Enterococcus faecalis with capsule polysaccharides type 2 and biofilm-forming capacity in Indonesians requiring endodontic treatment.

Bechter EV*1, Bechter BM*1, Dowell SP, Kumar A*2

© Author Information

Abstract

AIM: The aim of the present study was to evaluate the genetic diversity of Enterococcus faecalis (E. faecalis) strains isolated from saliva and infected root canal samples from Indonesians requiring endodontic treatment.

METHODS: A total of 50 isolates were genotyped using enterobacterial repetitive interspacerepetitive consensus-polymerase chain reaction (ERIC-PCR) and analyzed for locus polymorphisms of their capsule polysaccharides (CPS) and for biofilm-forming capabilities.

RESULTS: It was shown that all E. faecalis isolates shared >90% similarity. A higher degree of diversity of E. faecalis was observed in cluster 1 (31.29%) and cluster 2 (62.31%) from samples isolated from infected root canals. CPS type 0 was the dominant form observed in the clusters (C1-C5), but there was no relationship between the origins of these isolates. In contrast, all isolates in cluster C5 were of non-canal origin, and 50% were associated with a strong biofilm phenotype. Five unclassified strains were saliva isolates (>80% similarity). Most of these strains showed weak biofilm capability.

CONCLUSIONS: E. faecalis CPS type 0 is relatively common in Indonesians requiring endodontic treatment, and there are differences in the biofilm-forming abilities produced by CPS type 0 strains in all isolates depending on the source. In addition, there is no relationship between the ERIC-PCR profile and biofilm formation.

© 2015 Wiley Publishing Asia Pty Ltd.

KEYWORDS: Enterococcus faecalis; biofilm; capsule polysaccharides; endodontic; enterobacterial repetitive interspacerepetitive consensus-polymerase chain reaction (ERIC-PCR)
Enterococcus faecalis with capsule polysaccharides type 2 and biofilm-forming capacity in Indonesians requiring endodontic treatment

Endang W. Bachtiar¹, Boy M. Bachtiar¹, Sari Dewiyani² & Siti M. Surono Akbar³

¹ Department and Laboratory of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
² Department of Dental Conservation, Faculty of Dentistry, Universitas Prof. Dr. Moestopo (Beragama), Jakarta, Indonesia
³ Department of Dental Conservation, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia

Keywords: biofilm, capsule polysaccharides, endodontic, enterobacterial repetitive intergenic consensus–polymerase chain reaction, Enterococcus faecalis.

Correspondence
Dr Endang Winiati Bachtiar, Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia Jalan Salernba Raya No. 4, Jakarta 10430, Indonesia.
Tel: +6221-3910344
Email: endang04@ui.ac.id

Received 11 March 2014; accepted 29 October 2014.
doi: 10.1111/jcid.12143

Abstract
Aim: The aim of the present study was to evaluate the genetic diversity of Enterococcus faecalis (E. faecalis) strains isolated from saliva and infected root canal samples from Indonesians requiring endodontic treatment.
Methods: A total of 50 isolates were genotyped using enterobacterial repetitive intergenic consensus–polymerase chain reaction (ERIC-PCl) and analyzed for locus polymorphisms of their capsule polysaccharides (CPS) and for biofilm-forming capabilities.
Results: It was shown that all E. faecalis isolates shared >60% similarity. A higher degree of diversity of E. faecalis was observed in cluster 1 (C1, 28%) and cluster 2 (C2, 22%) from samples isolated from infected root canals. CPS type 2 was the dominant form observed in five clusters (C1–C5), but there was no relationship between the origins of these isolates. In contrast, all isolates in cluster C5 were of root canal origin, and 50% were associated with a strong biofilm phenotype. Five unclustered strains were saliva isolates (<60% similarity). Most of these strains showed weak biofilm capability.
Conclusions: E. faecalis CPS type 2 is relatively common in Indonesians requiring endodontic treatment, and there are differences in the biofilm-forming abilities produced by CPS type 2 strains in all isolates depending on the source. In addition, there is no relationship between the ERIC-PCR profile and biofilm formation.

