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Prevalence, phenotype and genotype of oral enterococci

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This study investigated the prevalence, phenotype and genotype of oral enterococci. Enterococci were detected in oral rinse samples from 11% of 100 patients receiving endodontic treatment and 1% of 100 dental students with no history of endodontic treatment ($P = 0.0027$). All enterococcal isolates were identified as *Enterococcus faecalis*. Viable counts ranged from 1×10 to 6×10^3 colony forming units per mL of oral rinse sample. Potential virulence traits expressed by oral *E. faecalis* strains included production of hemolysin ($n = 4$) and gelatinase ($n = 4$), and response to pheromones in *E. faecalis* culture filtrate ($n = 1$). Six strains produced bacteriocin. All strains were susceptible to ampicillin, benzylpenicillin, gentamicin and vancomycin. There was no evidence of metal-ion resistance. One isolate produced hemolysin, gelatinase and bacteriocin, was resistant to several antibiotics, and responded to the pheromone cPD1. Pulsed-field gel electrophoresis and plasmid analysis showed that oral *E. faecalis* exhibited widespread genetic polymorphism, with plasmids detected in seven strains.

Key words: oral enterococci; genotype; phenotype; prevalence

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Enterococci are gram-positive cocci that form part of the normal gastrointestinal tract flora in animals and humans (12). Clinical studies have shown that enterococci usually make up a small proportion of the flora in the untreated root canal (4, 27). However, enterococci have long been implicated in persistent root canal infections (3, 4, 11) and identified as the species most commonly recovered from root canals of teeth with failed endodontic treatment (20, 28).

It is feasible that the root canal species originate in the oral cavity, but data on oral prevalence of enterococci vary (14, 22). Enterococci were detected in samples from multiple oral sites in 60% of 20 laboratory personnel, 60% of 30 schoolchildren with high caries activity and 75% of eight endodontic patients (14). In contrast, subgingival enterococci were recovered from only 1% of 100 early-onset periodontitis patients and 5.1% of 545 adult periodontitis patients (22). In view of the limited data, the purpose of this study was to examine the oral prevalence, phenotype and genomic profiles of oral enterococci recovered from endodontic patients and from a cohort of subjects not requiring endodontic treatment.

Materials and methods

Study groups

The two study groups consisted of 100 endodontic patients attending the University of Michigan Graduate Endodontic Clinic and 100 dental students with no history of endodontic treatment enrolled at the University of Michigan. Approval to conduct the study was obtained from the University of Michigan Institutional Review Board. Written consent to participate in the study was obtained from all participants. In addition to obtaining an oral rinse sample, information was collected on age, gender, smoker status, medication and details of endodontic treatment. In cases where enterococci were detected in oral rinse samples from endodontic patients, data on active caries status, overall oral hygiene assessment and number of dental restorations were retrospectively obtained from patient records.

Oral rinse sampling and laboratory procedures

Oral rinse samples were obtained from all participants. The sampling and processing procedures were based on the concentrated oral rinse method (23). Patients were asked to rinse their mouths for 60 s with 10 ml sterile distilled water from a 50 ml Falcon polypropylene tube (Becton Dickinson Labware, Franklin Lakes, NJ). Denture-wearing subjects did not remove their prostheses. After 60 s the patients returned the oral rinse to the tube, which was then immediately transferred to the laboratory for processing or stored at 4°C before transfer. All samples were processed in the laboratory within 5 h of sampling. Each sample was centrifuged for 10 min at 10,000 r.p.m. and the supernatant

discarded. The pellet was resuspended in 1 ml of sterile distilled water and vortex mixed for 30 s, producing the final concentrated suspension, 50 μl of which was plated onto each of blood agar [Todd Hewitt Broth (THB, Difco; Becton, Dickinson and Company, Sparks, MD) supplemented with 1.5% agar and 4% horse blood (Colorado Serum Co., Denver, CO)] and bile esculin agar (Difco) using a Spiral Plater (Model D, Spiral Systems, Inc. Cincinnati, OH). The spiral plater mechanically dispenses an adjustable volume of the sample onto the agar plate in an Archimedian spiral, enabling quantification of microorganisms in a liquid sample, a technique found to correlate well with conventional methods of quantification (31). Following inoculation, agar plates were incubated aerobically for 24–48 h at 37°C. After incubation, all plates were examined, and colony forming units counted on bile esculin agar plates. No attempts were made to obtain quantitative data from blood agar plates that were included to serve as positive controls for the more selective bile esculin agar media.

