



Q-431: Genotypic Characterization of *Enterococcus faecalis* Isolates in Ocean Shoreline Water by Pulsed-Field Gel Electrophoresis

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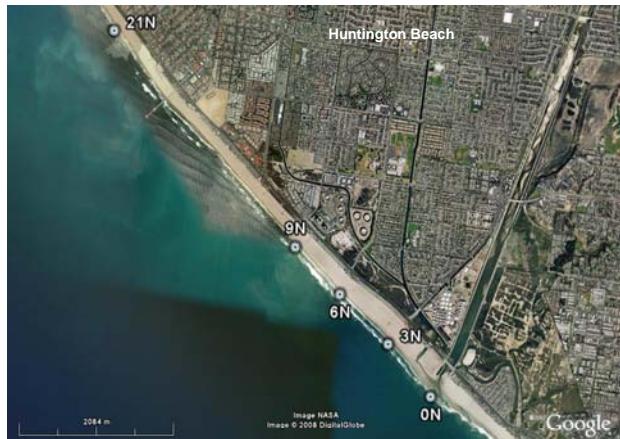
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ABSTRACT

Pulsed-Field Gel Electrophoresis (PFGE) was performed to determine the genetic relatedness of *E. faecalis* isolates from ocean shoreline water in a baseline study and a follow up study performed three years later. The 214 *E. faecalis* isolates formed a heterogeneous genetic distribution represented by 86 different PFGE types. While 30% of the PFGE types were unique 70% were shared by two or more isolates. Fifty-four percent of the isolates were part of a genetically related group of 13 PFGE types that were the most frequently isolated in both the initial and follow-up study. One PFGE type in this group contained 36% of the isolates. Overall, 105 (49%) of isolates were PFGE types that were isolated in both 2003 and 2006. The data indicates that a large percentage of *E. faecalis* isolated were members of a genetically related group that persisted in the environment for three years.



MATERIALS AND METHODS

Location

Huntington Beach, an ocean beach located in Southern California, spans approx. 9 km and is bounded on the southeast by the Santa Ana River (SAR) and Talbert Marsh and on the northwest by Bolsa Chica Beach.

Water Samples

Baseline study: Shoreline water samples were collected 5 times a week at 5 shoreline sites from the mouth of the SAR to 6.3 km northwest over a 6 week period in 2003.

Follow-up study: 128 water samples were collected over a 12 hour period from the 4 southeast sites in 2006.

Isolation

The concentration of enterococci in both studies was determined following EPA Method 1600 using Enterococcus Indoxyl- β -D-Glucoside Agar (mEI). Up to 5 presumptive enterococci colonies were picked from each sample for further analysis.