Introduction
Enterococcus faecalis (E. faecalis) is a predominant enterobacterial species in the human environment.¹ This bacterium is the most detected Enterococcus species in endodontic-related infections. However, data on the oral prevalence of E. faecalis and its virulence factors vary between studies.²⁻⁵ This variation might result from the different dental pulp conditions or from the varying geographic locations of the included individuals.⁶⁻⁸ Although, E. faecalis is only occasionally detected in an endodontic infection without previous endodontic treatment, it can be retrieved more frequently after a failed root canal treatment.⁶⁻⁸

In the present study, E. faecalis was recovered from saliva and infected root canals from Indonesian individuals who had not had previous endodontic treatment (i.e. primary or secondary endodontic infections).⁹ The isolates were assessed for clonal structure by analysis of genotypic diversity using polymerase chain reaction (PCR) amplification of short enterobacterial repetitive intergenic consensus (ERIC) sequences. The ERIC sequences are present in many copies in the enterobacterial genome, and their positions vary between different species.¹⁰ This technique is frequently used for the typing of bacteria or intraspecies profiling, as it is fast, simple, and informative.¹¹⁻¹³ Previous studies have suggested that the presence of a capsule is associated with pathogenic lineages of
E. faecalis. Despite these important findings, whether the presence of capsule correlates with biofilm formation and with the development of endodontic infections remains unclear. Additionally, a locus where the E. faecalis capsule was synthesized has been described. Based on this locus polymorphism, three capsule types can be differentiated. The locus of capsule polysaccharides (CPS) type 1 consists of cpsA and B, followed by the non-capsule-related hcp1. The cps locus of CPS type 2 consists of cpsA, B, C, D, E, F, G, H, I, J, and K, followed by hcp1. The cps locus of CPS type 5 consists of cpsA, B, C, D, E, G, H, I, J, and K, without cpsB, followed by hcp1. In this regard, we sought to determine whether the presence of a genetic locus polymorphism of CPS could be linked to the molecular typing data based on ERIC sequences.

Biofilm formation is a key virulence factor for many microbial species. Biofilm formation of E. faecalis and the role of genetic factors therein have been extensively studied. In addition, the formation of E. faecalis biofilm has been reported to be affected by environmental stimuli, suggesting that biofilm formation might be an adaptive response. However, the role of E. faecalis biofilm formation in endodontic infections and the genetic basis of biofilm formation have been less studied and have contradictory results.

The aim of the present study was to investigate a possible correlation between the ERIC-PCR, capsule type and biofilm formation capacity of E. faecalis isolates from primary or secondary endodontic infections. This bacterium is more frequently found in root canals of teeth with post-treatment disease. Thus, it seemed relevant to analyze the possible association between the CPS type of E. faecalis and biofilm formation with endodontic infection cases. We therefore determined whether saliva-derived or infected root canal-derived isolates could be differentiated based on ERIC-PCR genotypes and their capsule type.

Methods
Sample sources and selection of participants
Clinical samples were collected from Indonesian dental patients who were referred to the clinic for endodontic treatment. Twenty-seven needed primary endodontic treatment, and seven needed endodontic retreatment, as they had persistent apical periodontitis based on radiographic imaging. Only patients with overall good health, without disease (mean age: 26 years, range: 16–50 years) and who had not used antibiotics in the past 3 months, were included. Written, informed consent prior to the sampling procedure was obtained from all participants.

The protocol was approved by the Ethical Committee of the Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

After clinical, intra-oral, and radiographic examinations, two types of samples were collected. Microbial samples obtained from infected root canals without or with previous endodontic treatment were designated Rc-A and Rc-B, respectively. Saliva samples were also obtained from each patient and were designated Sa-A and Sa-B.

Aseptic procedures were performed throughout the clinical sample acquisition. After clinical diagnoses were made, the selected teeth were swabbed with sterile cotton rolls and polished with pumice, and were then isolated with rubber dams.