Colonies presumptively identified as enterococci based on bile esculin hydrolysis were purified. Conventional biochemical reactions were used to further characterize the organisms (13). Those isolates characterized as gram-positive cocci, catalase-negative, non-motile, pyruvate-positive, and capable of growth in THB supplemented with 6.5% NaCl at 42°C were identified using API 20 Strep identification kits (Analytical Profile Index; Bio Mérieux SA, France), with type strain *Enterococcus faecalis* ATCC 47077 serving as a positive control. All isolates were identified as *E. faecalis*.

Hemolysin and gelatinase production

Oral *E. faecalis* strains were assessed for hemolytic activity on THB agar supplemented with 4% horse blood. Samples from single colonies were inoculated onto blood agar plates and incubated at 37°C for 24 h. Hemolysin activity was determined as a clear halo around the colonies.

Gelatinase activity was assessed using samples from single colonies inoculated onto agar containing 3% gelatin and incubated at 37°C for 24–48 h. Gelatinase activity was evident as a clear halo around the colonies.

Pheromone response

Oral *E. faecalis* strains were assessed for pheromone response using microtiter dilution assays as previously described (9). Culture filtrate pheromones were prepared from culture filtrates of plasmid-free *E. faecalis* JH2-2. Pheromones cAM373, cCF10, cPD1 and cAD1 were synthesized at the Protein Structure Facility, University of Michigan (Ann Arbor, MI). *E. faecalis* DS16 pAD1, *E. faecalis* OG1X pAM373, *E. faecalis* OG1SS pCF10, *E. faecalis* 39-5 pPD1 and *E. faecalis* OG1X pAD1 were used as positive controls for pheromone response (5). Briefly, strains were grown to early stationary phase in THB and diluted 10-fold in fresh THB. All assays were performed in 8 × 12 microtiter trays. Pheromone solution (100 μl of 100 ng/ml in THB) was placed in the first well of the assigned row and serially diluted (twofold) into 50 μl THB. *E. faecalis* strains (50 μl bacterial suspension grown overnight in THB and diluted 1 : 10 in THB) were added to each well in the assigned row. Trays were incubated for 4 h at 37°C on an orbital shaker at 200 rpm. The pheromone titer was defined as the highest dilution of culture filtrate that induced aggregate formation (clumping) in responder cells.

Bacteriocin production

Oral *E. faecalis* strains were tested for production of bacteriocin activity essentially as previously described (18). Positive and negative control bacteriocin producer strains were *E. faecalis* DS16 and *E. faecalis* FA2-2, respectively (18). Indicator strains were *E. faecalis* FA2-2, *E. faecalis* DS16, *Enterococcus faecium* 409 and *Staphylococcus aureus* ATCC 6538 grown in THB, and *Escherichia coli* DH5α; and *E. coli* ATCC 29417 grown in LB broth (Difco) to stationary phase. An inoculum of 10 μl of each indicator strain was added to 3 ml of liquefied soft agar (0.75%) and poured on a THB agar plate. When this had solidified, samples from single colonies from appropriate producer strains were stabbed into the agar. After aerobic incubation overnight at 37°C, clear zones were visible around the stabs of bacteriocinogenic strains.

