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Development of quantitative PCR assays targeting 16S rRNA gene of *Enterococcus* spp. and their application to the identification of *Enterococcus* species in environmental samples

Hodon Ryu[1], Michael Henson[1], Michael Elk[1], Carlos Toledo-Hernandez[1], John Griffith, Denene Blackwood[2], Rachel Noble[2], Michèle Gourmelon[3], Susan Glassmeyer[4] and Jorge Santo Domingo[1]

[1]*US Environmental Protection Agency, National Risk Management Research Laboratory, Cincinnati, OH*
[2]*University of North Carolina, Institute of Marine Sciences, Chapel Hill, NC*
[3]*Ifremer EMP, Laboratoire de Microbiologie, Plouzané, France*
[4]*US Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH*

ABSTRACT

The detection of environmental enterococci has primarily been determined using culture-based techniques that might exclude some enterococci species as well as those that are nonculturable. To address this, the relative abundance of enterococci was examined by challenging fecal and water samples against a currently available genus-specific assay (Entero1). To determine the diversity of enterococci species, 16S rRNA gene group-specific qPCR assays were developed and evaluated against eight of the most common environmental enterococci species. Partial 16S rRNA gene sequences of 439 presumptive environmental enterococci strains were analyzed to further study enterococci diversity and to confirm the specificity of group-specific assays. The group-specific qPCR assays showed relatively high amplification rates with targeted-species (>98%), although some assays cross-amplified with non-targeted species (1.3 - 6.5%). The results with the group-specific assays also showed that different enterococci species co-occurred in most fecal samples. The most abundant enterococci in water and fecal samples were *E. faecalis* and *E. faecium*, although we identified more water isolates as *E. casseliflavus* than any of the other species. The prevalence of the Entero1 marker was in agreement with the combined number of positive signals determined by the groupspecific assays in most fecal samples, except in gull feces. On the other hand, the number of group-specific assays signals was lower in all water samples tested, suggesting that other enterococci species are present in these samples. While the results highlight the value of genus- and group-specific assays at detecting the major enterococci groups in environmental water samples, additional studies are needed to further determine the diversity, distribution, and relative abundance of all enterococci species in water.

Full Text

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