidase activity		0.034
DEG	Gene ID	Description	logFC	*P*-value
	WMC_RS07700	matrixin family metalloprotease	2.65	0.0051

Category	GO.ID	Term (DEGs/annotated gene numbers)		*P*-value
MF	GO:0051537	2 iron, 2 sulfur cluster binding (1/1)		0.017
DEG	Gene ID	Description	logFC	*P*-value
	WMC_RS09810	NAD(P)H-dependent oxidoreductase subunit E	3.10	0.0077
Category	**GO.ID**	**Term (DEGs/annotated gene numbers)**		***P*-value**
MF	GO:0005353	fructose transmembrane transporter activity (1/2)		0.034
MF	GO:0015145	monosaccharide transmembrane transporter activity (1/2)		0.034
MF	GO:0015149	hexose transmembrane transporter activity (1/2)		0.034
MF	GO:0022877	protein-N(PI)-phosphohistidine-fructose phosphotransferase system transporter activity (1/2)		0.034
MF	GO:0051119	sugar transmembrane transporter activity (1/2)		0.034
DEG	Gene ID	Description	logFC	*P*-value
	WMC_RS10100	PTS fructose transporter subunit IIB	1.21	0.023

GO, gene ontology; DEG, differentially expressed gene; BP, biological process; CC, cellular component; MF, molecular function; logFC, log2(fold change).

Eleven GO terms, including metalloendopeptidase activity (GO:0004222) and two related GO terms (GO:0031012, GO:0044421), were significantly enriched in the set of upregulated DEGs (Table 3). Sugar transmembrane transporter activity (GO:0051119) and four related GO terms (GO:0005353, GO:0015145, GO:0015149, GO:0022877), transposition (GO:0032196), DNA integration (GO:0015074), and 2 iron, 2 sulfur cluster binding (GO:0051537) were also enriched. The oriented graph of the category MF is shown as a representative in Fig. 2. In this category, metalloendopeptidase activity, 2 iron, 2 sulfur cluster binding, and five sugar transport-related GO terms were enriched.

Four GO terms, aminoglycan catabolic process (GO:0006026), glycosaminoglycan catabolic process (GO:0006027), lysozyme activity (GO:0003796), and peptidoglycan muralytic activity (GO:0061783) were significantly enriched in the set of downregulated DEGs (Table 4).

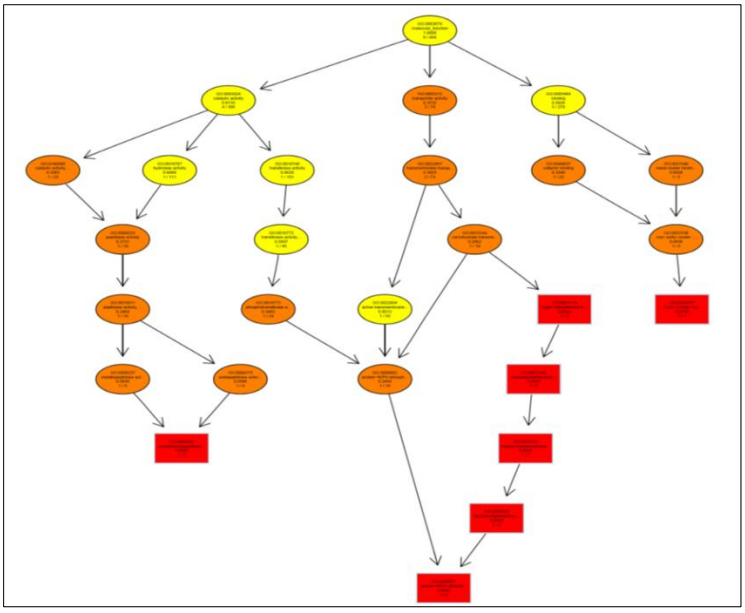

Figure 2 Directed acyclic graph of GO enrichment analysis of category MF (molecular function) and DEG functional annotation. Rectangles represent significantly enriched GO terms, including GO ID, *P*-value, and the numbers of DEGs/annotated gene numbers. The upstream GO function includes the downstream GO function (arrows) and the color intensity represents the degree of GO function enrichment

Table 4 GO enrichment analysis and functional annotation of DEGs (downregulated)

Category	GO.ID	Term (DEGs/annotated gene numbers)		*P*-value
BP	GO:0006026	aminoglycan catabolic process (1/1)		0.0062
BP	GO:0006027	glycosaminoglycan catabolic process (1/1)		0.0062
MF	GO:0003796	lysozyme activity (1/1)		0.0043
MF	GO:0061783	peptidoglycan muralytic activity (1/1)		0.0043
DEG	Gene ID	Description	logFC	*P*-value
	WMC_RS12280	LysM peptidoglycan-binding domain-containing protein	-1.20	0.00089

GO, gene ontology; DEG, differentially expressed gene; BP, biological process; MF, molecular function; logFC, log2(fold change).