Antimicrobial susceptibility

Minimal inhibitory concentrations (MICs) were determined using the E-test (AB Biodisk, Solna, Sweden) (2) following the procedures recommended by the manufacturer. Antibiotics tested were ampicillin, benzylpenicillin, chloramphenicol, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, rifampin, streptomycin, tetracycline and vancomycin. Bacterial suspensions were inoculated onto Mueller-Hinton agar (Difco). Following application of E-test strips, plates were incubated aerobically at 35°C for 16–20 h and MICs read from the interpretive scale at the point at which the ellipse of inhibition intersected the strip.

Metal-ion susceptibility

Minimal inhibitory concentrations of silver nitrate ($AgNO_3$), cadmium sulfate ($CdSO_4$), copper sulfate ($CuSO_4$), mercury chloride ($HgCl_2$) and zinc sulfate ($ZnSO_4$) were determined for oral *E. faecalis* and compared to laboratory strains *E. faecalis* FA2-2 and *E. faecalis* OG1RF in broth micro dilution susceptibility assays using microtiter trays.

Pulsed-field gel electrophoresis (PFGE) and plasmid DNA preparation

Genetic profiles were obtained using pulsed-field gel electrophoresis (PFGE). DNA isolation and digestion for PFGE was essentially as previously described (8). Small-scale plasmid DNA preparations were obtained using an alkaline lysis method as previously described (33). Ethidium bromide-CsCl gradient-prepared plasmid DNA was isolated as previously described (6). Restriction enzymes and buffers were obtained from Invitrogen and restriction analysis of samples was performed under conditions recommended by the manufacturer. Genomic DNA restriction fragments were separated on 0.8% agarose gels in TBE buffer. Plasmid DNA restriction fragments were separated on 0.7% agarose gels in TAE buffer. The gels were stained with ethidium bromide for 30 min and the bands were visualized by fluorescence under UV light.

Statistical analysis

The unpaired *t*-test was used to compare the mean ages of participants in each sampling group. Fisher's exact tests were used to compare prevalence of oral *E. faecalis*, smoking and medication usage in each sampling group and to analyze potential factors associated with oral prevalence of *E. faecalis* in endodontic patients. Significance was defined as $P < 0.05$.

Results

Sampling groups

There were 42 females and 58 males in the endodontic patient group. Reasons

for attendance were initial endodontic treatment ($n = 52$), mid-treatment ($n = 26$), endodontic retreatment ($n = 19$) and recall ($n = 3$). There were 47 females and 53 males in the dental student group. The mean age of endodontic patients (47.2 years, SD 15.9) was significantly higher than the mean age of dental students (23.9 years, SD 2.2) ($P < 0.0001$). There were significantly more smokers in the patient group ($n = 27$) than in the student group ($n = 5$) ($P < 0.0001$) and significantly more patients ($n = 24$) were taking antibiotics or had taken antibiotics within the past 2 months compared to students ($n = 13$) ($P = 0.0478$). There was no statistically significant difference in the numbers of subjects taking medication that had the potential to reduce salivary flow in the patient group ($n = 12$) compared to the student group ($n = 8$).

Oral prevalence of enterococci

Enterococci were detected in oral rinse samples from one dental student (C1) and 11 endodontic patients (E1–E11). All enterococci were identified as *E. faecalis*. Details of endodontic patients whose oral rinse samples carried *E. faecalis* are provided in Table 1. Enterococci were detected in significantly more samples from patients than students ($P = 0.0027$). Viable counts ranged from 1×10 to 6×10^3 colony forming units per ml of oral rinse sample.

Oral prevalence of *E. faecalis* in endodontic patients was not significantly affected by age, gender, smoker status, use of antibiotics within 2 months of sampling, dry mouth medication, symptoms at presentation, and radiographic evidence of periapical lesion associated with the tooth receiving endodontic treatment (Table 2). Data on active caries status, overall oral hygiene assessment and number of dental restorations obtained retrospectively from patient records showed that all patients practiced good oral hygiene and had 10 or more dental restorations. Seven patients had active caries at the time of sampling. The one dental student with oral enterococci was a male non-smoker (age 23 years), who had not taken antibiotics in the previous 2 months and did not take dry mouth medication.

