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detection and quantification

COMPILERS
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Preface

Nowadays molecular methods use in the microbiological diagnosis is booming because they allow microorganisms detection in less time with greater sensitivity and specificity; within these, the top technique is the polymerase chain reaction (PCR), that allows to generate multiple copies of a target DNA fragment and by this way to be able to detect it. The PCR has many variants, each of one of them have specific applications, the most used variant is the real-time PCR, which as its name implies, enables the detection of each DNA copy generated at the time, allowing it to be applied in the quantification of gene copies and its expression with a greater sensitivity.

Although traditional microbiological methods continue to be the reference, the use of the PCR technique in various areas such as clinical, agricultural, veterinary, food and environment has been implemented to detect microorganisms of interest, many of them pathogenic to humans, plants and animals. In environment and health study fields, this methodology has been applied, mainly around water quality investigation, leaving aside the opportunity to use it as a routine method, once validated could be used in surveillance and water quality control, whether it be drinking water, recreational water, sea water, water used in agriculture, among others. The advantage offered by the use of real-time PCR is that it allows quantitative data to be released and thus to know the state and evolution of the microbiological water quality with a high sensitivity, since many waterborne pathogens are diluted in high volumes and therefore making difficult its detection.

Many microbiologists, analysts and laboratory technicians find it very difficult to implement these methodologies in their laboratories, believing that they are still novel methods and require a high level of expertise for their implementation, even though many commercial kits are available in the market. Consequently, this book has been written, from the results of the project "Implementation and standardization of qPCR technique for public health importance bacterial pathogenic microorganisms detection as water quality bioindicators", funded by the National Regalias Fund of Colombia. The main aim of the project was the implementation of real time PCR for the water microbiological diagnosis using as model microorganisms *Escherichia coli* and *Salmonella* spp. The former bacterium was used for the development of a quantitative type method and the latter for a qualitative type method.

The present book is categorized as a research project result book and is divided into six chapters, the first is a brief problem introduction and the justification; showing a panorama about the use of the PCR technique in the water microbiological diagnosis; the second chapter corresponds to the theoretical framework and the state of the art and it is exposed initially the theoretical basis of the processes involved in the standardization as the methods of bacterial concentration, DNA extraction and PCR in real time and finally a description of the PCR implementation in the microbiological diagnosis. The third chapter presents the developed methodology for *Salmonella* spp. and *Escherichia coli* techniques standardization. The fourth chapter presents the results obtained from the tests performed according to the described methodology. In the fifth chapter the discussion and conclusion of the results are carried out. Finally, an additional chapter is presented, which compiles the experiences of a guest author on the PCR standardization applied to microbiological diagnosis.

The methodology and results are organized in three sections: the first describes the standardization process of concentration and bacterial elution for *Escherichia coli* DNA extraction from drinking water and sea water samples. In the second, the processes for the elaboration of the standard curve for *Escherichia coli* quantification by real-time PCR and in the third the method standardization for *Salmonella* spp. detection by real-time PCR in drinking water and sea water. At this point it should be clear out that the selected matrices are justified by their impact on public health and environmental quality, being of direct consumption in the drinking water case and for recreational purposes and environmental impact in the sea water case.

The presentation of the book is done in a simple way so that the reader understands the developed methodology and the obtained results. Presented methods can be easily repeated in other laboratories or used to compare the obtained results in order to select the most appropriate strategies for the development of the real-time PCR technique. The readers might be students, teachers, researchers from exact and natural sciences and medicine fields, working on the topic of water microbiological quality or bacterial pathogens molecular diagnosis.

The authors' view is that this book can be very useful for those who work in the biological sciences field, but especially for those who wish to implement the PCR technique in their laboratory, either for research purposes or for the purposes of control and diagnosis. Thus, it is expected that molecular biology techniques, especially PCR, will be considered in a near future a useful tool for routine use in microbiological quality control.