USE OF BACTERIOPHAGES AS A TOOL TO DETECT THE ORIGIN OF FAECAL POLLUTION IN WATER SOURCES.

Anicet R. Blanch*, Francisco Lucena, Maite Muniesa and Juan Jofre

Department of Microbiology. School of Biology. University of Barcelona. Barcelona. Spain.
e-mail address * ablanch@ub.edu

ABSTRACT

Water demand has been rising during the last years. Several factors are contributing to the water scarcity. New freshwater resources (harvested rainwater, reclaimed wastewaters and desalinated seawater) are considered to supply the increasing demands. Some of these water resources and many of the traditional ones frequently are polluted with faecal contaminants from different sources (human or animal), which determine the health risks when used. Determining the origin of faecal contamination sources in water is necessary for evaluation of health risks. A predictive model based on the enumeration of two groups of bacteriophages for the identification of faecal sources in waters is presented.

INTRODUCTION

Climate change, the intensive population growth, the non-optimized agriculture watering practices and the growth of cities and urban areas to unprecedented sizes are among the most important factors rising demands for water and causing this scarcity. The recycling of treated urban wastewater, rainwater harvesting and desalination of seawater are emerging as reliable contributions to water demands. However, a primary concern lies in the risk for human health of the faecal pollution related to traditional and alternative water resources. Faecal pollution comes from different sources: municipal sewage, slaughterhouse wastewaters, manure and biosolid disposal, wildlife and undetermined runoff. Determining the source of faecal contamination in waters is essential for estimating the health risks associated with pollution, facilitating measures to remediate polluted waterways, and resolving legal responsibilities. Microbial source tracking (MST) denotes procedures using different microbial indicators to establish the origin of faecal pollution in water. Recently MST predictive models -based on combinations of the most suitable and the lowest numbers of indicators- have been defined. A feasible MST model is based on the combined use of the enumeration of somatic coliphages and the enumeration of *Bacteroides*-phages using different host specific strains (one from humans and others from different animal species). The feasibility of this model based on the enumeration of both bacteriophages groups is presented and analysed.

THE DEVELOPMENT OF MST MODELS

Several statistical and inductive learning methods allowed defining predictive models using combinations of microbial or chemical indicators that provided different degrees of correct classification (Blanch et al., 2006). The best models are based on combinations of parameters involving at least one discriminating indicator and one universal faecal indicator. The universal (non-discriminating) faecal indicator provides quantitative information regarding the faecal load. The discriminating indicators contribute to the identification of a specific source. The optimum predictive model consisted of the pair of variables (ratio of numbers of somatic coliphages and phages infecting *Bact. thetaiotaomicron* GA17 strain, and numbers of somatic coliphages). This model allowed successful classification of faecal source in all cases tested. Alternative predictive models were also found, but they showed lower rates of correct classification and/or required more parameters.

MST PREDICTIVE MODEL BASED ON BACTERIOPHAGES

Somatic coliphages and bacteriophages infecting *Bacteroides* spp. are non-pathogenic, easily and quickly detectable, and quantifiable. These characteristics make them highly suitable for tracking faecal sources. Furthermore, standardized methods for detecting and enumerating both already exist. However, several questions related to their concentration in faecal sources, their persistence in the environment and resistance to inactivating treatments, the worldwide applicability, the host specificity and the potential use of their detection by molecular tools should be addressed.

Abundance in faecal sources. Both bacteriophages groups are sufficiently abundant in different water matrices in order to be detected even under high level of dilution. Values for between these phages maintain the same proportion in different water matrices with human faecal pollution and their relationship to faecal coliform. However, values of bacteriophages infecting *Bacteroides* spp in non-human faecal wastes are either zero or very low. No seasonal or year-to-year variations on concentration are detected in either of both groups of phages (Payán, 2006).

Persistence in the environment and resistance to inactivating treatments. Somatic coliphages and bacteriophages infecting strains of *Bacteroides spp.* persist reasonably and similarly in the water environment (wastewater, river water and seawater). Both persist much longer than faecal coliforms. They are found in the same proportions in river and sea water as in raw and treated sewage and tertiary treatments (Mocé et al., 2005; Payán, 2006).

Suitable *Bacteroides* hosts for different geographical areas. Different strains of *Bacteroides* spp. host strains could be easily isolated by a simple established method in order to obtain the most appropriate strain to enumerated bacteriophages associated to human or animal species faecal sources in each geographical area (Payán et al., 2005; Ebdon et al., 2007).

Suitable *Bacteroides* hosts for tracking non-human hosts. It is possible to find *Bacteroides* hosts that can differentiate the faecal sources of different animal species with a reasonable effort.

Molecular methods for the quantitative detection of specific phages in predictive models. Molecular methods based on genomic differentiation of both groups of bacteriophages could be expected in the future such as DNA microarrays or specific quantitative PCR analyses.

CONCLUSIONS

The use of the enumeration of somatic coliphages (non-discriminating parameter) and its ratio to bacteriophages infecting *Bacteroides* host specific strains emerged as a feasible approach when distinguishing faecal contamination sources in water. Both groups of bacteriophages fulfil the requirements for MST indicators in order to be used on the development of predictive models.

ACKNOWLEDGEMENTS

This study was supported by the Spanish Government, research project CGL2007-65980-C02-01/HID.

REFERENCES

Blanch et al. (2006). *Applied and Environmental Microbiology*, 72, 5915 - 5926.
Ebdon et al. (2007). *Water Research* 41, 3683 - 3690
Mocé-Llivina et al. (2005). *Applied and Environmental Microbiology*, 71, 6838-6844.
Payán et al. (2005). *Applied and Environmental Microbiology*, 71, 5659-5662.
Payán, A. (2006). PhD Thesis. University of Barcelona.