Phenotype

Details on phenotype and potential virulence traits expressed by oral *E. faecalis* are presented in Table 3. Four strains produced hemolysin, four strains produced gelatinase and six strains produced bacteriocin. Bacteriocin production was evident only using indicator strains *E. faecalis* FA2-2, *E. faecalis* DS16 and *E. faecium* 409 and not *S. aureus* ATCC 6538, *E. coli* DH5α and *E. coli* ATCC 29417.

The MIC ranges obtained for antibiotics are shown in Table 4. All strains were susceptible to ampicillin, benzylpenicillin, gentamicin and vancomycin. All strains except one were resistant to clindamycin. Two strains were resistant to erythromycin and one strain to chloramphenicol. Six strains showed intermediate resistance or resistance to tetracycline. The MICs obtained in metal-ion susceptibility tests using oral strains were comparable to those obtained using laboratory strains (Table 5). The strain recovered from a dental student, *E. faecalis* C1, was susceptible to all antibiotics except kanamycin and clindamycin, and expressed gelatinase and bacteriocin production and did not exhibit any particular phenotypic characteristics different from strains recovered from endodontic patients (Table 3).

E. faecalis E1 was the only strain to respond to pheromone in *E. faecalis* JH2-2 culture filtrate. Further analyses using synthetic pheromones showed that *E. faecalis* E1 responded specifically to the pheromone cPD1 but not to the other pheromones tested (Table 6). This strain produced hemolysin, gelatinase and bacteriocin and was resistant to chloramphenicol, clindamycin, erythromycin, kanamycin and streptomycin, and demonstrated intermediate susceptibility to tetracycline.

Table 1. Characteristics of endodontic patients with oral *E. faecalis* ($n = 11$)

Patient data						Viable counts	Oral status		Endodontic clinical status					
Sample	Gender	Age (yr)	Smoker	Antibiotics within 2 months	Dry mouth medication	CFU/ml of oral rinse	Overall oral hygiene	Restorations (*n*)	Active caries	Tooth number	Symptoms	Periapical radiolucency	Sinus tract	RCT stage
E1	M	66	No	No	No	3.6E+03	Good	20	Yes	30	Yes	No	No	Initial
E2	F	32	10 cigs/day for 10 years	No	No	5.0E+03	Good	22 (2 implants)	No	5	Yes	No	No	Initial
E3	F	63	No	Yes	Yes	1.0E+01	Good	14	Yes	3	Yes	No	No	Initial
E4	F	72	No	No	Yes	6.0E+03	Good	11	No	20	No	Yes	No	Initial
E5	M	45	10 cigs/day for 21 years	Yes	No	1.0E+01	Good	10	No	30	Yes	No	No	Initial
E6	F	56	No	No	No	4.0E+03	Good	17	Yes	2	Yes	No	No	Initial
E7	F	48	No	No	No	1.2E+01	Good	10 (full maxillary denture)	Yes	10	No	No	No	Retreatment
E8	M	60	No	No	Yes	3.4E+01	Good	17	Yes	6	No	No	No	Initial
E9	M	37	No	No	No	8.0E+02	Good	11	Yes	14	Yes	Yes	No	Initial
E10	M	63	No	Yes	No	1.4E+02	Good	14	Yes	12	No	Yes	No	Initial
E11	F	42	No	Yes	No	2.0E+03	Good	15	No	28	Yes	No	No	Midtreatment

Genotype

PFGE and plasmid evaluation showed that oral *E. faecalis* exhibited widespread genetic polymorphism (Fig. 1). Small-scale plasmid DNA preparation using an alkaline lysis method showed that seven *E. faecalis* strains had plasmid DNA (Table 3). Further evaluation using ethidium bromide-CsCl gradient-prepared plasmid DNA from *E. faecalis* E1, the oral strain exhibiting the greatest potential virulence activity, indicated that this strain has at least two plasmids (Fig. 2).