Samples from the infected root canals were collected, as described previously. The outer surface of each tooth and dam were first disinfected with 30% H2O2 (Sigma Chemical, Poole, UK) and decontaminated with 2.5% sodium hypochlorite (Sigma) for 30 sec, prior to being inactivated with 5% sodium thiosulphate (Sigma). After all canals were removed, the cavities were wiped using sterile cotton pellets moistened with 2.5% NaOCl, making sure that no excess NaOCl entered the canal. The disinfectant was inactivated using 5% sodium thiosulphate. A sterile #4 round bur without a water spray was further used to obtain access to the pulp chambers and root canals, and access to the working areas was gained using sterile saline irrigant. Subsequently, microbial samples were collected by means of a #15 H-type file (Dentsply-Maillefer, Ballaigues, Switzerland), with the handle cut off and using a discrete filing motion. This file was introduced 1 mm short of each tooth’s radiographic apex. Finally, sterile paper points were introduced into the root canals at the same level of the file for 1 min. Both the file and paper points were then transferred into Eppendorf tubes containing phosphate-buffered saline (PBS) (10 mmol/L potassium phosphate, 150 mmol/L NaCl, pH 7.2).

For the acquisition of bacterial samples from previously endodontically-treated teeth, the root canal fillings were removed with sterile Gates Glidden drills (Dentsply-Maillefer, Ballaigues, Switzerland) and 15–30 endodontic files (depending on the canal size), without the use of chemical solvents. The filling materials were further removed, and a small amount of sterile saline solution was then introduced into each root canal by syringe to remove any remaining material and to moisten the canal before collecting the sample. A sterile paper point was then introduced into each canal until working length, which was determined at 1 mm minus the radiographic length of the apex, was reached. After 60 sec, the paper points were transferred to Eppendorf tubes containing 1 mL each of sterile PBS. The root canal samples were homogenized, and 50-μL aliquots were plated on chromo-
Enterococcus faecalis and endodontics

Genomic agar plates (Brilliance VRE; Oxoid, Basingstoke, UK) to selectively isolate enterococci.

After 24 h of aerobic incubation at 37°C, typical blue colonies indicative of *E. faecalis* were picked and checked for oxygen tolerance, catalase activity, and microscope features after Gram staining. The initial confirmation of *E. faecalis* colonies were subcultured onto brain–heart infusion (BHI; Oxoid) agar plates, which were incubated overnight at 37°C until pure cultures could be obtained with further confirmation via PCR.

**Polymerase chain reaction confirmation of *Enterococcus faecalis* isolates**

Bacterial chromosomal DNA was extracted using a DNA test tissue extraction kit (Qiagen, Valencia, CA, USA), according to the recommendations of the manufacturer. The specific primer for 16S rDNA of *E. faecalis* was as follows: EF16SF 5'-CGAGTGGTCCAGCTCAGGTTG-3' and EF16SR 5'-CTCTTATGCCATGGCAGCATAAAC-3'.

The total volume of PCR reaction mixture was 50 μL, including 2.5 μL Tag buffer, 2 mMol/L dNTP 2.5 μL, 25 mMol/L magnesium chloride 2.5 μL, 0.625 μL sense and antisense primer at 0.01 mMol/L each, DNA template (50 μg/mL), and 2 U Taq DNA polymerase (Qiagen).

Sterile Milli-Q water (Millipore, Billerica, MA, USA) was added for a final volume of 50 μL. *E. faecalis* American Type Culture Collection (ATCC) 29212 served as the positive control, and sterile Milli-Q water served as the negative control. After initial denaturation of DNA at 94°C for 5 min, PCR amplification was performed in a thermocycler for 35 cycles of a denaturation step at 94°C for 1 min, an annealing step at 60°C for 1 min, and an extension step at 72°C for 1.5 min. The PCR amplification products were detected using agarose gel electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.5 mg/mL; Sigma, St Louis, MO, USA), and analyzed using an image analysis system in Gel Doc 2000 (Bio-Rad Life Science Group, Hercules, CA, USA). The 1-kb Plus DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as a molecular weight marker. Only isolates identified as *E. faecalis* via PCR were further characterized, and *E. faecalis* ATCC 29212 was included as a reference strain and used as positive control throughout the study. All *E. faecalis* isolates were maintained in BHI broth containing 30% (v/v) of glycerol and stored at −70°C.

