Capsule Locus Polymorphism among Distinct Lineages of *Enterococcus faecalis* Isolated from Canals of Root-filled Teeth with Periapical Lesions

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Abstract

Introduction: Although *Enterococcus faecalis* is a member of the normal microbiota, it is also a major cause of nosocomial infections. Some strains of *E. faecalis* produce capsule, which contributes to pathogenesis through evasion of host defenses, and its production is dependent on the capsule (*cps*) operon polymorphism. This study investigated *cps* locus polymorphism in distinct lineages of *E. faecalis* isolated from canals of root-filled teeth with periapical lesions. **Methods:** Twenty-two *E. faecalis* isolates were evaluated regarding the *cps* operon polymorphism and genetic diversity. The 3 known CPS types were determined by polymerase chain reaction. This information was correlated with multilocus sequence typing data, which were used to define genetic lineages. **Results:** *cps*A and *cps*B were the only detected genes within the *cps* operon in 62.5% of *E. faecalis* strains (14/22), indicative of genotype CPS 1, which lacks capsule expression. The essential genes in the *cps* operon for capsule production were detected in the remaining strains, whereas 3 belonged to genotype CPS 5 and 5 strains to genotype CPS 2. A total of 14 sequence types (STs) were resolved in 22 *E. faecalis* isolates. Comparison with the *E. faecalis* international multilocus sequence typing database revealed that 9 STs were previously found, and that the 5 STs were novel. **Conclusions:** Certain *E. faecalis* genotypes from canals of root-filled teeth with periapical lesions belong to lineages associated with capsule expression and production of multiple virulence factors, which might account for their increased pathogenic potential. *(J Endod 2012;38:58–61)*

Key Words

Capsule, *Enterococcus faecalis*, genotypes, root canals

Enterococcus faecalis is a common inhabitant of the human gastrointestinal tract; however, multidrug-resistant strains are leading causes of hospital-acquired infections. The ability of *E. faecalis* to cause serious infections has been linked to variable traits that enhance the virulence of the organism, including aggregation substance, enterococcal surface protein Esp, endocarditis-associated and biofilm-associated pili Ebp, cytolysin toxin, and gelatinase (1). Recent studies have explored further mechanisms that *E. faecalis* uses to circumvent the host's innate immune response and establish infection (2, 3). It was shown that capsule-producing *E. faecalis* strains are more resistant to complement-mediated opsonophagocytosis than noncapsulated strains (2). Moreover, the presence of the capsule has been associated with the pathogenic lineages of *E. faecalis* isolated from hospitalized patients (4).

E. faecalis is able to colonize a variety of sites in humans, including the oral cavity. It is the most common bacterial species isolated or detected from canals of root-filled teeth with periapical lesions (5–7). Nevertheless, the question remains whether *E. faecalis* plays a role in the pathogenesis of apical periodontitis or is a mere survivor because of ecological selective pressure in root-filled teeth (8).

The apical periodontitis of root-filled teeth might develop or persist as a result of complex immune responses against the bacterial infection in the root canal system. Bacterial structural components might shed into the periradicular tissues and stimulate the development of host immune reactions (9). To evade the host defenses in the inflamed tissue, the expression of capsule by *E. faecalis* masks bacterial surface antigens, allowing the bacteria to resist to opsonophagocytosis (2).

The biosynthesis of capsular polysaccharides by *E. faecalis* is encoded by the *csp* operon, which includes 11 open reading frames (*cps*A to *cps*K) (10). However, only 7 open reading frames in the *cps* operon are essential for capsule production (*cps*C, *cps*D, *cps*E, *cps*G, *cps*I, *cps*J, and *cps*K) (3). Previous genetic evidence demonstrated that *E. faecalis* isolates can be classified in 1 of 3 capsule operon polymorphisms. CPS 1 presents only *cps*A and *cps*B; CPS 2 presents all 11 genes in the *cps* operon; and CPS 5 presents all genes except for *cps*F. Furthermore, CPS 2 and 5 express the capsular polysaccharide, whereas CPS 1 does not (4).

Because of the frequent detection of *E. faecalis* in cases of endodontic failure, this study aimed to investigate capsule-producing *E. faecalis* strains among isolates from canals of root-filled teeth with periapical lesions by genetic analysis of the capsule (*cps*) operon.

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Materials and Methods

Clinical Material, Bacterial Strains, and DNA Extraction

The 22 *E. faecalis* strains used in this study were previously isolated from the canals of teeth with failed root canal treatments (5). The clinical data of these teeth were recorded, so that they could be correlated with the *cps* operon polymorphism: clinical symptoms, presence or absence of a sound coronal restoration, status of the root canal in terms of whether dry or wet (the term *wet canal* in this study means presence of exudate), and the radiographic quality of the root canal filling. Coronal restorations were categorized as defective if there were open margins, fracture, or recurrent decay. Initial root fillings were classified as good if no voids were present and were within 2 mm of the radiographic apex. If 1 or more of these criteria were not met, they were classified as poor.

All *E. faecalis* strains were identified at the species level by using rapid ID 32 STREP galleries and partial 16S rDNA sequencing (11). For chromosomal DNA isolation, Wizard Genomic DNA solutions (Promega, Madison, WI) were used with the modifications previously described by Ulrich and Hughes (12).