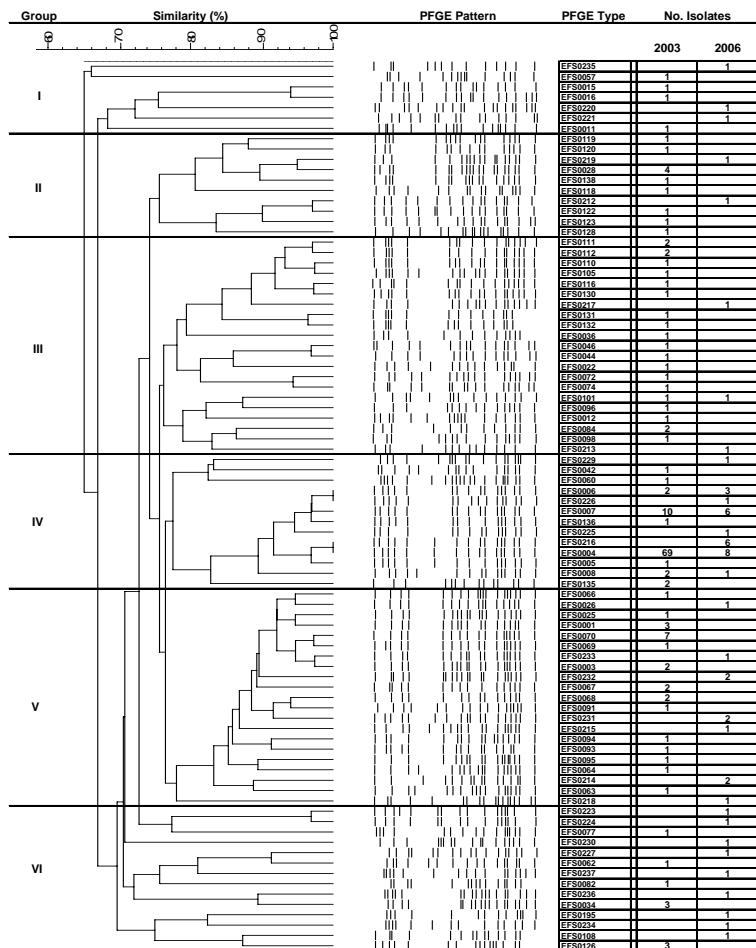
Identification

Baseline study: 160 isolates were identified as *E. faecalis* with the API™ 20 Strep and additional biochemical tests (carbohydrate fermentation, motility test medium, and pigment production). 74 isolates were API biotype 7142711 and 86 isolates were API biotype 7142711.

Follow up study: 54 isolates were identified as *E. faecalis* with the Microscan Walk Away system and additional biochemical tests.

PFGE Analysis

The PFGE procedure followed previously published techniques that were modified to enhance reproducibility and band clarity (Murray et al. 1990, McDougal et al. 2003) with SmaI restriction enzyme. A subset of the isolates were also restricted with a second restriction enzyme – NotI. Tiff images were analyzed using BioNumerics software, version 4.0 (Applied Maths Kortrijk, Belgium).



Small PFGE dendrogram and patterns showing relatedness, frequency, and year of 214 *E. faecalis* isolates. Dendrogram was determined by Dice coefficient and the UPGMA (unweighted pair group with arithmetic averaging) analysis of PFGE patterns.

RESULTS

- The 214 *E. faecalis* isolates grouped into 86 different PFGE types. 6 Groups were differentiated at similarity levels ranging from 66% to 75% with each group containing between 7 and 21 different PFGE types.
- The overall distribution of PFGE types was heterogeneous with a percent similarity of the PFGE patterns ranging from 64% to 100%.
- 30% (65) of the PFGE types were unique with one isolate. 70% (149) were shared with two or more isolates.
- 54% of the isolates were part of a genetically related group (Group IV) of 13 PFGE types that were the most frequently isolated in both the baseline and follow-up study.
- One PFGE type, EFS0004, contained 36% (77/214) of all isolates. EF0004 represented 43% (69/160) of the isolates from the baseline study and 15% (8/54) of the isolates from the follow-up study.
- The 74 isolates from biotype 7142711 were differentiated into 55 different PFGE types that were distributed in all groups. The 86 isolates from biotype 7142711 were differentiated into 5 different PFGE types (EFS0004, EFS0007, EFS0022, EFS0068 and EFS0069) in groups III, IV and V only.
- Overall, 105 (49%) of the isolates were PFGE types that were isolated in both 2003 and 2006.
- 13 Isolates of EFS0004, from both the baseline and follow-up study, had 100% similarity with a second PFGE restriction enzyme, NotI.
- In this study PFGE types present in samples failing water quality standards were also heterogeneous, however the highest percentage of failure isolates were from Group IV due to isolates of that group being the most numerous.

DISCUSSION and CONCLUSIONS

- While the overall distribution of PFGE types was heterogeneous, several PFGE types were isolated multiple times and one related group (Group IV) represented a majority of isolates.
- One PFGE type (EFS0004) represented 36% of all isolates and was the most frequently isolated PFGE type in both studies.
- A subset of highly related PFGE types isolated in the baseline study persisted three years and were the most frequently isolated types during the follow-up study.

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