**Genomic subtyping of strain using enterobacterial repetitive intergenic consensus–polymerase chain reaction**

The strains were subtyped using the ERIC–PCR technique, which generates a unique genotype-dependent band pattern for the different *E. faecalis* isolates. The ERIC–PCR primers were used as described previously: ERIC1 5'-ATGTAAGCTCGGGGATTACAC-3' and ERIC2 5'-AAGTAAGTGGACGGGATTACCG-3'. Amplification reactions were performed with 5 min of initial denaturation at 95°C, 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 3 min, followed by a final elongation for 5 min at 72°C. Amplification products were subjected to electrophoresis for 150 min at 100 V in 1.5% agarose gels. Gels were stained with 0.5 mg/mL ethidium bromide, and the 1-kb DNA ladder was used as molecular weight for ERIC–PCR profiles. The band profiles were visualized under UV light and digitalized using Gel Doc 2000.

For the genotypic analysis, all amplified products revealed after electrophoresis were converted to binary codes based on the presence (1) and absence (0) of *E. faecalis*, and then an initial matrix was constructed. A dendrogram was created using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm. Two strains were considered different when their profiles differed in at least one band.

**Capsule locus polymorphism detection**

A previous study demonstrated that *E. faecalis* isolates can be classified based on three capsule operon polymorphisms. Isolates that belong to CPS type 1 do not express the CPS. They possess only the first two *cps* genes (*cpsA* and *cpsB*) and lack all remaining genes of this locus (*cpsC–cpsK*), which are essential for capsule production. Conversely, CPS type 2 serotypes possess all genes (*cpsA–cpsK*) in the cps-operon, whereas in the CPS 5 serotypes, the *cpsF* gene is absent, which differentiates them from CPS 2 serotypes.

To determine the CPS type of the *E. faecalis* in the present study, we used the primers and PCR conditions reported by McBride and coworkers. CPS type 1 in non-capsulated strains was confirmed using a 950-bp product, whereas the generation of a 1098-bp product indicated the occurrence of CPS type 2 with the presence of *cpsF*. The production of 199 bp was characteristic of the CPS type 5 polymorphism.

**Biofilm assay**

The ability of *E. faecalis* strains to form biofilm on microtiter plates was assayed, as previously described. Briefly, isolates were grown overnight in Luria–Bertani (LB) broth at 37°C. Cultures were diluted at 1:20 in fresh LB broth at room temperature, and 200 μL of this suspension was used to inoculate sterile, 96-well polystyrene microtiter plates (Iwaki, Tokyo, Japan). After 24 h at 37°C, the biofilms
grown on microtiter plates were washed with PBS, dried in an inverted position, and stained with 1% crystal violet for 15 min. After rinsing with deionized water, the crystal violet was solubilized in 200 μL of ethanol acetone (80:20, vol/vol), and quantitated by adding 95% ethanol followed by measurement of the absorbance at 595 nm. Biofilm-forming ability was scored according to Stepanovic. Strains were classified as non-biofilm producers if there was no change in absorbance over the control or the change was weak (up to a twofold change), moderate (up to fourfold change), or strong (greater than a fourfold change). All experiments were carried out in duplicate and repeated three independent times. Absorbance values from wells without bacteria were used as negative controls, and the E. faecalis standard strain (ATCC 29212) was used as a biofilm-positive control.

Statistical analysis

The associations between the ERIC-PCR with the origin of the strains, capsule type, and biofilm formation capacity of E. faecalis isolates from primary or secondary endodontic infections were assessed with χ²-test. Differences were considered statistically significant at P < 0.05.

Results

A total of 50 E. faecalis colonies were collected and consisted of 33 and 17 colonies from individuals diagnosed as having primary (27 patients) and secondary endodontic infections (7 patients), respectively. In the present study, we collected 24 E. faecalis isolates from root canals with primary endodontic infections, and 16 E. faecalis isolates from the saliva of all patients. Ten further isolates were obtained from the root canals with secondary endodontic infections. In general, E. faecalis was present in 72.7% (24/33) of the root canals (Ra-A) and 27.3% (9/33) of the saliva (Sa-A) samples derived from patients with primary endodontic infections.