Polymorphism of the *cps* Operon

The 3 known capsule operon polymorphisms previously described were investigated as described by McBride et al (4). The *cps* locus of CPS 1 consists of *cpsA* and *B*, followed by the non–capsule-related *hcp1* gene. The *cps* locus of CPS 2 consists of *cpsA, B, C, D, E, F, G, H, I, J, K*, followed by *hcp1*. The *cps* locus of CPS 5 consists of *cpsA, B, C, D, E, G, H, I, J, K*, followed by *hcp1*, with *cpsF* absent. The primers used in this study are listed in Table 1. Primers pair cpsB5-F/hcp1-R generated an amplification product of 950 base pairs (bp) from CPS 1 type strains. Primer pair cpsEend-F/59cpsG-R was used to amplify the region between *cps*E and *cps*G to detect the presence of *cps*F, which distinguishes CPS 2 and 5 polymorphisms. An amplification product of 1098 bp indicated the presence of *cps*F characteristic of CPS 2. Generation of a product of 199 bp indicated the presence of *cps*E and *cps*G but the absence of *cps*F, as is characteristic of the CPS 5 polymorphism. Polymerase chain reaction (PCR) conditions for all amplification reactions were as follows: initial denaturation at 94°C for 5 minutes; 30 cycles at 94°C for 30 seconds, 52°C for 2 minutes, and 72°C for 2 minutes; and final extension at 72°C for 7 minutes. PCR products were submitted to electrophoresis in agarose gels and stained with ethidium bromide for visualization under UV light.

TABLE 1. Primers Used in This Study

Primer name	Primer sequences (5′ to 3′)	Size (bp)
CPS types		
cpsB5-F	CCAGGACATGGTGGTATTTTAGATC	950
hcp1-R	CGCCAATAAACAATCTTTACCAGAGC	
cpsEend-F	GAACCTACAACAATTAAAAAAGC	199/
cpsG-R	GCATAGTATGTTAAGATTGATCCA	1098
MLST		
aroE-1	TGGAAAACTTTACGGAGACAGC	459
aroE-2	GTCCTGTCCATTGTTCAAAAGC	
gdh-1	GGCGCACTAAAAGATATGGT	530
gdh-2	CCAAGATTGGGCAACTTCGTCCCA	
gki-1	GATTTTGTGGGAATTGGTATGG	438
gki-2	ACCATTAAAGCAAAATGATCGC	
gyd-1	CAAACTGCTTAGCTCCAATGGC	395
gyd-2	CATTTCGTTGTCATACCAAGC	
pstS-1	CGGAACAGGACTTTCGC	583
pstS-2	ATTTACATCACGTTCTACTTGC	
xpt-1	AAAATGATGGCCGTGTATTAGG	456
xpt-2	AACGTCACCGTTCCTTCACTTA	
yqiL-1	CAGCTTAAGTCAAGTAAGTGCCG	436
yqiL-2	GAATATCCCTTCTGCTTGTGCT	

Multilocus Sequence Typing Analysis

Multilocus sequence typing (MLST) of *E. faecalis* isolates was performed as described previously (13). The 7 genes evaluated were *gdh* (glucose-6-phosphate dehydrogenase), *gyd* (glyceraldehyde-3-phosphate dehydrogenase), *pstS* (phosphate adenosine triphosphate binding cassette transporter), *gki* (putative glucokinase), *aroE* (shikimate 5-dehydrogenase), *xpt* (shikimate 5-dehydrogenase), and *yiqL* (acetyl-coenzyme A acetyltransferase). PCR conditions for all amplification reactions were as follows: initial denaturation at 94°C for 5 minutes; 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1 minute; and final extension at 72°C for 7 minutes (13). Reactions were performed in 25-μL volumes by using *Taq* DNA polymerase (Invitrogen, São Paulo, Brazil). PCR products were purified with a kit from QIAGEN, Inc (Hilden, Germany) and sequenced with PCR forward and reverse primers, an ABI PRISM Big Dye cycle sequencing ready reaction kit (Perkin-Elmer, Applied Biosystems, Foster City, CA), and an ABI 3700 DNA sequencer (Perkin-Elmer).

For each isolate, each gene was amplified and sequenced with the specific forward or reverse primer. For each locus, a distinct allele number was assigned to every different sequence, in accordance with the *E. faecalis* MLST database (http://efaecalis.mlst.net). The allelic profile or sequence type (ST) was assigned in the order *gdh*, *gyd*, *pstS*, *gki*, *aroE*, *xpt*, and *yqiL* to a total of 7 integers corresponding to the allele numbers at the 7 loci. The relatedness between the different STs was investigated by using BioNumerics software (version 6.0; Applied Maths, Sint-Martens-Latem, Belgium) by the unweighted pair group method with arithmetic averages and the categorical coefficient of similarity. Isolates with the same allelic profile, and therefore the same ST, are regarded as members of a single clone or lineage (13).

Statistical Analysis

The data collected for each *E. faecalis* strain (CPS type, ST, and clinical data) were typed onto a spreadsheet and statistically analyzed by using SPSS for Windows (SPSS Inc, Chicago, IL). The Pearson χ^2 test or the one-sided Fisher exact test, as appropriate, was chosen to test the null hypothesis that there was no relationship between endodontic symptoms and signs and the presence of capsule-producing *E. faecalis* strains.

Results

All *E. faecalis* strains tested yielded 1 of the 3 CPS polymorphisms, on the basis of characteristic PCR products (Fig. 1). The most common type among the root canal isolates was CPS 1 (62.5%), indicating that these strains do not have in their chromosome the essential genes in the *cps* operon for capsule production. The CPS 2 and 5, which are associated with capsule-producing strains, were found in a lower prevalence, 16.7% and 20.8%, respectively.

A total of 22 *E. faecalis* isolates were resolved into 14 STs, of which 9 STs comprised only 1 isolate (Fig. 2). Nine STs were already reported in the database, comprising strains isolated from hospitalized patients, human community, animal reservoirs, and food in Europe and North and Latin America. In addition, 5 STs were demonstrated for the first time in this study, ST 394–398. Capsule (*cps*) locus polymorphisms were correlated with MLST data, and