3.3. MMP activity

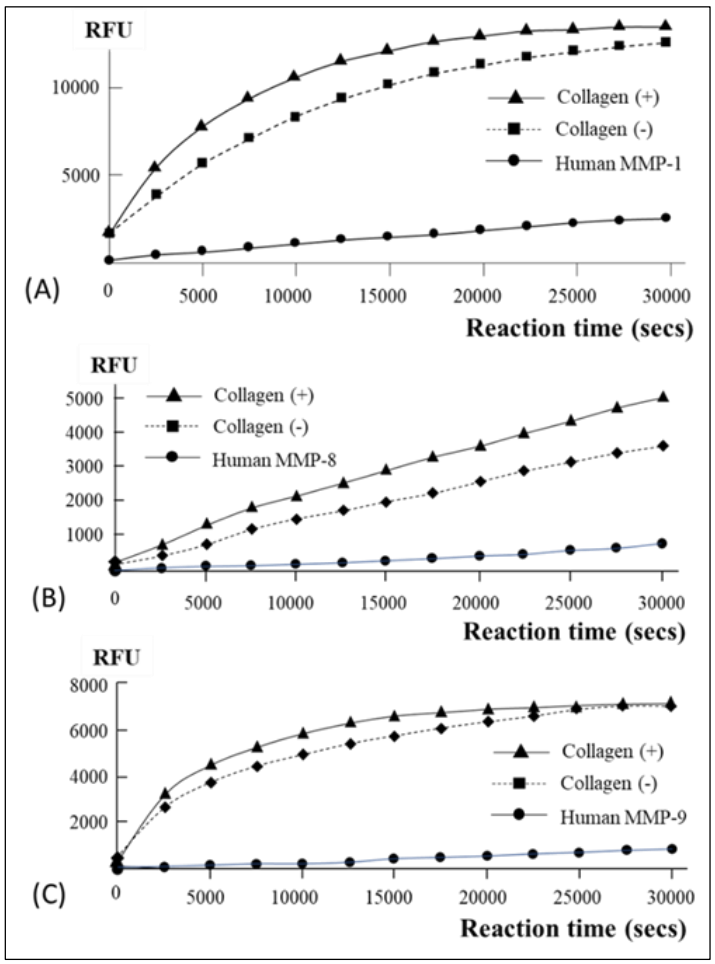

Figure 3 Kinetics of generic MMP activity (A), MMP-8 activity (B), and MMP-9 activity (C) of collagen(+) and collagen(−) *E. faecalis*. RFU denotes the fluorescence intensity resulting from degradation of the substrates and is shown after normalization relative to the negative control. Recombinant human MMP-1, MMP-8, and MMP-9 were used as positive controls

The results of DEG and GO enrichment analysis identified a matrixin family metalloprotease gene that may upregulate MMP activity in *E. faecalis* in contact with type I collagen. Based on the results of transcriptome analysis, phenotypic alterations of *E. faecalis* in MMP activity were examined.