Of the seven cases of persistent endodontic infections associated with secondary endodontic infections, E. faecalis was found in 58.8% (10 of 17) and 41.2% (7/17) of the root canals (Rc-B) and saliva (Sa-B) samples, respectively. There was no statistically-significant association (P > 0.05) found between the presence of E. faecalis in root canals and its presence in saliva.

Enterobacterial repetitive intergenic consensus–polymerase chain reaction analysis of the E. faecalis isolates and its association with the origin of samples

The genetic analysis of the E. faecalis strains via ERIC-PCR disclosed five different patterns. Figure 1 shows the

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Unweighted pair-group method with arithmetic averages dendrogram of enterobacterial repetitive intergenic consensus (ERIC). Dendrogram shows the relationship between the 50 Enterococcus faecalis (E. faecalis) strains analyzed by ERIC–polymerase chain reaction (PCR). All isolates shared >60% similarity. Despite some clustering of E. faecalis isolates, relatively high diversity was observed among them. Relatively high heterogeneity was observed with the ERIC–PCR profiles, both in E. faecalis strains belonging to different capsule polysaccharides (CPS) type and those within a given CPS type. Numbers correspond to isolates, and strain K is American Type Culture Collection 29212. UC, unclustered.

dendrogram of the ERIC–PCR profiles created by the UPGMA. Two main clusters (C1 and C2) and three minor clusters (C3, C4, and C5) were grouped with 0.64 simple match similarities. Five isolates could not be grouped with a minimum of 60% similarity.

As shown in Table 1, cluster C1 was highly diverse, containing 14 isolates (28%) collected from all sources (root canals and saliva), but it was dominated by E. faecalis from the root canal samples, Ra-A (6, 12%) and Rc-B (4, 8%), followed by the saliva samples, Sa-A and Sa-B, each containing two isolates (4%). Cluster C2 contained 11 isolates (22%) and was dominated by those of root canal origin; Ra-A (7, 14%) and RC-B (2, 4%); those from the saliva samples were Sa-A (1, 2%) and Sa-B (1, 2%). Cluster C3 and C4 each contained seven isolates and were dominated by root canal isolates. Although six isolates in cluster C5 were exclusively of root canal infection origin, the remaining five isolates were unclustered and all came from saliva samples. There was no correlation between the sample origin and
the fingerprinting profiles of the isolates. For example, in addition to the isolates in cluster C5, only three strains (43, 46, and 41, Figure 1), which were isolated from root canal infections, showed identical fingerprint profiles at a correlation level of 0.64. The three isolates were clustered in cluster C1. The present study indicates the high genotype diversity among isolates that might be associated with the different origins of *E. faecalis* in the oral niches.

Distribution of capsule polysaccharides type based on sample origin and enterobacterial repetitive integenic consensus–polymerase chain reaction pattern

The five genotypically-distinct *E. faecalis* isolates were further studied for their CPS types. Overall, CPS type 2 was found in 39 (78%) of the samples. The respective values for the other CPS types were as follows: CPS type 1 with five isolates (10%), and CPS type 5 with four samples (8%). Two (4%) isolates were not detected with the primers used. Of CPS type 2, 14 (28%) isolates were distributed in cluster C1. They all came from the root canal samples (data not shown). The two unidentified isolates, which came from the root canal samples, were found in clusters C3 and C4. Interestingly, four of the five of unclustered isolates that were CPS type 2 (Table 2) came from the saliva samples (not shown), suggesting that the related phenotype could be a prerequisite for colonization in saliva. As shown in Table 2, when infected root canal-acquired strains were examined, CPS type 2 was significantly correlated with the Rc-A sample (18, 36%, *P* < 0.05), whereas only eight (16%) were found in the Rc-B samples. Compared with the CPS type 2, CPS type 5 occurred less, with two (4%) in the Rc-A and one (2%) in the Rc-B samples, respectively. However, when only saliva-acquired strains were examined, only CPS type 2 was observed in the saliva samples collected from patients with primary infection cases (Sa-A), whereas in the Sa-B samples (saliva from patients with post-endodontic treatment), all CPS types were detected; there were two CPS type 1 (4%), six CPS type 2 (12%), and one CPS type 5 (2%) cases identified.