Discussion

The 1% and 11% prevalence of oral *E. faecalis* in oral rinse samples in this cross-sectional study is comparable to the 1–5% previously reported in plaque samples from periodontal patients (22) but considerably lower than the 75% (cumulative) in multi-site samples collected from eight

Table 2. Potential factors associated with prevalence of *E. faecalis* in oral rinse samples from endodontic patients

		Present, *n* (%)	Absent, *n* (%)	*P*
Number of individuals		11 (11)	89 (89)	–
Age (mean)		53 years (SD 13)	45 years (SD 15)	n.s.
Gender	Female	6 (55)	36 (40)	n.s.
	Male	5 (45)	53 (60)	
Smoker	Yes	2 (18)	25 (28)	
	No	9 (82)	64 (72)	n.s.
Antibiotics within 2 months				
	Yes	4 (36)	20 (22)	n.s.
	No	7 (64)	69 (78)	
Dry mouth medication				
	Yes	2 (18)	10 (11)	n.s.
	No	9 (82)	79 (89)	
Endodontic symptoms at presentation				
	Yes	7 (64)	37 (42)	n.s.
	No	4 (36)	52 (48)	
Endodontic periapical lesion				
	Yes	3 (27)	49 (55)	n.s.
	No	8 (73)	40 (45)	

n.s., Not significant ($P > 0.05$, Fisher's exact test).

Table 3. Phenotypic characteristics of oral *E. faecalis*

Strain*	Haemolysin	Gelatinase	Bacteriocin	Pheromone response	Plasmid DNA
E1	+	+	+	+	+
E2			+		
E3		+			
E4					+
E5					+
E6	+				
E7			+		+
E8	+		+		
E9	+	+	+		+
E10					+
E11					+
C1		+	+		

*Oral strains E1–11 from endodontic patients, C1 from dental student.

endodontic patients (14). One reason for this disparity could be the different sampling methods used in each study. The present study used a concentrated oral rinse technique in an attempt to quantitatively evaluate enterococci using one sample of the overall oral cavity (23), in effect combining saliva, soft tissue and dental plaque samples, in contrast to the individual site sampling employed by Gold et al. (14). While the oral rinse method allows a degree of quantification not available using other sampling methods, the precise source of *E. faecalis* in the oral cavity and the role of these organisms, if any, in dental caries and endodontic infections remains unestablished. Further, it should be noted that cross-sectional studies are limited, being single views of a microflora existing in a complex dynamic environment and not reproducible within the same individual on different occasions (25, 26). Future studies could examine the prevalence and characteristics of enterococci recovered in the proximity of dental caries or endodontic infections in longitudinal studies.

E. faecalis was detected in significantly more endodontic patients than dental students with no history of endodontic treatment ($P = 0.0027$). Total numbers of enterococci isolated did not exceed 6×10^3/ml of transport medium, in agreement with previous findings (14). Apart from endodontic treatment experience, there were several differences between the two sampling groups, the most significant being smoking habits and age (both $P < 0.0001$), in addition to antibiotic usage ($P < 0.0478$). However, while there were significantly more smokers and antibiotic consumers in the patient group than in the student group, these factors did not appear to influence the presence of *E. faecalis* in endodontic patients (Table 2). In contrast, increased age has previously been shown to be associated with higher oral prevalence of other enteric microorganisms, for example Enterobacteriaceae (24). To determine if age is a significant factor in the oral prevalence of enterococci, future studies could examine the prevalence in different age groups but would need also to consider other variables such as number and status of restorations, caries incidence, salivary flow rate and periodontal status. The potential role of salivary bacteriophages in the low prevalence of *E. faecalis* could also be considered in view of a recent

