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DEVELOPMENT OF MOLECULAR AND ENZIMATIC KITS FOR THE DETECTION OF TOTAL COLIFORMS AND *ESCHERICHIA COLI* IN WATER SAMPLES

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KEYWORDS

Total coliforms, Faecal coliforms, *Escherichia coli*, Water quality, Detection, Enzymatic methods, Molecular tools.

ABSTRACT

The drinking water is one of the main sources of infectious diseases. It is of major importance to keep a good water quality monitoring. The need for more rapid, sensitive and specific tests is essential; not only for water industry, but for a better public safety. Therefore, detection of microbial indicators of potential pathogens in water is the solution to the prevention and recognition of problems related to human health and safety. The main purpose of this work is to develop commercial kits for the detection of the extensively used as indicator organisms: *Escherichia coli* and total coliforms. An enzymatic method of detection of these microorganisms is being developed based on the enzymes β-D-glucuronidase and β-D-galactosidase, respectively. The results are visible in 18 h for 1 CFU. In order to achieve a higher level of sensitivity and specificity, molecular detection using the Polymerase Chain Reaction (PCR) technique is being investigated. Three primers were selected for identification of total coliforms, *E. coli* and *E. coli* with other enteric pathogens. At this moment, we achieved a sensitivity level of 676 CFU in 8 h, which is already a good achievement but there is still more research to be done in order to accomplish the 1 CFU detection.

INTRODUCTION

Outbreaks of waterborne diseases remain a major challenge to public health providers causing diseases, claiming millions of lives annually, worldwide.

Faecal coliforms (particularly *E. coli*) and total coliforms have been widely used as an indicator of the microbiological quality of drinking water, as well as in surface and ground water, seawater and recreational water. This group of bacteria is commonly found in the gastrointestinal tracts of humans and all warm-blooded animals. Human sources of faecal pollution can contain human pathogens such as Salmonella spp., Shigella spp., pathogenic *E. coli*, and enteroviruses (McLellan 2004). Agricultural animals can also serve as a vector for important pathogens including *Crysptosporidium parvum* and *E. coli* O157:H7(McLellan 2004). These can give origin to a series of diseases such as Typhoid Fever (*Salmonella typhi*), Cholera (*Vibrio cholerae*), Hepatitis, Gastroenteritis, and others (World Health Organization 2004)

E. coli is widely accepted as a faecal indicator bacterium (Khan et al. 2007) since it is an enteric organism but not normally pathogenic, it is easy to detect and culture, it is found at concentrations much higher than other pathogens in surface waters and its persistence in water and in the extent to which is removed by water treatment is similar to that of waterborne pathogens (Ahmed et al. 2005). Total coliforms include organisms that are found normally in nature, with the ability to survive and grow in water. Therefore, are not useful as an indicator of faecal pathogens, but may be used as an indicator of the efficacy of treatment and to evaluate the cleanliness and integrity of the systems of distribution as well as the potential presence of biofilms (Stevens 2003).

The aim of this research is, then, to design commercial kits (enzymatic and molecular), at request and in collaboration with a Portuguese company, with the ability to detect *E. coli* and total coliforms in water samples. In order to be competitive in the market, these kits should be distinguished for being fast to obtain the results, of simple handling, with low cost, high efficiency and sensitivity (1 CFU). In all kits to be developed, it will be explored the qualitative and quantitative possibilities of detection.

METHODS

The kits to be developed based on the enzymatic method have advantage over conventional methods (Multiple-Tube Fermentarion, Membrane Filtration) due to short time response (18 h to 48 h), very simple handling and of relative low cost. Therefore, these kits are based on the detection of the enzymes β-galactosidase, which catalyses the hydrolysis of a chromogenic substrate into a yellow coloured product indicating the presence of total coliforms; and β-glucuronidase (specific for *E. coli*), which catalysis the hydrolysis of a fluorogenic

substrate into a blue fluorescent product, indicating the presence of faecal coliforms and, consequently, the possible presence of pathogenic organisms.
A research is also being done regarding molecular methods, using PCR and its variants (Multiplex PCR, Real-Time PCR) as a possible detection method. Until now, the primers have been chosen and optimization of the DNA extraction procedure was studied. Further investigation on molecular methods is to be done. The challenge is developing a protocol of simple steps and enabling faster results than the existing in order to be competitive and to allow a timely action by those in charge of monitoring water quality.

In both methods (enzymatic and molecular), it will be investigated the hypothesis of conciliate the qualitative detection with the possibility of quantification, as well as its application and adaptability to environmental samples.

RESULTS

This is a work in progress but, until now, it was possible to obtain an enzymatic method of detection, with successful results of 1 CFU in 18h, with optimal applicability to environmental samples. Using PCR as a method of detection, we achieved a sensitivity of 676 CFU in 8 h. Even being satisfactory, further work is being done to improve these results since we aim for sensitivity as close as possible of 1 CFU. These results are for qualitative identification of the microorganisms, quantification will be included on the next steps of this research.

Molecular technology represents a turning point for the future when introduced in routine analysis of microbiological quality of waters. The availability of methods for effective treatment and detection of pathogens in environmental samples would reduce the incidence of human illnesses by preventing the transmission of bacterial pathogens to humans, saving millions in health-related medical costs.

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DIANA MENDES was born in Penafiel, Portugal, and went to the University of Minho (Braga), where she studied Biological Engineering and obtained her degree in 2008. She worked in 2009 on the “Network of Food Innovation North of Portugal – Galicia”, before beginning her PhD research work, where she develops the present investigation.