Studying the frequency of the detected CPS subtypes of bacterial serotypes, CPS type 2 was found to be positively associated with the microbial samples collected from the root canals with primary infections (Rc-A, *P* < 0.05). In contrast, a significant association was shown between the occurrence of CPS type 2 and samples taken from saliva (Sa-A and Sa-B, *P* < 0.05, *χ*²-test) (Table 2).

**Table 2. Distribution of Enterococcus faecalis CPS type based on sample origin and phylogenic group**

<table>
<thead>
<tr>
<th>CPS type</th>
<th>Sample origin no. (%)</th>
<th>Phylogenic group no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rc-A</td>
<td>Rc-B</td>
</tr>
<tr>
<td>1</td>
<td>3 (6)</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>18 (36)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>5</td>
<td>2 (4)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Non-typeable</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (48)</td>
<td>10 (20)</td>
</tr>
</tbody>
</table>

Data are presented no. and percentage. Rc-A, Rc-B are root canal samples from primary and secondary endodontic infections, respectively. Sa-A and Sa-B are saliva samples from primary and secondary endodontic infections, respectively. C1–C5 are clusters based on enterobacterial repetitive integenic consensus–polymerase chain reaction genotyping. CPS, capsule polysaccharides.
Association of enterobacterial repetitive intergenic consensus–polymerase chain reaction, capsule polysaccharides types, and biofilm-forming capability

Biofilm-forming ability was examined in 96 well microtitre plates. We observed a wide variation in the quantity of biofilm biomass among the isolates tested (Table 3). In the teeth with primary infections (RC-A), all *E. faecalis* isolates (24, 48%) had biofilm-forming capabilities, whereas one (2%) of the 10 isolates had no biofilm formation in endodontically-failed teeth (RC-B). Our data found that the biofilm-formation ability of *E. faecalis* isolated from root canals with primary infections (RC-A) was better than those isolated from root canals with secondary infections (RC-B, *P* < 0.05). There was no significant relationship between the ability of *E. faecalis* to form biofilms and the source of the isolates (root canal and saliva, *P* > 0.05).

As shown in Table 3, the highest numbers of medium-to-strong biofilm-forming isolates were found in cluster C1 (9, 18%), followed by cluster C2 (7, 14%). The percentage of medium-to-strong biofilm-forming isolates in clusters C3 and C4 was only 6% and 10%, respectively. It is possible that the strong and medium production of biofilm could be a virulent phenotype, and five of the six (1%) isolates grouped in cluster C5 that came from the root canal-infection samples were of the biofilm-forming phenotype (Figure 1, Table 3). This suggests that biofilm-forming capability might favor *E. faecalis* strains as opportunist pathogens in root canal infections. Among the strong and medium biofilm-producer strains (34 strains), only 27 (54%) were CPS type 2 positive, whereas 11 (22%) isolates categorized as weak biofilm producers were CPS type 2 positive (Table 3).

When the correlation between CPS type and quantitative biofilm production was evaluated, clear differences between the three CPS types were observed. In general, biofilm production was higher for CPS type 2 isolates than for the other two CPS types (Table 3). Differences in the biofilm-forming capabilities of CPS type 2 strains in all isolates based on the source (root canal and saliva) were statistically significant (*P* < 0.05). However, there was no relationship detected between the ERIC-PCR profile and biofilm formation (*P* > 0.05).

**Discussion**

*E. faecalis* has been implicated in post-endodontic treatment root canal infections, and occasionally in cases of intraradicular infections. The occurrence of *E. faecalis* in both primary and persistent endodontic infections has also been reported. Our finding is in agreement with most of studies that regard *E. faecalis* as a relevant endodontic pathogen.

In the present study, ERIC-PCR was used to study *E. faecalis* isolates from Indonesians who required endodontic treatment. Furthermore, pathogenic lineages of *E. faecalis* isolated from root canals and systemic infections have been associated with the presence of a capsule. Thus far, data that combine genomic diversity and CPS type have not been available for saliva isolates. Our data show that genetic diversity, as revealed with ERIC-PCR, was not only found among different CPS types, but also among isolates belonging to the same CPS type. This suggests that the ERIC-PCR method might be better than capsular-based typing in differentiating *E. faecalis* isolates and in studying the relationship between primary and post-endodontic treatment cases. Potential applications include identification of isolates that appear to have a broad oral niche distribution, suggesting interoral site transfer.

