Detection of viable toxigenic *Vibrio cholerae* and virulent *Shigella* spp. in environmental waters by pit-stop seminested polymerase chain reaction assays

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Abstract

A rapid and sensitive assay was developed for the detection of low numbers of viable *Vibrio cholerae* and *Shigella* spp. cells in environmental and drinking water samples. Water samples were filtered, and the filters were enriched in a non-selective medium. The enrichment cultures were prepared for polymerase chain reactions (PCR) by a rapid and simple DNA extraction procedure consisting of boiling. Seminested PCR, based on specific amplification of the cholera toxin operon of *V. cholerae* and the invasion plasmid antigen gene (*ipaH)* of virulent *Shigella* spp., was performed and the PCR products were visualised by agarose gel electrophoresis. The assay allowed the detection of as few as 1 cfu/100 ml of *V. cholerae* and 8 cfu/100 ml of *Shigella* cells. A comparison of the PCR method and culturing methods by using environmental water samples showed that the PCR method has a higher level of sensitivity than culturing methods. As an application of the PCR detection protocol, environmental water samples were screened for the presence of *V. cholerae* and *Shigella* spp. Positive amplifications resulted from *V. cholerae* and *Shigella* species in environmental samples. The results obtained indicate that the described seminested PCR has the advantage of a rapid turn-around time and fulfils the requirements of sensitivity for use in an environmental laboratory.

Key words: *Vibrio cholerae*, *Shigella*, water-borne pathogens, polymerase chain reaction, environmental waters, drinking water, detection

Introduction

Protection of public health requires the rapid detection of water-borne pathogens which often occur in relatively low levels in environmental waters. Consequently, some form of concentration procedure will usually be the first step in traditional, culture-based methods for pathogen detection (Oyofo and Rollins, 1993; Graczyk et al., 1997). Following concentration of the sample, bacteria retained on the filters can be detected by culturing in or on selective media (Toro et al., 1995; Høi et al., 1998; Cerdà-Cuéllar et al., 2000). Some methods may give reliable results in one single step, but usually additional steps are necessary such as resuscitation to allow detection of sublethally injured cells, selective incubation, and confirmatory tests (Kang and Siragusa,1999; Reissbrodt et al., 2000). The sensitivity and specificity of these methods depend strongly on the number of confirmatory tests performed and such tests may take several days to complete.

Given these drawbacks of culturing techniques, molecular biology methods have emerged as a primary alternative for detecting pathogenic bacteria in water samples. Unlike traditional culturing techniques, these methods are based on the detection of a fraction of the genetic material of the targeted bacteria. By using such techniques, the selectivity and sensitivity problems associated with culture techniques can be overcome. The polymerase chain reaction (PCR) is one such molecular technique. It relies on the *in vitro* amplification of a DNA fragment and due to its simplicity and rapidity, a result is obtained in a short period of time after receiving the sample. Various detection protocols have thus been developed based on cell filtration and PCR amplification of target sequences (Bej et al., 1991; Juck et al., 1996). A disadvantage of these direct detection assays is that inhibitors may hamper the PCR reaction. Such inhibition phenomena have often been described for different types of matrices, including water concentrates (Kreader, 1996; Wilson, 1997). In addition, it is not possible to assess the viability of the detected bacteria (Josephson et al., 1993). Yet, the viability concept is fundamental for interpreting the results in terms of public health when dealing with water samples. The PCR technique must consequently be associated with a viability test.

South Africa has recently experienced outbreaks of *V. cholerae* and *S. dysenteriae* that have resulted in 288 and 13 fatalities, respectively (Pegram et al., 1998; Dept. of Provincial and Local Government, 2001). Both these pathogenic bacteria are usually transmitted to humans by ingestion of contaminated water and foods. The genus *Shigella* is composed of four species, *Shigella dysenteriae*, *S. boydii*, *S. sonnei* and *S. flexneri*. The infective dose of *Shigella* spp. is very low, varying from 10^1 to 10^4 organisms (Rowe and Gross, 1984). Virulent *Shigella* organisms cause bacillary dysentery (shigellosis) which may lead to death in some cases if effective intervention strategies are not used. Toxigenic *V. cholerae* is responsible for causing cholera, a highly epidemic diarrhoeal disease which continues to devastate many developing countries where socio-economic conditions are poor, sanitary systems and public hygiene are rudimentary, and safe drinking water is not available. The ingestion of approximately 10^4 to 10^6 *V. cholerae* O1 organisms is likely to produce clinical cholera (Cash et al., 1974). The detection of these microbial contaminants in drinking water supplies and source waters should thus be viewed as a high priority.

In this paper, protocols have been developed for the detection of viable *Shigella* spp. and toxigenic *Vibrio cholerae*. The detection protocol described here, consists of a non-selective enrich-

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