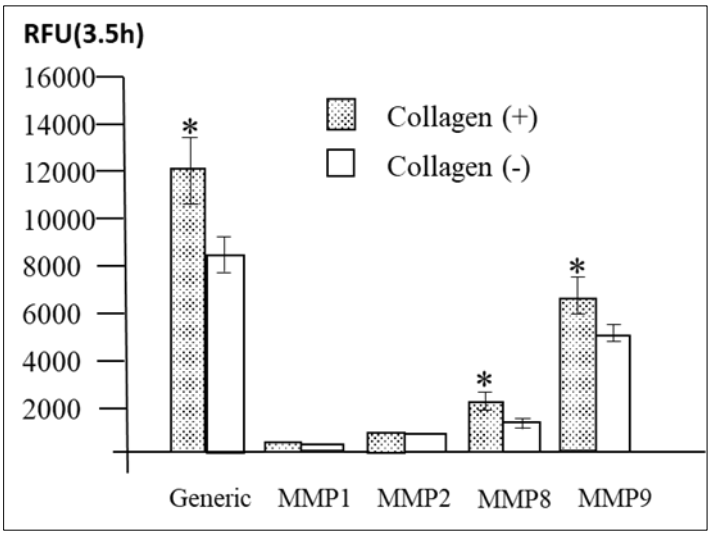

Figure 4 MMP activities of collagen(+) and collagen(−) E. faecalis at 3.5 h. Triplicate measurements were performed twice, and the data are shown as the mean ± SD. *P < 0.05, significant differences in generic MMP, MMP-8, and MMP-9 activities between collagen(+) and collagen(−) E. faecalis (Mann–Whitney U test). RFU denotes the fluorescence intensity resulting from degradation of the substrates

Generic MMP, MMP-1, 2, 8, and MMP-9 activities of the bacterial cell lysate were examined and were compared between collagen(+) and collagen(−) *E. faecalis*. Representative kinetics curves of generic MMP, MMP-8, and MMP-9 activities of the bacterial cells are shown in Fig. 3A – C. The curves of generic MMP and MMP-9 activities reached a plateau at the end-point of the measurements, while that of MMP-8 activity did not reach a plateau at the end-point. The rates of increase in RFU resulting from generic MMP, MMP-8, and MMP-9 activities of collagen(+) *E. faecalis* were higher than those of collagen(−) *E. faecalis*. There were no differences in MMP-1 or MMP-2 activities between the two different culture conditions (data not shown).

The RFU at 3.5 h were compared between collagen(+) and collagen(−) *E. faecalis* (Fig. 4). The RFU resulting from generic MMP, MMP-8, and MMP-9 activities of the collagen(+) *E. faecalis* were significantly higher than those of collagen(−) *E. faecalis* ($P < 0.05$).

4. Discussion

E. faecalis, a common commensal of the gastrointestinal tract of humans and other animals, has emerged as an important opportunistic pathogen that causes a wide variety of systemic diseases [1,2]. The bacterium has also been implicated in the pathogenesis of root canal infection, especially in persistent periapical periodontitis [3]. *E. faecalis* can adhere to the root canal walls, invade into the dentinal tubules, and form a biofilm that makes this bacterium resistant to disinfectants [12]. For bacteria to act as pathogens, they first need to adhere and colonize the host tissue. As *E. faecalis* can colonize both systemic organs and dentin, collagen is the possible host target of this bacterium. Among the collagens, type I collagen is the key molecule in endodontic infections, and a number of studies have investigated the underlying pathogenic mechanisms [12 – 14]. To our knowledge, however, there have been no reports of comprehensive gene expression analyses of *E. faecalis* induced by type I collagen. Our goal was to identify common virulence factors of *E. faecalis* between systematic disease and dentin infections for the prevention of these diseases. As the first step, transcriptome analysis of *E. faecalis* cultivated with type I collagen was performed.

The upregulated genes included three virulence-associated genes (LPXTG cell wall anchor domain-containing protein, WxL domain-containing protein, and matrixin family metalloprotease). The LPXTG cell anchor domain is conserved among the virulence proteins of gram-positive cocci. A collagen-binding protein of *Staphylococcus aureus*, Can, contains the same domain [15]. In addition, Cnm, a collagen-binding protein of *Streptococcus mutans*, contains the LPXPTG domain [16]. Ace, an adhesin with an LPXPTG domain, has been reported to be a collagen-binding protein in *E. faecalis* [17]. These adhesins have been reported to be closely related to the virulence of these bacteria [14 – 17]. The functions of the LPXTG cell wall anchor domain-containing protein identified in this study are still unclear, and further studies are required to elucidate its functions. The WxL domain-containing protein has also been reported to bind to type I collagen and fibronectin in *Enterococcus faecium* [18,19]. *E. faecalis* and *E. faecium* are closely related species, and the WxL domain-containing protein identified here probably has the same function. Although Ace is now recognized as a central

virulence adhesin in *E. faecalis*, further studies of the WxL domain-containing protein are required. The Ace was not upregulated in this study.

