Use of real-time PCR to detect and enumerate *Vibrio vulnificus* strains pathogenic to human health

C. Baker-Austin1, E. Lemm1, R. Rangdale1, C. Amaro3, J. D. Oliver2 and D. N. Lees1.

1Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Weymouth, Dorset, England (louise.stockley@cefas.co.uk)

2Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina, USA (jdoliver@uncc.edu)

3Departamento de Microbiología, Facultad de Biología, Universidad de Valencia, Valencia, Spain (Carmen.Amaro@uv.es)

Abstract

*V. vulnificus* is a common inhabitant of marine and estuarine environments, and a serious human pathogen. The species is present in high numbers in filtering organisms, such as oysters, especially in warmer months (Oliver et al. 2006). *V. vulnificus* is a potent human pathogen, and is responsible for more than 95% of all seafood-related deaths (Oliver 1989). Unfortunately, molecular methods for the detection and enumeration of pathogenic *V. vulnificus* are hampered by the highly genetically diverse nature of this pathogen, the range of different biotypes capable of infecting humans and aquatic animals, and the fact that *V. vulnificus* contains pathogenic as well as non-pathogenic variants. Numerous targets have been suggested as potential molecular targets to distinguish strains capable of causing infections in humans with non-pathogenic variants, with varying degrees of success. These include differences in the sequence of the small subunit 16S rRNA gene, as correlating with either clinical (pathogenic) and environmental (non-pathogenic) origin (Aznar et al. 1994, Nilsson et al. 2003, Vickery et al. 2006); polymorphisms based on a virulence-correlated gene (*vcg*) (Rosche et al. 2005), and more recently a polymorphism in the pilus-type IV assembly protein of *V. vulnificus* (Roig et al. 2010).

Here we report the comparison of several previously published real-time PCR assays used recently for the detection of pathogenic *V. vulnificus* strains. These included assays targeting the virulence correlated gene, *vcgC*, 16S rRNA and *pilF*. When screened against a wide library of pathogenic and non-pathogenic *V. vulnificus* strains encompassing biotypes 1, 2 and 3, we found that the *pilF* and *vcgC* assays were extremely accurate in correctly identifying pathogenic biotype 1 *V. vulnificus* strains (>97% accuracy). 16S-based real-time PCR assays were substantially less accurate (~ 70%). All real-time PCR assays demonstrated that it did not amplify any distantly related bacteria, or closely related non-pathogenic. Significantly, many human infections caused by *V. vulnificus* are attributed to non-biotype 1 strains, such as serovar E biotype 2 and biotype 3 isolates. Of the three analyzed real-time PCR assays, the *pilF* method appeared the most robust for identifying these pathogenic strains. We were able to detect as few as 10 genome copies of target per reaction using both the *vcgC* and *pilF* assays, however the 16S rRNA assay was substantially less sensitive with consistent detection achieved only with ca. 100 genome copies of target per reaction. Overall, the *pilF* assay was found to represent the most accurate, reliable and sensitive real-time PCR approach to distinguish pathogenic and non-pathogenic *V. vulnificus* strains,
irrespective of biotype. This tool will enable early detection capability in a range of different applications, such as food processing, regulatory and clinical settings.

References


