Q-500: Long-term Occurrence of Clonally Related *E. faecalis* Strains in Marine Water and Sediments

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ABSTRACT

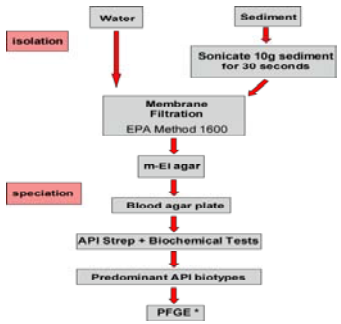
Recreational water is considered contaminated with fecal matter and therefore unsafe for public use when enterococci levels exceed 104-colony forming units (CFU)/100 ml. However, the reliability of this standard to indicate fecal contamination has been questioned. Although enterococci are commonly found in the gastrointestinal tract of humans, birds and animals, they are also ubiquitous in the environment.

To better understand the sources of enterococci in beach water, 230 *Enterococcus faecalis* isolates from shoreline water and marine sediments at Huntington Beach, California, were subjected to molecular typing by Pulsed-Field Gel Electrophoresis (PFGE).

Cluster analysis revealed 46 clusters at the 85% similarity level. Within these, a cutoff of 100% similarity revealed 26 clusters. Twenty of these "clonal groups" included isolates found in more than one sample. Clonal groups found in shoreline water were also found weeks and even several months later in offshore sediments and intertidal sediments in a river adjacent to the beach. Seventy-five *E. faecalis* isolates, mostly from shoreline water, were part of one large clonal group. The source of this group has not yet been determined. The occurrence of clonal groups in shoreline water samples with enterococci concentrations meeting or exceeding enterococci standards was also compared. Clonal isolates found in these samples were present in samples collected when enterococci levels were below standards. No specific clonal groups were predominant in samples exceeding standards.

Highly related strains of *E. faecalis*, particularly those that are genetically indistinguishable, indicate a high likelihood that these strains originated from a common source. The continued presence of clonally related *E. faecalis* strains in shoreline water and marine sediments indicate a persistent source and/or possible replication of *E. faecalis* strains in the marine environment.

MATERIALS AND METHODS



Shoreline water samples were collected at ankle depth from the mouth of the Santa Ana River (SAR) and at shoreline sites 3N, 6N, 9N and 21N corresponding to distances of 914, 1829, 2743 and 6384 meters northwest of the SAR (Figure 1).

SAR sediment was collected every 30 – 90 m along a transect from the mouth of the river to about 3000 m upstream.

Offshore sediment were obtained at 10 m depths, 330 m offshore to Huntington State Beach every 160 m along a 3200 m long transect.

Water samples were processed by membrane filtration (EPA Method 1600). Sediment (10g) were sonicated for 30 s. The supernatant was filtered similarly as water. Presumptive enterococci isolates from mEI agar were identified using API 20 STREP and additional biochemicals. Predominant API biotypes were subjected to PFGE using the *Staphylococcus aureus* procedures used by CDC (McDougal et al. 2003) with the following changes: (1) mutolysin was added to the lysis solution, (2) plugs were lysed and washed for additional times and (3) the switched times in the running conditions were modified.

To verify the ability of a Sma 1 to distinguish clonal groups, 42 isolates were analyzed using a second enzyme, Not 1.

RESULTS (1)

Figure 1. Map of study site

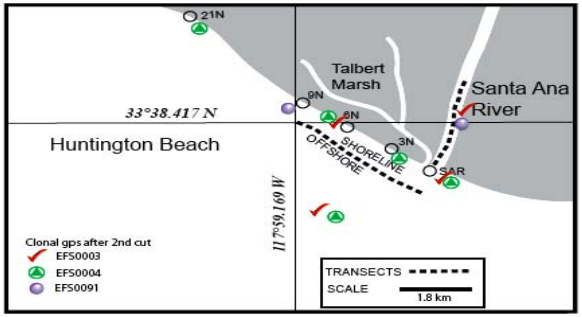


Table 1. Summary of *E. faecalis* samples, isolates and PFGE patterns

Sample Type	No. samples	No. isolates	No. patterns	No. clonal* patterns	No. isolates in clonal patterns
Shoreline water	127	158	58	18	115
River sediment	26	35	23	9	20
Offshore sediment	25	37	19	6	22
Total	178	230	87**	20**	157

*Patterns found in multiple samples
**The total no. does not add up because some patterns were seen in more than one sample type

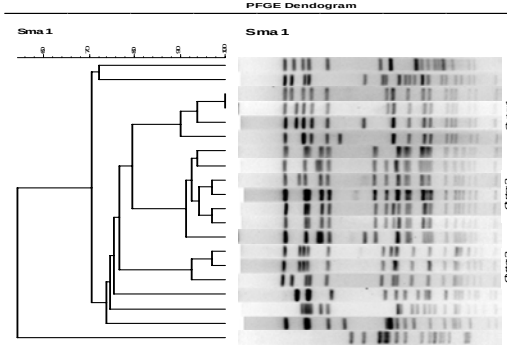
Figure 3. Distribution of 5 *E. faecalis* PFGE types found in 3 or more marine water samples* meeting or failing enterococci single sample standard (104 CFU/100-ml).