Table 4. Antibiotic susceptibilities (MICs) of oral *E. faecalis**

	NCCLS MIC standards*			Laboratory strains		Oral strains†											
	Susc	Inter	Res	JH2-2	OGI	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	C1
Ampicillin	<0.5	1–4	>8	0.75	0.75	0.38	1	0.75	0.75	0.5	0.5	0.75	0.38	0.5	1	0.5	0.75
Benzylpenicillin	<8	–	>16	2	3	1	2	2	2	1.5	2	1.5	1.5	1	1.5	2	4
Chloramphenicol	<4	8	>16	3	3	32	4	3	4	1.5	3	2	1.5	8	4	2	4
Clindamycin	<0.5	1–2	>4	8	32	>256	8	24	12	12	16	3	12	>256	>256	8	24
Erythromycin	<0.5	1–4	>8	0.75	4	>256	3	4	3	1.5	3	0.094	4	32	2	1	0.19
Fusidic acid	No standards available			>256	3	4	3	3	2	3	3	4	1.5	3	3	2	3
Gentamicin	<500	–	>500	16	16	12	8	16	12	12	12	6	12	12	12	16	16
Kanamycin	No standards available			>256	>256	>256	48	>256	48	48	48	16	48	>256	128	96	>256
Rifampin	No standards available			>256	4	0.75	4	3	2	3	4	0.5	2	1.5	2	3	4
Streptomycin	No standards available			96	96	>1024	128	96	96	96	128	64	32	128	128	96	128
Tetracycline	<4	8	>16	0.19	0.19	12	48	0.25	16	0.64	0.094	0.094	16	8	16	0.064	0.19
Vancomycin	<4	8–16	>32	1.5	3	3	3	3	1.5	1	2	2	1.5	3	1.5	1.5	3

*E-test, antibiotic concentrations in μg/ml.

†Oral strains E1–11 from endodontic patients, C1 from dental student.

Table 5. Metal-ion susceptibility (MIC) of oral *E. faecalis*[*]

	Laboratory strains		Oral strains[†]											
	FA2-2	OGIRF	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	C1
AgNO3 (μM)	78	39	39	78	78	39	78	78	39	39	39	78	39	78
CuSO4 (mM)	16	16	31	31	16	8	16	16	16	16	8	16	8	16
CdSO4 (mM)	0.6	0.6	1.2	0.6	0.6	0.6	0.6	0.6	0.6	0.6	1.2	0.6	1.2	1.2
HgCl2 (μM)	16	8	8	16	16	16	16	31	16	16	4	16	8	31
ZnSO4 (mM)	16	16	8	16	16	8	16	8	16	8	16	8	16	16

[*]Broth dilution, MICs recorded as μM or mM as indicated.
[†]Oral strains E1–11 from endodontic patients, C1 from dental student.

Table 6. Response of *E. faecalis* E1 to pheromones

Strains tested for induced clumping	Pheromone	Dilution factor 1:2											Negative control (no pheromone)
		2	4	8	16	32	64	128	256	512	1024	2048	
OG1X/pAM373[*]	cAM373	+	+	+	+								−
E1	cAM373	−											−
OG1SS/pCF10[*]	cCF10	+	+	+	+	+	+	+	+	+			−
E1	cCF10	−											−
39-5/pPD1[*]	cPD1	+	+	+	+	+							−
E1	cPD1	+	+	+	+	+							−
OG1X/pAD1[*]	cAD1	+	+	+	+	+	+						−
E1	cAD1	−											−

[*]Known pheromone responsive strains serving as positive controls (5).
E1, oral strain.

report of *E. faecalis* bacteriophages in 22% of human saliva samples (1).

Failed endodontically treated teeth have been associated with the presence of intracanal enterococci (20, 28) although their role, if any, in the infection process has not been definitively established. Therefore, of particular interest in this study was whether oral enterococci, which could feasibly enter the root canal system due to close proximity, carried potential virulence factors. Four of 11 strains recovered from endodontic patients produced hemolysin, a cytolytic protein capable of lysing erythrocytes. While hemolysin activity (often plasmid-encoded) has been associated with 60% of 97 human clinical isolates (17), and virulence in mice (16), the role, if any, of hemolysin production by *E. faecalis* in the human oral cavity remains unclear.