In GO enrichment analysis, the GO term metalloendopeptidase activity (GO:0004222) was enriched, and the matrixin family metalloprotease gene identified as an upregulated DEG was also suggested to be a putative virulence factor of *E. faecalis*. This molecule consists of 185 amino acids, and contains a zinc-binding domain. The genome database of *E. faecalis* ATCC 19433 includes six metalloprotease genes and another six metallopeptidase genes. The results of the present study suggested that the identified matrixin family metalloprotease may be a key molecule for elucidating the virulence associated with type I collagen among these proteases. The functions of this molecule have not been reported. Although the details are unclear, the results of MMP assay in this study suggested that the matrixin family metalloprotease may have MMP-8- or MMP-9-like activity. Although gelatinase (GelE) of *E. faecalis* [20] has been reported to be a major protease that acts as a virulence factor, this gene was not upregulated in this study.

The results of GO enrichment analysis and the list of upregulated DEGs suggested the alteration of nutrient uptake of *E. faecalis* through contact with type I collagen. Among the 35 upregulated DEGs, 15 were related to sugar transport or metabolism.

GutM is a DNA-binding transcription factor that regulates an operon involved in transport and utilization of glucitol [21], and permease and aldolase genes were also included in the upregulated DEGs. In addition, three genes for amino acid and peptide metabolism were included in the upregulated DEGs (pyridoxal phosphate-dependent aminotransferase, amino acid ABC transporter ATP-binding protein, and bifunctional lysozyme/C40 family peptidase) [22]. It has been reported that gene expression in *E. faecalis* is regulated to ensure cell survival under altered environmental conditions [23]. The upregulated DEGs associated with nutrient uptake and metabolism identified here may play important roles in the adaptation and survival of *E. faecalis* in host tissue with type I collagen.

The downregulated enriched GO terms and DEG may be related to the membrane structure. LysM modules recognize polysaccharides containing *N*-acetylglucosamine residues, including peptidoglycan, an essential component of the bacterial cell wall [24]. However, the molecular mechanisms underlying LysM–peptidoglycan interactions and their functions remain unclear. Five phage-related genes were included in the downregulated DEGs for unknown reasons.

Our attention was focused on the enriched GO term, metalloendopeptidase activity, and the phenotypic alterations of MMP activity of the cells reacting to type I collagen were examined. In the present study, generic MMP, MMP-8, and MMP-9 activities were enhanced in *E. faecalis* exposed to type I collagen. The results suggested that these MMP activities of *E. faecalis* may play important roles in colonization and virulence in host tissue containing type I collagen. The matrixin family metalloprotease may be a key molecule for enhanced activity. Marashdeh *et al*. [25] reported that the MMP activities of *E. faecalis* showed different activity profiles among the strains, but the greatest activity was seen for MMP-8 or MMP-9, consistent with our results. They also reported that MMP activities of *E. faecalis* were 50 times higher than those of dentinal and salivary enzymes. MMP-8 is a collagenase that cleaves native triple-helical type I, II, and III collagens [26], and MMP-9 is a gelatinase that can degrade collagens [27]. These types of enzymes of *E. faecalis* may play important roles in degradation of dentinal collagen rather than salivary or dentinal enzymes, and may be common virulence factors between dentin and systemic infections.

5. Conclusion

A set of genes in *E. faecalis* induced by type I collagen were identified. The gene for matrixin family metalloprotease, LPXTG cell wall anchor domain-containing protein, WxL domain-containing protein, and a series of genes for energy uptake were upregulated. These molecules may be involved in the pathogenesis of dentin infection. MMP-8 and MMP-9 activities of *E. faecalis* were enhanced by stimulation with type I collagen, suggesting that they play enzymatic roles in dentin degradation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflicts of interest to declare.

References

[1] Raza T, Ullah SR, Mehmood K, Andleeb S. Vancomycin resistant Enterococci: A brief review. J Pak Med Assoc. 2018; 68: 768-772.

[2] Rice LB. Emergence of vancomycin-resistant enterococci. Emerg Infect Dis. 2001; 7: 183-187.

[3] Sedgley C, Buck G, Appelbe O. Prevalence of Enterococcus faecalis at multiple oral sites in endodontic patients using culture and PCR. J Endod. 2006; 32: 104-109.

[4] Salah R, Dar-Odeh N, Abu Hammad O, Shehabi AA. Prevalence of putative virulence factors and antimicrobial susceptibility of Enterococcus faecalis isolates from patients with dental Diseases. BMC Oral Health. 2008; 8: 17.

[5] Zhang C, Du J, Peng Z. Correlation between Enterococcus faecalis and Persistent Intraradicular Infection Compared with Primary Intraradicular Infection: A Systematic Review. J Endod. 2015; 41: 1207-1213.

