Monitoring the water through molecular techniques

Marina Carrasco-Acosta[1], José Alberto Herrera-Melián[2], Rafael Robaina Romero[1] & Pilar García-Jiménez[1]*

[1] Department of Biology. Faculty of Marine Sciences. University of Las Palmas de Gran Canaria.
[2] Department of Chemistry. Faculty of Marine Sciences. University of Las Palmas de Gran Canaria.
* pilar.garcia@ulpgc.es

ABSTRACT

This research proposes the establishment of new methodologies based on the genome of microorganisms to control quality water. Monitoring of quality of water is mainly carried out through biochemical techniques with a consuming-time cost. The development of molecular techniques and specific probes to determine microorganisms has allowed a breakthrough in the validation of water quality not only for the technique sensitivity but also for its precision. In this work two attempts were carried out i) Design of specific molecular probes to identify *FIB* (*Fecal Indicator Bacteria*) in seawater and sewage; ii) Molecular characterization of structure of the bacterial communities from a wetland of bioremediation. The specific probes were designed based on the 16S rRNA of main bacterial groups, included in the current legislation: *Escherichia coli*, *Enterococcus intestinalis*, *Salmonella spp.* and *Legionella spp.* The genus *Shigella spp.* and *Citrobacter spp.* were also determined by their correlation with health conditions. The amplification of fragments was carried out by conventional PCR. The specific sequences obtained were phylogenetically analysed and were used to fine-tuning the *fingerprint* method T-RFLP. This method has been employed to determine the effectiveness of diverse wastewater treatments from wetland bioremediation of Tafira University Campus (ULPGC). We conclude that the results represent an advance in the assessment of water quality of coastal environments as our islands, being the basis for future work on the optimization of the time-cost of early diagnosis and also to determine the effectiveness of natural treatment of wastewater and the reused water quality from wetland bioremediation.

INTRODUCTION

There is a close relationship between the quality of recreational water and human health. The origin of this risk for public health comes to the confluence of numerous anthropogenic uses and natural process that occurs in coastal zones. Some examples are rain runoff, urban runoff [1], farm runoff [2], wastewater [3]; and pollution caused by the bather himself, including the corresponding excreta [4]. The nature of pollution inputs in marine media can involve a variety of pollutants into recreational beaches, often including bacterial pathogens and indicators of fecal contamination [5]. Ignorance of the presence of fecal contamination in recreational water could suppose a particular health risk to small children, potentially immune compromised persons and short-term visitors who may be susceptible to endemic bacteria strains [4], important factor to consider in tourist areas, as ours islands.

Approved traditional methods of *FIB* detection include different specific media and incubation conditions. These methods have limitations, such as duration of incubation, antagonistic organism interference, lack of specificity and poor detection of slow-growing or viable but non-cultivable microorganisms [5]. Methods based on the amplification of organism-specific nucleic acids are faster than culturing methods and yield high precision and sensitivity to reliably and simultaneously detect waterborne pathogens [6]. The implementation of molecular techniques is important since it has been shown that concentrations of *FIB* can change substantially over a time scale of hours [7]. Meanwhile through conventional culture methods beaches can remain open during the processing of samples in the laboratory, with the collateral effect of failing to report on time to the beachgoers [8].

The aim of the present work was to design specific probes in order to determine bacterial bioindicators, included in the current legislation: *Escherichia coli*, *Enterococcus* intestinalis, *Salmonella spp.* and *Legionella spp.* in seawater and sewage. The genus *Shigella spp.* and *Citrobacter spp.* were also determined by their correlation with health conditions. All probes recognized 16s rRNA region, widely used as molecular chronometer for identification of prokaryotes. The specie/specific probes from *E.coli* and *Enterococcus intestinalis* also have been used to develop the *fingerprint* method T-RFLP to determine the effectiveness of diverse wastewater treatments from wetland bioremediation of Tafira University Campus (ULPGC).

