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Abstract

In this narrative review, the potential reasons for the high occurrence of enterococci in filled root canals are explored. The pulpless root canal appears to be a habitat for these bacteria, particularly for *Enterococcus faecalis*. However, re-surveying the literature in caries research, it can be concluded that, contrary to earlier belief, enterococci are rarely if ever found at the advancing front of dentinal lesions. The same is the case for true primary endodontic infections, but some uncertainty remains, because the coronal seal and the history of teeth harbouring enterococci have rarely been accurately investigated. Furthermore, from longitudinal studies with a known infection at the initiation of treatment, which was carried out under controlled asepsis, it is questionable whether enterococci are as difficult to eliminate from the canal system as is commonly held. A more likely explanation for the high occurrence of enteroccci in filled root canals is that they enter after treatment, but from which source? The intriguing finding in this context is that enterococci do not appear to be colonizers of the oral cavity. They are merely transient oral bacteria, unless there is a predilection site such as the unsealed necrotic or filled root canal. The origin of this infection is most likely food. Using the example of enterococci in filled root canals, this paper highlights the possible importance of transient microorganisms in the oral cavity and changes in a microenvironment that can create favourable conditions for infection.

Introduction

The search term "enterococcus and root canal" yielded 353 related articles from the PubMed database on May 29, 2008, when the work on this manuscript was initiated. This indicates a high interest in this bacterial genus, especially the species *Enterococcus faecalis*. However, other enterococcal species such as *E. faecium*, *E. casseliflavus* and *E. durans* have also been identified in root canals (Mejàre 1975, Ferrari *et al.* 2005). When talking to clinicians, there appears to be a general consensus that enterococci are hardy inhabitants of the necrotic root canal system, which are more difficult to eliminate than other taxa and are likely to survive chemomechanical root canal treatment (Stuart *et al.* 2006). However, this assumption may be wrong, or at least not entirely correct. Whilst there are some excellent reviews on microbiological aspects of enterococci and their elimination via antimicrobials (Portenier *et al.* 2003), there appears to be a lack of knowledge when it comes to the question *why* enterococci are likely to be found in filled root canal systems. The necrotic or improperly filled root canal system appears to be a habitat for enterococci, especially *E. feacalis*. Contrary to most other species, *E. faecalis* can survive on its own and appears to tolerate the ecological stress in the root canal niche better than most other taxa, which, in turn, may profit from its presence (Fabricius *et al.* 1982a). There can be no doubt that in those regions of the world where such analyses have been performed, enterococci appear to be common colonizers of filled root canals (Molander *et al.* 1998, Peciuliene *et al.* 2000, Hancock *et al.* 2001, Pinheiro *et al.* 2003, Rocas *et al.* 2008). Enterococci have been identified in a considerable proportion of teeth with persisting periapical lesions that had technically adequate (Sundqvist *et al.* 1998) as well as in counterparts that had insufficient root fillings (Peculiene *et al.* 2000). They appear to occur frequently in root filled teeth both in regions where calcium hydroxide is commonly used as an interim dressing (Molander *et al.* 1998) and in countries where this topical antiseptic is usually not applied (Hancock *et al.* 2001). In one study, the occurrence of viable enterococci in root filled teeth with apical periodontitis was as high as 64% of the

culture-positive cases (Peciuliene *et al.* 2001). In theory, there are two possibilities to explain this finding: 1) enterococci are primary colonizers of the root canal system as it becomes necrotic and then survive endodontic treatment including root filling better than other taxa; or 2) enterococci are opportunistic coronal invaders of the improperly sealed necrotic or filled root canal system and hence enter this system during or after treatment. The aim of this review is to investigate which of the above possibilities is more likely to explain the high occurrence of enterococci recovered from filled root canals.

Is it really difficult to eliminate enterococci from the root canal?