[6] Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85: 86-93.

[7] Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol. 1990; 6: 142-9.

[8] Fabricius L, Dahlén G, Holm SE, Möller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res. 1982; 90: 200-2006.

[9] Sood S, Malhotra M, Das BK, Kapil A. Enterococcal infections & antimicrobial resistance. Indian J Med Res. 2008; 128: 111-121.

[10] Kayaoglu G, Ørstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Crit Rev Oral Biol Med. 2004; 15: 308-320.

[11] Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol. 2019; 37: 907-915.

[12] Kayaoglu G, Erten H, Ørstavik D. Possible role of the adhesin ace and collagen adherence in conveying resistance to disinfectants on Enterococcus faecalis. Oral Microbiol Immunol. 2008; 23: 449-454.

[13] Love RM. Enterococcus faecalis--a mechanism for its role in endodontic failure. Int Endod J. 2001; 34: 399-405.

[14] Hubble TS, Hatton JF, Nallapareddy SR, Murray BE, Gillespie MJ. Influence of Enterococcus faecalis proteases and the collagen-binding protein, Ace, on adhesion to dentin. Oral Microbiol Immunol. 2003; 18: 121-126.

[15] Gillaspy AF, Patti JM, Pratt FL Jr, Iandolo JJ, Smeltzer MS. The Staphylococcus aureus collagen adhesin-encoding gene (cna) is within a discrete genetic element. Gene. 1997; 196: 239-248.

[16] Nomura R, Ogaya Y, Nakano K. Contribution of the Collagen-Binding Proteins of Streptococcus mutans to Bacterial Colonization of Inflamed Dental Pulp. PLoS One. 2016; 11(7): e0159613.

[17] Rich RL, Kreikemeyer B, Owens RT, LaBrenz S, Narayana SV, Weinstock GM, Murray BE, Höök M. Ace is a collagen-binding MSCRAMM from Enterococcus faecalis. J Biol Chem. 1999; 274: 26939-26945.

[18] Galloway-Peña JR, Liang X, Singh KV, Yadav P, Chang C, La Rosa SL, Shelburne S, Ton-That H, Höök M, Murray BE. The identification and functional characterization of WxL proteins from Enterococcus faecium reveal surface proteins involved in extracellular matrix interactions. J Bacteriol. 2015; 197: 882-892.

[19] Brinster S, Posteraro B, Bierne H, Alberti A, Makhzami S, Sanguinetti M, Serror P. Enterococcal leucine-rich repeat-containing protein involved in virulence and host inflammatory response. Infect Immun. 2007; 75: 4463-4471.

[20] Wang L, Dong M, Zheng J, Song Q, Yin W, Li J, Niu W. Relationship of biofilm formation and gelE gene expression in Enterococcus faecalis recovered from root canals in patients requiring endodontic retreatment. J Endod. 2011; 37: 631-636.

[21] Yamada M, Saier MH Jr. Positive and negative regulators for glucitol (gut) operon expression in Escherichia coli. J Mol Biol. 1988; 203: 569-583.

[22] Nemoto TK, Ohara Nemoto Y. Dipeptidyl-peptidases: Key enzymes producing entry forms of extracellular proteins in asaccharolytic periodontopathic bacterium Porphyromonas gingivalis. Mol Oral Microbiol. 2021; 36: 145-156.

[23] Giard JC, Hartke A, Flahaut S, Boutibonnes P, Auffray Y. Glucose starvation response in Enterococcus faecalis JH2-2: survival and protein analysis. Res Microbiol. 1997; 148: 27-35.

[24] Mesnage S, Dellarole M, Baxter NJ, Rouget JB, Dimitrov JD, Wang N, Fujimoto Y, Hounslow AM, Lacroix-Desmazes S, Fukase K, Foster SJ, Williamson MP. Molecular basis for bacterial peptidoglycan recognition by LysM domains. Nat Commun. 2014; 5: 4269.

[25] Marashdeh MQ, Gitalis R, Lévesque C, Finer Y. Endodontic pathogens possess collagenolytic properties that degrade human dentine collagen matrix. Int Endod J. 2019; 52: 416-423.

[26] Perumal S, Antipova O, Orgel JP. Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis. Proc Natl Acad Sci U S A. 2008; 105: 2824-2829.

[27] Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand. 2007; 65: 1-13.