MATERIAL & METHODS

Sampling. 2L of two types of matrix were sampled namely seawater and wastewater. All wastewater samples (influent and effluent) were collected from wetland bioremediation of Tafira University Campus (ULPGC), sampling in three different periods between December2014-April2015. Seawater samples were collected according the method of reference described in RD 1341/2007.

Recovery of DNA. Two methods were used to recover DNA from bacterial cells. The membrane filter (MF) technique was employed to concentrate the samples in volumes of 100mL. Half of filters were individually crushed with liquid nitrogen and stored at -20ºC until DNA extraction. The other half part of filters were cultured in specific media. Moreover DNA was extracted from filters using modifications from LiOAc protocol described by Lõoke [9]. The integrity of DNA was checked by

electrophoresis on 0,8% agarose gel with Ethidium Bromide in 1× Tris-acetate-EDTA (TAE) buffer.
Primer design. Specific molecular probes to identify *FIB* and pathogenic bacteria were designed using the 16S rRNA gene as a marker. A software of multiple alignment, (CLUSTALW), was enable the location of the most conserved regions.
PCR amplification. PCR amplification was carried out using 50µl as a total volume, for each sample, containing 0.5U *TaKaRa Ex Taq* DNA polymerase (TaKaRa Shuzo Co., Shiga, Japan), 2.5mM of each dNTP, 10µL of *Takara Ex Taq PCR buffer* w/$MgCL_2$, 10pmol of each probe and 20–30ng of template. Template DNAs were initially denatured at 96°C for 1 to 4 min. Then a total of 40 PCR cycles were run under the following conditions: denaturation at 94°C for 1 min, primer annealing at 45ºC for 1 min, DNA extension at 72°C for 5 min. Fragments were sequenced (Sistemas Genómicos, Valencia, Spain) and phylogenetically analysed.
T-RFLP analysis. The evolution of the bacterial community structure from wetland bioremediation was carried out with T-RFLP. The specific primer for *E.coli* and *Enterococcus intestinales* were 5′-end FAM-labelled and 5'-end HEX-labelled, respectively. The PCR products were purified using Wizard SV Gel and PCR Clean-Up kit as directed by the supplier (PROMEGA). Purified PCR products (100ng) were digested with the restriction enzyme MseI at 37°C for 3h and subjected to T-RFLP analysis. The precise length of T-RFs was determined by capillary electrophoresis using the Applied Biosystems DNA Sequencer 3130 (Secugen, Madrid, Spain). T-RFLP patterns and quality have being analysed using the GeneMarker (version 1.85).

RESULTS & DISCUSSION

We were obtained specific sequence for all specie/specific probes designed (*E.coli*, *Enterococcus intestinalis*, *Salmonella spp.*, *Legionella spp.*, *Shigella spp.* and *Citrobacter spp.*). Sequencing and phylogenetic analysis confirmed the specificity of these probes.
Currently we are analysing the electropherograms obtained from T-FRLP through (Fig.1.). GeneMarker software, and clearly points difference between structural patterns from the influent and effluent bioremediation wetland.

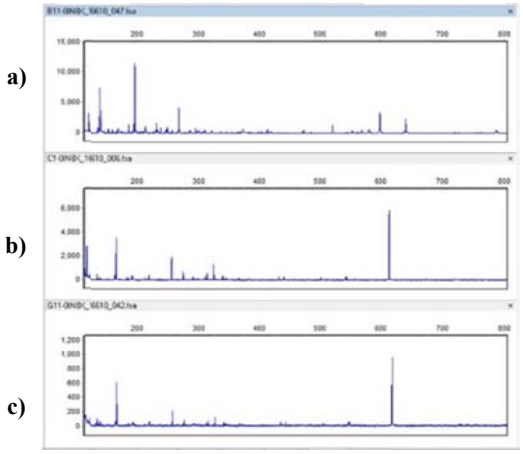

Fig.1. Electopherograms from wetland bioremediation samples; a) Influent; b) Effluent 1; c) Effluent 2.

The results obtained in this work emphasizes the need to develop molecular techniques for specific, sensitive, and especially fast identification of microbial contamination of coastal environments and all those waters that represent pollution inputs to marine environment.

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