Four oral strains produced gelatinase. Gelatinases are extracellular zinc endopeptidases capable of hydrolyzing gelatin, collagen and other peptides, and produced by a large proportion of *E. faecalis* isolated from hospitalized patients and patients with endocarditis (7). *E. faecalis* strains capable of gelatinase production were shown to contribute to increased severity of endocarditis in animal models (15). Recent data have shown that expression of GelE may contribute to increased dissemination of *E. faecalis* in high-density environments (32). Previously Gold et al. (14) described *E. faecalis* 2 SaR (now known as OG1) as a strain recovered from a human oral cavity that exhibited gelatinase activity capable of inducing caries in germ-free rats. In the present study, three strains recovered from endodontic patients produced gelatinase. A retrospective review of patient records showed that these three patients had active caries at the time of sampling. Further analysis of gelatinase activity in oral *E. faecalis* is ongoing. While oral *E. faecalis* from another four patients with active caries did not produce gelatinase, future studies could examine the relationship between caries status and gelatinase production by oral enterococci.

The pathogenicity of enterococci has been long established in animal models (19). Further, at the molecular level, it is recognized that *E. faecalis* participate in plasmid-mediated horizontal transfer of virulence determinants, thus emphasizing the importance of these organisms in nosocomial infections (5). Plasmids are covalently closed circular, double-stranded, supercoiled DNA that can replicate autonomously. They range in size from ~1 kb to >200 kb, in contrast to the chromosomal DNA size of 3,218 kb recently reported for *E. faecalis* V583 (21). Plasmids frequently

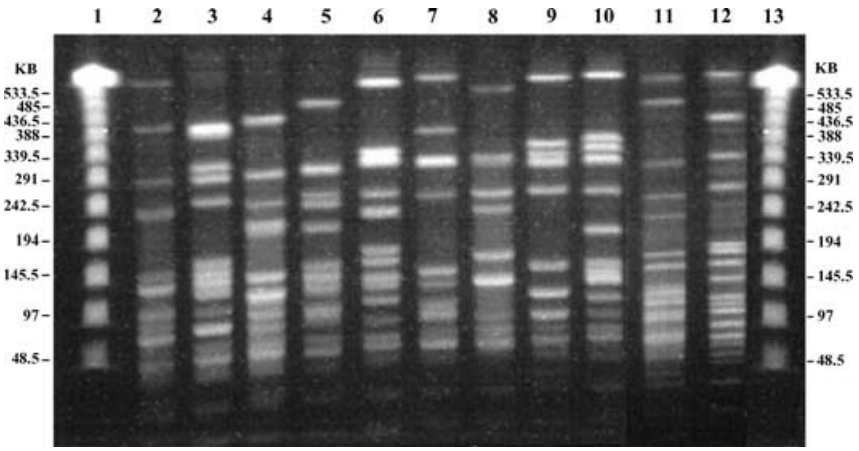

Fig. 1. Pulsed field gel electrophoresis of *SmaI*-digested genomic DNA from oral *E. faecalis* strains. Lanes: 1, lambda phage (PFG) DNA standard; 2, *E. faecalis* E1; 3, *E. faecalis* E2; 4, *E. faecalis* E3; 5, *E. faecalis* E4; 6, *E. faecalis* E5; 7, *E. faecalis* E6; 8, *E. faecalis* E7; 9, *E. faecalis* E8; 10, *E. faecalis* E9; 11, *E. faecalis* E10; 12, *E. faecalis* E11.

