Development of a direct in-situ extraction procedure coupled to real-time PCR for the detection and enumeration of pathogenic vibrios in shellfish matrices

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Abstract

Since the 1970's, bacteria of the genus *Vibrio* have emerged as important pathogens associated with shellfish consumption, mediated in particular, by two bacterial pathogens, *V. vulnificus* and *V. parahaemolyticus*. Both bacteria are found naturally in temperate and tropical marine and estuarine waters and are serious human pathogens. *V. vulnificus* causes gastroenteritis and wound infections with severe symptoms, and are fatal in approximately 50–60% of cases. *V. parahaemolyticus* is the most prevalent bacterium associated with outbreaks of seafood consumption causing acute gastroenteritis with a global distribution, with an estimated 4500 cases annually in the USA.

Unfortunately, there are significant technical constraints placed on the direct analysis of shellfish matrices for the simultaneous detection and enumeration of bacterial pathogens. Culture-based methods are routinely used for the isolation and enumeration of bacteria from such foodstuffs, but the approaches are often labour intensive, cumbersome, time consuming, and often lack sensitivity. Most studies that detect and enumerate vibrios from shellfish samples require pre-enrichment, and there are concerns regarding this approach for quantitation purposes. A separate major technical obstacle regarding the direct analysis of shellfish matrices is that PCR-based approaches often fail due to the abundance of PCR inhibitors present in shellfish matrices. These issues have limited approaches to adequately identify and enumerate bacterial pathogens directly from such samples, with ramifications from both a regulatory and public health perspective. Here we describe the development and application of a simple, rapid and efficient direct nucleic acid extraction procedure for use on shellfish matrices, targeting the digestive gland. The approach allows the simultaneous extraction of DNA and RNA from shellfish tissues, followed by real-time PCR, and can be horizontally applied for the detection of both bacterial as well as viral nucleic acids. Several advantages to this methodology are evident. Firstly, the method requires no pre-enrichment or culture-based step, significantly speeding up the procedure of detecting and enumerating vibrios in artificially amended shellfish samples. In our hands were able to shuck, gland, DNA extract and PCR analyse samples within 3 hours, which compares favourably with conventional approaches used in regulatory and public health laboratories. These methods have been compared to more traditional approaches used for the detection and semi-quantitation of vibrios in shellfish matrices (colony hybridisation, conventional PCR), with highly promising results. We found that the extraction procedure coupled to real-time PCR was substantially more sensitive than both colony hybridisation and conventional PCR. The findings from this work will be of significance in a range of different applications concerned with vibrios in shellfish, in particular food processing, regulatory and clinical settings.