The eradication of enterococci from the root canal system and its tolerance to most antimicrobials is not the primary topic of this review. As indicated above, excellent reports on this issue have been published (Portenier *et al.* 2006). It should be realized, however, that the only study design that can conclusively assess the resistance of enterococci to chemo-mechanical root canal treatment is a longitudinal study in humans with a known infection at the initiation of the treatment and controlled asepsis during all the steps that follow. *Ex vivo* studies using models with infected dentinal tubules are certainly helpful for comparing the effectiveness of certain antimicrobials, but on the other hand may be misleading when it comes to estimating the ability of enterococci to survive chemo-mechanical root canal treatment *in situ*. Enterococci are found in filled root canals regardless of the antimicrobials that were used during treatment (Hancock *et al.* 2001). Furthermore, the ability of enterococci to enter dentinal tubules observed in bovine teeth is probably clinically irrelevant, because tubules of the apical root dentine in the adult human tooth are sclerotic (Vasiliadis *et al.* 1983, Mjör *et al.* 2001). Sclerotic dentine is not invaded by microorganisms (Shovelton 1964). Infected ramifications of the root canal system that are in direct contact to the periodontium are clinically more important (Nair *et al.* 2005), yet the microorganisms therein have not been identified.

Clinical studies with samples that were sent in from private practitioners are prone to another type of systematic error, namely that samples collected by individuals that are not primarily involved in microbiological research are likely not to represent the true root canal microbiota (Bender *et al.* 1964, Morse 1971). Last but not least, studies that found enterococci in filled root canals with persisting apical lesions cannot elucidate whether the target bacteria have survived treatment, entered during treatment, or even accessed the canal space after the root filling procedure.

In terms of properly controlled longitudinal studies, the classical work on enterococci in root canals was reported by Engström (1964) who investigated the culturable microorganisms from root canals of 223 teeth that were either root filled or had a necrotic pulp. In this material, *E. faecalis* (no other enterococci were identified) was found in 20 teeth, i.e. in 9% of the cases. The canals were then treated chemo-mechanically using an iodophor or a quartenary ammonium compound in aqueous solution in combination with Dakin's solution (0.5% NaOCl buffered with bicarbonate) to irrigate the canals. Between visits, 5% iodine potassium iodide was sealed into the canal system. The first antibacterial treatment failed in 13/20 cases infected with *E. faecalis*, compared to 45/114 cases infected with other taxa. Whilst Engström stated that this difference was not statistically significant based on a non-disclosed test, it actually is (Fisher's exact test, two-tailed, $P = 0.049$). On the other hand, the cases with enterococcal infection were relatively few, and it would thus not be justified to make clinical conclusions based on these observations. Moreover, the true clinical outcome (i.e. the healing rate) was not assessed in Engström's study. In a more recent study (Ferrari *et al.* 2005), enterococci were identified in 6/25 (24%) of single-rooted teeth with intact pulp chamber and apical periodontitis. The teeth were instrumented and rinsed with 0.5% NaOCl and then EDTA. At the end of the first treatment, no canals harboured culturable enterococci, whilst 5/25 canals still contained other species. The canal was then left empty and sealed with a zinc oxide cement for 7 days. At the initiation of the second visit, 14/25 canals harboured

enterococci including *E. faecalis*, *E. faecium* and *E. casseliflavus*. The authors explained the occurrence of enterococci at the second visit in canals that did not show these bacteria at the beginning with an initial number of enterococci that was below the detection limit. However, despite the unusually high occurrence of enterococci in 24% of the primarily infected cases reported by these authors, there were too few observations to allow any general conclusions from this material. In all other longitudinal studies on root canal disinfection published thus far, too few cases contained enterococci to allow any statistical analysis (see below: "Enterococci in primary root canal infections").

Studies with a controlled infection in monkey teeth draw a slightly different picture regarding the survival of enterococci in root canals. When canals containing necrotic pulp tissue were autogenously infected with oral microorganisms and then sealed, enterococci, if present, were recovered in similar absolute numbers during an experimental period of 1060 days, but were gradually outnumbered by strict anaerobes (Fabricius *et al.* 1982b). In a later study using a five-strain combination including *E. faecalis*, the latter could be re-isolated from 24/24 monkey root canals 8-12 months after closure (Möller *et al.* 2004). *E. faecalis* appeared to also survive chemomechanical treatment using 1% NaOCl and 10% H_2O_2 slightly better than the anerobes in the five-strain combination; however, only in 3/24 cases *E. faecalis* was the only culturable strain, compared to 14/24 root canals, in which *E. faecalis* survived treatment together with other taxa. Nevertheless, in 21/24 root canals inoculated with the five-strain combination one or more strains survived, compared to 99/160 inoculated with the four-strain combination (Fisher's exact test, two-tailed, $P = 0.02$). Furthermore, the presence of *E. faecalis* in the original inoculum made it more likely that some bacteria remained viable after the root filling procedure in the long term (Fabricius *et al.* 2006).