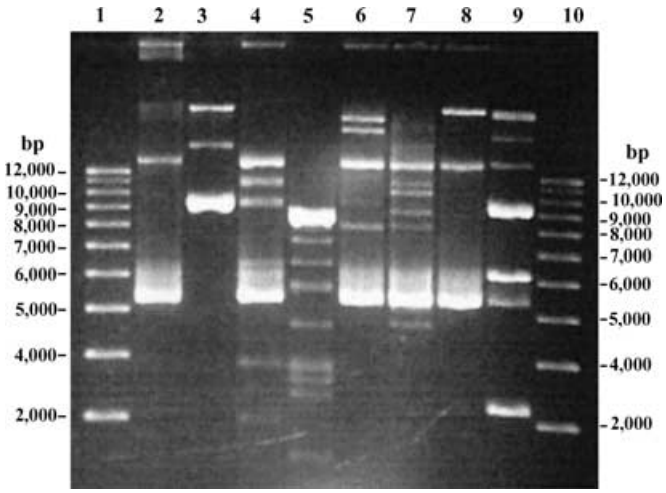

Fig. 2. Agarose gel electrophoresis of *E. faecalis* E1 plasmid DNA prepared using ethidium bromide-CsCl gradients. Lanes 1 and 10: 1 Kb Plus ladder. Lane 2. Uncut plasmid DNA; Lanes 3 to 9, overnight digestion with restriction enzymes: Lane 3, *Bam*HI; Lane 4, *Eco*RI; Lane 5, *Hin*DIII; Lane 6, *Kpn*I; Lane 7, *Pst*I; Lane 8, *Sma*I; Lane 9, *Xba*I.

confer traits that facilitate growth/survival under atypical conditions, for example resistance to antibiotics and metal-ions. In the present study, plasmid DNA was recovered from seven oral strains. None of the oral strains demonstrated increased resistance to metal-ions compared to laboratory strains *E. faecalis* FA2-2 and *E. faecalis* OG1RF. With the exception of *E. faecalis* E1, the oral strains with plasmid DNA were generally highly susceptible to the antibiotics studied apart from clindamycin and aminoglycosides, which are associated with intrinsic resistance (29). Two strains exhibited low-level tetracycline resistance (MIC = 16 μg/ml). While plasmid-mediated virulence activity associated with these seven strains remains to be elucidated, *E. faecalis* E1, which demonstrated hemolysin, gelatinase and bacteriocin production and was resistant to several antibiotics, responded to the pheromone cPD1. Pheromones are small linear peptides secreted by plasmid-free *E. faecalis* strains. Horizontal transfer of plasmid-encoded virulence determinants can be initiated by pheromones in *E. faecalis* (5) and some pheromones are chemotactic for human neutrophils (10). *E. faecalis* E1 clumped in the presence of cPD1, a pheromone known to encode bacteriocin production (34). Bacteriocins are any of a wide variety of protein or peptide antibiotics, commonly encoded by plasmids. In this study, in addition to *E. faecalis* E1, five other oral strains produced bacteriocin, indicating a capacity for bacteriostatic or bacteriocidal activity directed specifically towards organisms closely related to the bacteriocin-producing strain and not to other genera. Further analysis of *E. faecalis* E1 plasmid DNA is ongoing.

Pulsed field gel electrophoresis (PFGE) allows separation of DNA fragments produced by rare-cutting restriction enzymes, too large to be resolved using conventional agarose gel electrophoresis. The technique has been utilized in the identification of enterococci (8) and in molecular epidemiological typing of nosocomial enterococci (30). In the present study PFGE of genomic DNA demonstrated the genotypic polymorphism of the oral strains.

In conclusion, enterococci were detected in oral rinse samples from 11% of 100 patients receiving endodontic treatment and 1% of 100 dental students with no history of endodontic treatment ($P = 0.0027$). Enterococcal isolates, all identified as *E. faecalis*, exhibited widespread genetic and phenotypic polymorphism. Potential virulence traits expressed by oral *E. faecalis* strains included production of hemolysin and gelatinase, and response to pheromones in *E. faecalis* culture filtrate.

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