PFGE Type	Week #	Sites SAR	3N	6N	9N	21N
EFS0004	1					
	2					
	3					
	4					
	5					
	6					
EFS0007	1					
	2					
	3					
	4					
	5					
	6					
EFS0070	1					
	2					
	3					
	4					
	5					
	6					
EFS0001	1					
	2					
	3					
	4					
	5					
	6					
EFS0126	1					
	2					
	3					
	4					
	5					
	6					

*between 3 - 46 samples over the 6 week period

samples meeting enterococci single sample standard
samples failing enterococci single sample standard

Figure 2. Pulsed-field gel electrophoresis patterns and frequency distribution of *Enterococcus faecalis* found in multiple samples.



Pattern No.*	No. samples with isolates of indicated pattern: Marine Water Location SAR†	3N	6N	9N	21N	Sediment Location SAR†	Offshore	Total
EFS0126	0	0	1	1	1	0	0	3
EFS0028	0	1	1	0	0	0	0	2
EFS0007[a]	1	0	3	2	3	0	0	9
EFS0006[a]	0	0	1	1	0	0	0	2
EFS0004	3	8	12	10	13	0	4	50
EFS0008	0	1	0	1	0	0	0	2
EFS0068	0	0	2	0	0	0	0	2
EFS0026	0	0	0	1	0	1	0	2
EFS0069	0	0	1	0	0	1	2	4
EFS0070	0	1	1	3	0	1	0	6
EFS0003	1	0	1	0	0	1	2	5
EFS0001	0	1	1	1	0	3	0	6
EFS0091	0	0	0	1	0	1	0	2
EFS0111	0	0	2	0	0	0	1	3
EFS0112	0	1	0	0	1	1	0	3
EFS0116	0	0	0	1	0	0	1	2
EFS0024	0	0	0	0	0	2	0	2
EFS0034	2	0	0	0	0	0	0	2
EFS0044	0	1	0	0	0	1	0	2
EFS0054	0	0	0	0	0	0	2	2

* Pattern numbers were assigned by visual inspection. Isolates that were indistinguishable by visual inspection were assigned different pattern numbers. Patterns EFS0006 and EFS0007 were assigned different pattern numbers even though they were indistinguishable by computer analysis.
† Santa Ana River

RESULTS (2)

Of 230 presumptive enterococci isolates tested, 38% were identified as *E. facealis*.

87 *E. faecalis* PFGE pattern types representing groups of one or more isolates were identified (Table 1).

157 *E. faecalis* isolates from shoreline water were clonal. Most clonal groups consisted of a few isolates per group.

20 PFGE pattern types represented isolates found in multiple samples (Fig 2). Fig. 1 shows the geographic distribution of 3 clonal groups (verified with a 2nd enzyme).

Clonal types were present in shoreline water over a 6 week period (Fig 3). Isolates from the largest group, EFS0004, were most frequently detected at sites spanning a distance of 6.4 km. We are uncertain as to the source origin. No single clonal group dominated samples exceeding standards.

PFGE typing using a 2nd enzyme resulted in 4 groups if isolates that were still 100% similar and 8 groups that had at least one isolate that differed from others by 1 to 3 bands.

DISCUSSION AND CONCLUSION

There are still no set criteria for how many band differences constitute a clone. In this study, *E. faecalis* isolates with PFGE patterns that were 100% similar using Sma 1 (verified visually) were considered clonal.

Finding isolates that were still very similar after a 2nd enzyme is suggestive that the isolates are very closely related. However, it is also possible that there is little genetic diversity among these *E. faecalis* subtypes.

Finding isolates in the river and offshore sediments that were either identical or closely related to isolates found in shoreline water 3 months earlier, indicate long term persistence. The widespread distribution and persistence of these *E. faecalis* strains suggests that these may be environmentally adapted or strains originating from a widely distributed source.

ACKNOWLEDGEMENTS

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