*E. faecalis* is the most frequently-isolated species, and is sometimes the only isolate found in root canals. However, the involvement of this species in the pathogenesis of endodontic infection is still not fully understood. To the best of our knowledge, limited information is available on the epidemiology of Indonesian *E. faecalis* isolates recovered from saliva and infected root canals.

<table>
<thead>
<tr>
<th>Biofilm ability</th>
<th>Sample origin no. (%)</th>
<th>Phylgenic group no. (%)</th>
<th>CPS type no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RC-A</td>
<td>RC-B</td>
<td>Sa-A</td>
</tr>
<tr>
<td>Strong</td>
<td>15 (30)</td>
<td>3 (6)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Moderate</td>
<td>6 (12)</td>
<td>–</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Weak</td>
<td>3 (6)</td>
<td>6 (12)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>NF</td>
<td>–</td>
<td>1 (2)</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>24 (48)</td>
<td>10 (20)</td>
<td>7 (14)</td>
</tr>
</tbody>
</table>

Biofilm abilities of each capsule polysaccharides (CPS) type was classified as strong (greater than fourfold change), moderate (up to fourfold change), weak (up to a twofold change), and no biofilm producers (NF, no change in absorbance over the control). Wells containing uninoculated medium served as negative controls and to determine background optical density.

© 2015 Wiley Publishing Asia Pty Ltd
using the DNA-based approach. In general, the results of the present study demonstrate the high genetic diversity of *E. faecalis* obtained from saliva and infected root canals of primary and secondary endodontic infection patients. As observed in Figure 1, after ERIC-PCR genotyping, we found that the two large clusters (C1 and C2) contain a mixture of isolates originating from all samples. Some isolates within C1, such as *E. faecalis* no. 41 and no. 46 from root canals of post-endodontic-treatment patients (RC-B), were closely related to isolates from infected root canals (RC-A) of primary endodontic-infection patients (no. 43). Moreover, our results show that the *E. faecalis* isolated from root canal samples and from the saliva samples are quite similar in their biofilm-forming capabilities. This suggests the possibility of either saliva or the root canal as the common origin of the *E. faecalis* strains that we studied, but the present study did not allow for confirmation of this hypothesis.

The oral cavity is considered a transient location for *E. faecalis*, which might originate from the human food chain. We assumed that as a transient bacterium that triggers endodontic-related infections, *E. faecalis* must colonize, and that it has the ability to trigger periapical inflammation. Thus, *E. faecalis* strains must survive in saliva and have the capacity to form biofilm in root canals in addition to the tongue surface. As reported by Sedgley et al., *E. faecalis* is detected more frequently on the tongue, whereas this species was present in oral rinse samples from patients who had endodontic treatment. This means that there are strains of this species that might have the potential to maintain root canal infection and are protected by host-response surveillance.

This study found that the majority of *E. faecalis* isolated from both saliva and root canals, either in primary or post-endodontic treatment-infection patients, are of the CPS type 2 genotype. Thus, they are CPS producing. This indicates that the presence of CPS type 2 in the *E. faecalis* strain that we studied might enable it to survive in saliva and root canals. Therefore, the expression of the *E. faecalis* capsule could be considered a major determinant in triggering a periapical lesion.

The importance of CPS type 2 strains is well known among other *E. faecalis* human isolates, but the significance of endodontic isolates is still unknown. The results from this study show that only the CPS type 2 isolates were found in all clinical samples, and this strain was most frequently found in the infected root canal samples of primary endodontic patients (RC-A), followed by the root canals samples of the secondary-infection patients (RC-B). However, no significant differences were found between the *E. faecalis* CPS type 2 from either the saliva or root canal samples. This indicates that the genotypic diversity, other than CPS type 2, found in the present study might be related to the origin of different isolates from different patients. There are some reasons to support this notion. First, within oral environments, the root canal and the oral bacteria, including *E. faecalis*, are interconnected. Unlike other bacteria found in the oral cavity, *E. faecalis* has the ability to persist in the root canal, as it can bind to the main organic component of dentin (collagen type 1). While living in the root canal, *E. faecalis* strains might inflict damage to the periodontal tissue caused by their gelatinase activity and their enterococci surface protein. Second, the occurrence of *E. faecalis* in saliva and root canal samples shows the bacterium’s capacity to integrate with other oral bacteria as biofilm. Lastly, *E. faecalis* might have the ability to regulate capsule expression in a phase shift-like manner, as observed in other bacteria. The ability to phase shift might be of particular interest for *E. faecalis*, because biofilm-forming capability is an important factor in the pathogenesis of endodontic infections, and the ability of *E. faecalis* isolates to form biofilm, as demonstrated in the present study, seems to be proportional to the prevalence of the CPS type 2 strain.

