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Occurrence and characterisation of enterococci
in terrestrial and aquatic environments

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Abstract

The use of enterococci as a microbiological indicator of water quality requires an understanding of the sources, persistence, and ecology of this group of bacteria in the environment. This research describes a series of investigations undertaken to describe the abundance, occurrence, and diversity of enterococci from aquatic and terrestrial environments.

A screening protocol for environmentally sourced enterococci was developed to describe species and sub-species variability. This protocol combined classical microbiological methods of selective culture and biochemical characterisation, with molecular techniques including gene probe screening for identity and RAPD-PCR for genotypic diversity.

Preliminary studies on the occurrence of enterococci in terrestrial and aquatic environments suggested that these organisms are ubiquitous. However, abundance varied between individual samples taken from terrestrial (e.g. leaf litter, sand, seaweed, animal faeces) or aquatic (marine or freshwater) environments, complicating the ability to predict the enterococci load from these sources.

Enumeration of enterococci from bathing beach environments indicated occasionally high levels from seaweed and sand, where levels of up to 660 CFU/100 g (wet weight) were recorded from aged and degrading seaweed but not from fresh seaweed samples. Restriction enzyme analysis (REA) of isolates from degraded seaweed indicated a dominance of clonal populations and supported the notion of replication or survival of strains.

Laboratory studies conducted to investigate enterococci persistence and growth on seaweed were not conclusive, although there was some evidence to suggest enterococci replication was occurring. This was indicated by molecular fingerprinting (REA analysis), which showed that the inoculated strain persisted for the full duration of experiments (up to 28 days). The isolation of non-inoculum strains from seaweed treatments, combined with increased abundance of these strains with incubation, suggested the persistence or replication of enterococci that were naturally occurring on seaweed.

To investigate the occurrence of enterococci in bathing environments a statistical analysis of Auckland Regional Council (ARC) bathing water quality data was undertaken. This analysis indicated a strong positive correlation between enterococci and turbidity, and hence turbidity may serve as a useful physical measure to indicate deteriorating water quality. Surveys of three marine bathing beaches on Auckland’s North Shore (Long Bay, Mairangi Bay, and Rothesay Bay) indicated the abundance of enterococci in all bathing beach environments surveyed. These included marine and fresh water, sand, seaweed, and stream sediment, and a significant association between enterococci levels found in the sand and in the seaweed.
Enterococci screening protocols were evaluated for use in describing enterococci species and sub-species diversity in bathing beach environments. This investigation showed a diversity of enterococci from all beach environmental sources, with highest levels of species diversity from marine and stream water. Enterococci diversity did not provide clues as to the sources in marine water samples. RAPD-PCR analysis and phenotypic screening of enterococci isolates did not indicate a pattern of niche-specific associations of enterococci strains, and there was no strong evidence from this study that enterococci sub-species associate with specific environments. However, the presence of identical genotypes indicated that enterococci can persist and possibly replicate in environments such as sand and seaweed. Calculation of similarity coefficients from *Ent. faecalis* and *Ent. casseliflavus* sub-species groupings indicated a greater level of sub-specific similarity between selected environments, for example, seaweed:sand, marine water:stream water, seaweed:marine water, although this was not a guarantee that environments were biologically or ecologically associated.

Where an elevated level of enterococci is measured in the absence in identifiable pollution source the separation of pigmented from non-pigmented enterococci was proposed as an indicator of the environmental or faecal nature of the enterococci within the sample. Although not tested under controlled conditions, this concept was found to have good utility for discriminating sources from elevated marine bathing water samples. Enterococci from one of 13 elevated ARC marine water samples examined was shown to be environmentally derived, with 5 of the 13 samples attributed to putative human or animal faecal sources. With further validation, this concept may be a useful means of determining source.
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For Ben.
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**List of abbreviations**

Système International d’Unités (SI units) of measurement, standard notations for describing chemical formulae, and standard conventions for biological nomenclature were used in this work. Commonly used abbreviations cited in the text are listed below:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;260&lt;/sub&gt;</td>
<td>absorbance at 260 nm</td>
</tr>
<tr>
<td>A&lt;sub&gt;280&lt;/sub&gt;</td>
<td>absorbance at 280 nm</td>
</tr>
<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphism</td>
</tr>
<tr>
<td>ARC</td>
<td>Auckland Regional Council</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>DIG</td>
<td>digoxigenin</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxyribonucleoside triphosphate</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine-tetra-acetate acid (disodium salt)</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>hrs</td>
<td>hours</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase pairs</td>
</tr>
<tr>
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<td>millilitre(s)</td>
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<tr>
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<td>millimetre(s)</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>nm</td>
<td>nanometres</td>
</tr>
<tr>
<td>PBW</td>
<td>Phosphate buffered water</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet light</td>
</tr>
<tr>
<td>RAPD</td>
<td>Randomly amplified polymorphic DNA</td>
</tr>
<tr>
<td>RE</td>
<td>Restriction enzyme</td>
</tr>
<tr>
<td>REA</td>
<td>restriction enzyme analysis</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNAse</td>
<td>ribonuclease</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>rDNA</td>
<td>ribosomal DNA</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>-------------</td>
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<tr>
<td>rRNA</td>
<td>ribosomal RNA</td>
</tr>
<tr>
<td>R/T</td>
<td>room temperature</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SSC</td>
<td>standard saline citrate</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-acetate EDTA buffer</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-borate EDTA buffer</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA buffer</td>
</tr>
<tr>
<td>Tris</td>
<td>[2-amino-2-(hydroxymethyl) propane-1,3-diol, (tris)]</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet light</td>
</tr>
<tr>
<td>V</td>
<td>volts</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
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1.1 Overview
1.2 Taxonomy of the genus *Enterococcus*
1.3 Enterococci habitats and sources
1.4 Survival and persistence of enterococci in the environment
1.5 Enterococci/Faecal streptococci as faecal source indicators
1.6 Microbiological indicators of water quality
1.7 Development of water quality criteria
1.8 Objectives of this study

This image of enterococci shown on each chapter title page was created by imaging a Gram-stain of enterococci viewed by light microscopy at 1,000x magnification. The final image was created in Adobe Photoshop using the emboss function to create the final cell impression.