In summary, it may indeed be so that once a root canal is infected with enterococci, proper disinfection may be harder to achieve. However, the relative importance of enterococci in this context appears to be over-estimated, and the high occurrence of enterococci in filled

root canals cannot be explained based simply on a higher resistance to antimicrobials of this compared to other species. So the question remains as to *when* enterococci most likely enter the root canal. Furthermore, the source of this infection is of interest. The rest of the review will thus focus on the possible entry of enterococci during the necrotizing process of the pulp, the root filling process and the restorative phase.

Enterococci in dentinal caries

There appears to be consensus that the primary causes of pulpal necrosis and the subsequent occurrence of apical periodontitis are dental caries and its sequelae. The progressive infection of dentine eventually leads to microabscesses in the pulp and tissue breakdown mediated by proteolytic enzymes (Langeland 1987, McLachlan *et al.* 2004). It has been known for some time that, as a carious lesion progresses into the dentine close to the pulp, the microbial infiltrate therein resembles the one in primary root canal infections (Edwardsson 1974). This was recently reconfirmed in a study using contemporary molecular biology methods (Martin *et al.* 2002).

In this context, it would be of interest to know whether enterococci are present in dentinal caries and consequently would be among the early invaders of the necrotizing pulp space. In 1933, Wohlfeil speculated that enterococci could cause dental caries based on the observation that they occurred more frequently around carious teeth and in individuals with bad oral hygiene than in orally healthy patients (Wohlfeil 1933). However, at that time, proper methods for the identification of oral streptococci (enterococci were included in the streptococci) were not available (Isenberg *et al.* 1970). A prominent example for this is the fact that *Streptococcus mutans*, although first detected and associated with caries in 1924 (Clarke 1924), could not be properly discriminated from other oral streptococci until the 1960s (Carlsson 1967, Guggenheim 1968). Enterococci were traditionally identified by their characteristic morphology, Gram staining, haemolysis, catalase test and their ability to grow

in methylene blue milk or on eosin-methylene blue agar (Sherman 1938, Isaacs & Scouller 1948). However, these methods do not differentiate between enterococci and oral streptococci (Guggenheim 1968). Moreover, non-enterococcal streptococci bearing Lancefield's group-D antigen, such as *Streptococcus bovis*, have even more similarities with enterococci than other oral streptococci when identified with phenotypic identification methods (Facklam 1973). The genus Enterococcus was proposed only in 1984 based on genomic differences between *Streptococcus faecalis* and *Streptococcus faecium* and other streptococci bearing the Lancefield group-D antigen (Schleifer & Klipper-Baelz 1984). *S. bovis* is found in the oral cavity (Crawford & Russell 1983) and is more cariogenic in the gnotobiotic rat than *E. faecalis* (Drucker & Green 1981, Willcox *et al.* 1990).

The first experimental study that found an association between enterococci and dental caries was undertaken in the cotton rat (Wakeman *et al.* 1948). However, the identification tests used were far from sufficient to properly identify enterococci (Guggenheim 1968, Facklam 1973). In 1951, Burnett and co-workers published a study on the culturable microorganisms from the advancing front of dentinal lesions (Burnett & Scherp 1951). They isolated aciduric streptococci, which they presumed to be enterococci because they were non-haemolytic, catalase-negative and grew in methylene-blue milk. Furthermore, these bacteria grew on 40% bile agar and at pH 9.6. The latter, but not the former feature appears to make it likely that they were correctly identified as enterococci and not *S. mutans*-like strains (Edwardsson 1968). However, the production of extracellular polysaccharides in 5% sucrose agar was not tested, which would have provided a clearer differentiation (Guggenheim 1968). Based on the Burnett and Scherp study, the first dental caries in a gnotobiotic rat model was produced by a "*Streptococcus faecalis*-like strain in combination with a proteolytic bacillus" (Orland *et al.* 1955). Later, when proper biochemical identification tools for enterococci were available, Gold and co-workers showed that indeed some of the enterococcus species isolated from human oral cavities were able to cause caries in germ-free rats (Gold *et al.* 1975).

However, they also observed that in the oral cavity of conventionally maintained rats, these strains could not survive and were not detectable 2 weeks after inoculation. Hence, interest was lost to study caries induction by enterococci any further in conventionally maintained rats. Later studies confirmed the low cariogenic potential of different *E. faecalis* strains under laboratory conditions (Drucker & Green 1981, Chestnutt *et al.* 1994).

A further bias in early studies on caries close to the pulp was the fact that proper anaerobic techniques for the recovery of strict anaerobes were not available before 1969 (Aranki *et al.* 1969). Consequently, as was shown later (Hoshino 1985), the relative number of facultative anaerobes was over-estimated in early caries studies. Interestingly, none of the researchers who controlled contamination of caries samples from the tooth surface and used modern culture and identification techniques found enterococci in carious dentine close to the pulp (Hahn *et al.* 1991, Hoshino *et al.* 1992, Martin *et al.* 2002). In a well-controlled study on the microorganisms related to early fissure caries in naval recruits, cultures from 48 carious fissures and 20 healthy control fissures yielded only 4 *E. faecalis*-positive samples from carious and 0 from non-carious sites, as compared to 48 and 17 positive cultures for *S. mutans*, respectively (Meiers *et al.* 1982). Even in positive samples, the counts of *E. faecalis* were three orders of magnitude below those of *S. mutans*.

In conclusion, early studies erroneously linked enterococccci with caries, because this genus was known from other fields of microbiology, was easy to culture, but could not be properly differentiated from oral streptococci that were later identified in carious lesions and dental plaque. Given the evidence we have today it is rather unlikely that enterococci occur at the forefront of carious lesions.

Enterococci in primary root canal infections

Even if not present in caries, enterococci may theoretically still enter the necrotizing pulp space at an early stage. Not all primary endodontic infections are caused by caries

(Abbott 2004). Many teeth contain cracks (Ratcliff *et al.* 2001), and thus it is conceivable that enterococci found in primary root canal infections entered via that route rather than dentinal tubules in carious teeth. Furthermore, inadequate coronal restorations with open margins have been linked to the occurrence of apical periodontitis (Kirkevang *et al.* 2007). However, the issue of studying primary root canal infections is difficult, especially when trying to determine how and when the microorganisms that eventually lead to pulpal breakdown entered the endododontic system. One of the most prominent problems in this context is again contamination from saliva or plaque from the outer tooth surface (Möller 1966). Because root canal infections are usually polymicrobial (Sundqvist 1994), and the most common invaders of the *endodontium* can be found in other sites of the oral cavity, it is impossible in the laboratory to discern between the microorganisms that were actually present in the root canal at the time of sampling and contaminants. As already highlighted by Engström, many of the teeth that harbour enterococci in the root canal system also show positive enterococcal growth on outer tooth surfaces (Engström 1964). False positive results are even more likely when polymerase chain reaction (PCR) is used to detect specific DNA sequences of microorganisms suspected to be present in the root canal (Nair 2007). Hence, a meticulous sampling technique including disinfection of the tooth and the access cavity with the respective sterility checks from both sites is a prerequisite to yield meaningful results. Whilst such protocols have been validated and published for both culture and molecular methods (Möller 1966, Ng *et al.* 2003), relatively few studies have complied with these guidelines. In studies on the culturable micrbiota of primary endodontic infections with proper sterility checks of the access cavity, enterococci have usually been found in a rather low proportion of infected canals or not at all (for review, see Portenier *et al.* 2003). This is in contrast to the high frequency of enterococci encountered in filled root canal systems associated with treatment failure (Molander *et al.* 1998, Sundqvist *et al.* 1998, Peciuliene *et al.* 2000, Hancock *et al.* 2001). On the other hand, studies employing DNA-DNA hybridization or PCR

techniques (without checking the access cavity) found a somewhat higher occurrence of *E. faecalis* in primary root canal infections as compared to those investigations, which were performed using culture techniques (Siqueira *et al.* 2002, Pirani *et al.* 2008). Nevertheless, the occurrence of *E. faecalis* in a PCR assay was still significantly lower in teeth containing necrotic pulps compared to root filled counterparts with apical lesions (Pirani *et al.* 2008). Contrary to these findings, a relatively high occurence of *E. faecalis* in primary root canal infections has been reported when nested PCR was used to identify this taxon and was compared to a conventional culture technique: *E. faecalis* was identified in 82% versus 4%, respectively (Gomes *et al.* 2006). The authors concluded that *E. faecalis* could be present in numbers below the detection level for culturing or that they could be in a viable but non-culturable state. On the other hand, the authors also conceded that 49/50 of their sampled teeth had coronal leakage. Consequently, proper decontamination of the access for PCR was a difficult, if not impossible task, and enterococcal DNA could thus have originated from sources outside the root canal. Furthermore, nested PCR is notoriously difficult to perform; the paper describes only sequencing a single band from the gels – this would afford little confidence in the band identification. Moreover, it is well-known that *E. faecalis* is one of the easiest species to culture, and it does not enter a viable but non-culturable state (Bogosian *et al.* 1998).

In summary, as already suspected from the low or non-existing occurrence of enterococci in caries, members of the genus *Enterococcus* probably exist relatively rarely in primary root canal infections. To the best of our knowledge, only one study thus far has specifically targeted the difference in the microbiota recovered from necrotic root canals between teeth with an exposed and counterparts with a non-exposed pulp space. However, in that investigation, cracks were not taken into consideration. Furthermore, the incidence of *E. faecalis* was merely 0/45 versus 2/43 in pulps from teeth that had a visible contact to the oral

cavity compared to those that did not, respectively (Chu *et al.* 2005). These numbers are again too low to allow any conclusions.

The low occurrence of enterococci in primary root canal infections makes one possible pathway for the colonization of necrotic pulp tissue that cannot be terminally excluded unlikely: *anachoresis*, i.e. the transfection of microorganisms via the blood stream (Tunnicliff & Hammond 1937). Animal experiments with high numbers of microorganisms injected in the blood stream have shown that a colonization of necrotizing pulp tissue is possible (Gier & Mitchell 1968, Tziafas 1989). In the case of enterococci, it is conceivable that these bacteria could enter the bloodstream from the large intestine and then enter necrotic areas of a tooth with terminal pulpitis. However, as indicated above, the low occurrence of enterococci in primary root canal infections and the low likelihood of any bacterium to colonize necrotic pulp tissue via the blood stream make the pathway of direct oral entry more likely.

Enterococcal invasion of the root canal during or after treatment

Few data exist in the literature to support or contradict the theory that enterococci could enter the root canal system during or even after endodontic treatment. It has been surmised from longitudinal studies that culture reversals with the sudden occurrence of enterococci after the initial treatment session could be due to leakage through the temporary filling (Sjögren *et al.* 1991, Sundqvist *et al.* 1998). However, again these observations were too infrequent to allow generalization. The only study thus far that has addressed the correlation between the clinical occurrence of enterococci and other enteric bacteria and the root canal seal was based on samples that were sent in by private practitioners, accompanied by a questionnaire regarding the treatment steps that had been performed (Sirén *et al.* 1997). As it turned out, there was a significant positive correlation between the occurrence of the target species and the number of visits, as well as leaving the canal unsealed between treatment sessions.

Studies on the occurrence of enterococci in root filled teeth (Engström 1964, Molander *et al.* 1998, Kaufman *et al.* 2005) also suggest that these bacteria could have entered the canal system after the root filling procedure. Enterococci are able to induce and maintain an apical lesion as monoinfectants (Fabricius *et al.* 1982a, Ferrari *et al.* 2005). On the other hand, they have been found more frequently in filled canals *without* a radiographic lesion compared to counterparts with a lesion (Kaufman *et al.* 2005). It appears rather unlikely that enterococci from a primary infection survived treatment and root filling procedures only in the coronal aspect of the canal and not in the apical portion (which would, with a high degree of certainty, result in a lesion). Hence, it may be assumed from everything that we know at this point that enterococci in filled root canals without apical rarefaction are likely to have entered after the root was filled. In this context, it should be stated that one almost general shortcoming in endodontic articles is the lack of information regarding the restoration and the history of the teeth under investigation. Depending on the coronal restoration, filled root canal systems may invariably have been exposed to the oral cavity at one point during treatment. This is especially the case with teeth that receive an indirect restoration, which undergo a phase of temporization. However, clinical studies that investigated microbial leakage around temporary fillings or crowns are few, and the involved microorganisms have not been identified (Beach *et al.* 1996).

Taken together, the little evidence currently available points in the direction that enterococci enter the root canal system at some time after the root canal treatment has been initiated. The source of infection for pulpless root canals appears to be the oral cavity with its currently more than 700 identified bacterial species or phylotypes. However, whether the oral cavity is a habitat for enterococci is the next question.

Are enterococci colonizers of the oral cavity?

This question may appear somewhat stupid, because common sense would dictate that, of course, if enterococci can maintain in necrotic root canals, why should they not be present at other oral sites? However, microorganisms that can enter a specific niche in the oral cavity that is not present in all individuals (in our case the unsealed pulpless root canal) need not necessarily be consistent inhabitants of adjacent sites. The issue that is often overlooked in this context is *infections caused by transient oral microorganisms* that have recently gained some interest in connection with the survival of probiotics in the oral cavity (Meurman 2005).

It should be realized that humans live in an environment surrounded by a complex microbiota, continuously inhaling and ingesting microorganisms that are unable to colonize epithelial and tooth surfaces permanently (Pamer 2007). The healthy gastrointestinal mucosal surfaces are inhabited by microbial populations that, in aggregate, are called the commensal "flora" (outdated term) or microbiota (Ley *et al.* 2006). The local composition of the commensal microbiota is site-specific, and can vary considerably. The main habitat of enterococci, as the name indicates, is the gastrointestinal tract. In humans, *E. faecalis* and *E. faecium* are the predominant species, whilst *E. faecium* is the predominant species in poultry and pigs. However, *enterococci are not part of the typical commensal microbiota of the oral cavity*, that has recently been defined using culture-independent techniques (Aas *et al.* 2005). Sampled sites included tongue dorsum, lateral sides of tongue, buccal epithelium, hard palate, soft palate, supragingival plaque of tooth surfaces, subgingival plaque, maxillary anterior vestibule, and tonsils. Interestingly, enterococci were not found in any of the sites of the five individuals that were screened. Instead, most sites were covered with protective microorganisms such as *Streptococcus mitis* and *S. salivarius*, but the bacterial richness was striking, with well over 100 predominant bacterial taxa representing six phyla that were identified, almost half of which had never been cultured.

As reviewed by Engström, early studies that specifically screened the oral cavity for the presence of viable enterococci were inconsistent (Engström 1964). The most thorough of

these early studies was probably the one by Williams and co-workers (1950). The authors were looking for enterococci and yeasts in saliva based on an earlier finding in their related dental clinic that these microorganisms were frequently recovered from root canals that had been dressed with a penicillin/streptomycin mix (Grossman & Steward 1949). Saliva samples from 206 individuals were collected and analyzed for enterococci. From most donors, saliva was collected more than once. Overall, 45 persons (21.8%) had enterococci (mostly *E. faecalis*) in their saliva at least once. However, the carriage was not consistent, meaning that on one experimental day an individual tested positive, on another day the same individual tested negative. This correlates rather well with the above-mentioned observation in rats with a normal oral microbiota, namely the finding that enterococci that were introduced into the oral cavity did not maintain over time (Gold *et al.* 1975). In a recent study, the prevalence, phenotype and genotype of oral enterococci was studied (Sedgley *et al.* 2004). Enterococci were detected in oral rinse samples from 11/100 patients receiving endodontic treatment and 1/100 dental students with no history of endodontic treatment ($P = 0.0027$). All enterococcal isolates were identified as *E. faecalis*. The data from that study could be interpreted in the direction that the occurrence of *E. faecalis* could have something to do with oral hygiene, as dental students should technically have a better oral hygiene than endodontic patients. This, however, was not assessed. In an even more recent study no viable entrococci were found in any of the oral rinse samples from 50 dental students with good oral hygiene and few fillings (Razavi *et al.* 2007).

Taken together, it could be stated that the most common enterococcal species found in the oral cavity occasionally is *E. faecalis*. This is consistent with the predominance of this species in the root canal. However, enterococci are rarely found in the oral cavity of healthy subjects with a good oral hygiene, and are not consistently recovered from the oral cavity of "average" patients. How can this be? The most logical explanation for this is that enterococci

are just transient bacteria in the oral cavity. And the most likely source of enterococci that can be found in the oral cavity at times is *food*.

Enterococci in food

E. faecalis is an indicator for contamination of food, water and inanimate objects from human sources (Reuter 1992). On the other hand, enterococci are ubiquitous in food products for raw consumption (Franz *et al.* 2003). Only few health problems have been reported related to this fact (Kayser 2003). Enterococci are even used as veterinary food supplements. Since February 2004, 9 different strains of *E. faecium* are authorized as additives in feeding stuff in the European Union (Foulquie Moreno *et al.* 2006). Furthermore, *E. faecium* SF 68 (Cylactin, Hoffmann-La Roche, Basel, Switzerland) is a probiotic used in humans, which was shown to be effective against different types of diarrhoea (Foulquie Moreno *et al.* 2006).

The resistance of enterococci to high temperatures and their ability to grow under a wide array of conditions is the reason why they can be found in food not only manufactured from raw materials but also in heat-treated merchandise. Milk products, meat products for raw consumption, vegetables and olives commonly contain enterococci (Franz *et al.* 2003). *E. faecalis* is mostly found in milk products such as cheese and in fermented sausages for raw consumption, but can also commonly be isolated from various other food products such as minced beef, minced pork and fish/crustacea (Klein 2003, Peters *et al.* 2003). The presence of enterococci in food is not necessarily always unwanted, as they can add to the specific taste of, for instance, Mediterranean cheeses. Enterococci have even been used as starter cultures to ferment olives and cheese (Giraffa 2002).

The possibility that enterococci could cause a transient oral infection has only been identified recently (Razavi *et al.* 2007). In a study with healthy volunteers, the clearance of *E. faecalis* from a highly contaminated cheese (Brie de Meaux containing a mean of 4.8×10^4 *E. faecalis* colony-forming units g^{-1}) was investigated. The volunteers refrained from eating or

drinking between cheese ingestion and the collection of an oral rinse sample 1, 10, or 100 min after cheese ingestion. Between experimental days, the volunteers maintained their normal oral hygiene and diet habits for one week. One minute after ingestion, a median of 5480 *E. faecalis* colony-forming units was recovered. Bacterial counts were reduced after 10 min, dropped after 100 min to levels that were significantly ($P < 0.005$) different from 1 and 10-min scores and were below the detection limit after one week. These findings suggest that enterococci do adhere to oral tissues of healthy subjects but fail to grow when competing with the normal oral microbiota and are thus gradually eliminated. However, the rate of clearance of the food-derived enterococci from the oral cavity was such that infection of predilection sites cannot be excluded. This adds weight to the hypothesis that enterococcal root canal infections could be food-borne. In a follow-up study, the possibility that *E. faecalis* from food could enter the *endodontium* was explored in an artificial oral environment in a mastication apparatus (Kampfer *et al.* 2007). It was shown that viable enterococci could indeed leak through a calcium sulphate-based temporary filling material, if its thickness was below 4 mm.

Concluding remarks and call for future studies

Enterococci, especially *E. faecalis* strains, appear to adapt to the habitat of a treated root canal better than other taxa. From the information available, it may be concluded that these bacteria are not among the early invaders of the necrotizing root canal system. They may enter the root canal at any point in time during or after treatment if the coronal seal is inadequate. Their source is most likely food. In individuals with an adequate level of oral hygiene, they do not colonize the oral cavity. However, they may enter the unsealed root canal system, where they find a habitat that allows their growth and survival.

Future studies should be directed at several issues. First, virulence factors should be identified that favour the occurrence of enterococci in filled root canals. This work has already started and has yielded some interesting results (Sedgley 2007). Gelatinase production

is one of the virulence factors that may be associated with the survival of *E. faecalis* in filled root canals. The *E. faecalis* strains producing gelatinase were termed *Streptococcus faecalis var. liquefaciens* in early studies and were frequently recovered from root canal systems (Guthof 1953, Winkler & van Amerongen 1959, Engström 1964, Mejàre 1975). Interestingly and perhaps obviously, gelatinase production is also a factor that promotes the presence of enterococci in fermented food products (Franz *et al.* 2003). The origin of enterococci in root canals should be further identified by comparing the scheme of highly preserved genes between clones found in root canals and counterparts from food products, preferably from a similar area (Ruiz-Garbajosa *et al.* 2006). On a less sophisticated level, contamination of different enterococcal species in typical regional foods could be compared with the recovery of these species from root canals in a given country or area. Last but not least, it has been known for a long time that the healthy microbiota of the oral cavity can defend against potential pathogens (Deyloff & Sanders 1980). It would be interesting to identify the mechanisms preventing the colonization of the oral cavity by enterococci.

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