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

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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

Aznar, R., W. Ludwig, R.I., Amann, and Schleifer, K.H., 1994. Sequence determination of rRNA genes of pathogenic *Vibrio* species and whole-cell identification of *Vibrio vulnificus* with rRNA-targeted oligonucleotide probes. *Int. J. Syst. Bacteriol.* 44: 330-337.

Baker-Austin, C., A. Gore, J. Oliver, R. Rangdale, and D. N. Lees. 2010b. Rapid in situ detection of virulent *Vibrio vulnificus* strains in shellfish matrices using real-time PCR. *Environ. Micro. Rep.* 2:76–80.

Baker-Austin, C., E. Lemm, R. Rangdale, J. Lowther, R. Onley, C. Amaro J. Oliver, and D. N. Lees. 2010b *pilF* Polymorphism-Based RT-PCR To Distinguish Pathogenic Biotypes of *Vibrio vulnificus*. *In review*.

Nilsson, W.B., R.N. Paranjpye, A. DePaola, and Strom, M.S. 2003. Sequence polymorphism of the 16S rRNA gene of *Vibrio vulnificus* is a possible indicator of strain virulence. *J. Clin. Microbiol*. 41:442–446.

Oliver, J.D. 2006. *Vibrio vulnificus*. In: Biology of Vibrios. F.L.Thompson, B. Austin, and J. Swing. (eds.). Amer. Soc. Microbiol. Press, Washington, D.C. pp. 349-366.

Oliver, J. D. 1989. *Vibrio vulnificus*, p. 569-599. *In* M. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc., New York, N.Y.

Roig, F.J., Sanjuán, E., Llorens, A., Amaro, C. 2010. PilF polymorphism-based PCR to distinguish *Vibrio vulnificus* strains potentially dangerous to public health**.** *Appl. Enviro. Micro.* 76 (5):1328-1333.

Rosche, T. M., Yano, Y. and Oliver, J. D. 2005. A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. *Microbiol. Immunol*. 49:381-389.

Vickery, M.C., W.B. Nilsson, M.S. Strom, J.L. Nordstrom, and Depaola, A. 2007. A real-time PCR assay for the rapid determination of 16S rRNA genotype in *Vibrio vulnificus*. *J. Microbiol. Meth.* 68:376-384.