*E. faecalis* CPS type 2 was the most common serotype isolated from the samples collected in this study, indicating that the *E. faecalis* strain that produces capsules is highly prevalent in the human oral cavity in the Indonesian population. In contrast, in a study of *E. faecalis* isolated from root canals in a Brazilian population, *E. faecalis* CPS 2 strains were rare, and CPS 1 was the most common isolate. One explanation for this could be that there are geographic differences, because the varying oral environmental conditions could lead to genetic determinants of microbial colonization, including colonization by *E. faecalis*. Additionally, the involvement of CPS type 1 in the biofilm-forming capability of *E. faecalis* strains was not reported by the authors. In the present study, an *in vitro* biofilm assay was performed to analyze the ability of *E. faecalis* strains to form biofilm. We found that the detection of the *E. faecalis* strains with a high capability to form biofilm was remarkable, especially because most belong to the CPS type 2 genotype. We also demonstrated that within the group of patients with primary endodontic infections, *E. faecalis* with the medium-to-strong biofilm phenotype was more frequently isolated in the samples from root canaled teeth with primary endodontic infections (RC-A) than in the samples from root canaled teeth from post-endodontic treatment (RC-B). This observation was corroborated by the finding that most of the *E. faecalis* isolates obtained in the RC-A samples belonged to the CPS type 2 genotype and were more likely to possess multiple virulence factors. This led to the conclusion that in addition to other virulence
Enterococcus faecalis and endodontics

E.W. Bachtiar et al.

determinants, the CPS expressed by the CPS type 2 genotype might give E. faecalis the advantage of persisting in the root canal. As reported by Ziebuhr et al., in order to survive under environmental stress, bacteria, including E. faecalis, have to adapt to continuously-changing conditions. This also supports the report regarding the important contribution of the CPS as a virulent factor in the pathogenesis of E. faecalis infections. However, we are not excluding the possibility that our E. faecalis CPS type 2 consisted of the encapsulated and unencapsulated strain. As previously reported by Gaspar, there is a strain of E. faecalis CPS type 2 with a cps gene that might not be expressed due to the presence of an insertion sequence in the cpsC-cpsK promoter region.

In conclusion, the present study shows that E. faecalis with CPS type 2 and biofilm-forming capacity is relatively common in saliva and infected root canals of Indonesians with primary and post-endodontic treatment cases. Such genotypes and phenotypes might represent a predilection site of endodontic-relevant E. faecalis strains in the oral cavities of the investigated individuals. To clarify our results, more studies are needed to investigate the genotypic and phenotypic comparison of non-oral isolates of E. faecalis within the Indonesian population.

Acknowledgments

This work was supported partly by the World Academy of Sciences grants (research grant agreement no. 11-104 RG/Bio/AsG-UNESCO FR:340262673 to Boy M. Bachtiar for supporting laboratory equipment, and Universitas Indonesia Research Grant 2011/12 to Endang W. Bachtiar. We acknowledge Dr Bastiaan P. Krom from the Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam, University of Amsterdam and Free University Amsterdam, Amsterdam, the Netherlands and Dr Suzette V. van der Waal from the Department of Endodontology, Academic Centre for Dentistry Amsterdam, University of Amsterdam and Free University Amsterdam, Amsterdam, the Netherlands, for their time and effort in previewing this article. Their help is greatly appreciated. Additionally, we thank Maysyaroh and Dessy Sulistya As-hari for their technical assistance.

References

20 Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic


