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Detection of somatic coliphages as indicators of faecal contamination in estuarine waters

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ABSTRACT

The appearance of enteric disease outbreaks associated with the use of waters considered bacteriologically suitable, calls for the search of new and more precise indicators. Samples of estuarine water were collected in order to quantify *E. coli* and *E. coli* ATCC 13706 somatic coliphages and to compare the usefulness of the latter to detect faecal contamination when the concentration of traditional indicators is not quantifiable. Statistical analyses suggested the division of sampling sites into two groups: group I and group II, according to the minor or major level of faecal pollution respectively registered. In group II a high correlation between the coliphages and *E. coli (*r: 0.73 p<0.01) was detected. *E. coli* always exceeded coliphage abundance. In group I, this relationship was statistically significant (r: 0.55 p< 0.05), coliphage counts were higher than those of *E. coli* and were detected in the absence of the latter. In summary, the use of *E. coli* ATCC 13706 somatic coliphages is proposed as a complementary tool for the diagnosis of the level of faecal contamination of estuarine waters, especially in areas of low pollution

Key words: Somatic coliphages, faecal contamination, estuarine water

RESUMEN

Detección de colifagos somáticos como indicadores de contaminación fecal en aguas estuarinas. La aparición de brotes de enfermedades víricas entéricas asociadas al uso de aguas bacteriológicamente aptas impone la búsqueda de nuevos y más precisos indicadores de contaminación. Se recolectaron muestras de agua estuarina, donde se cuantificaron simultáneamente la bacteria *E. coli* y los colifagos somáticos de *E. coli* ATCC 13706, a fin de evaluar la utilidad de estos últimos para detectar contaminación fecal cuando la concentración de los indicadores tradicionales no es cuantificable. Los resultados estadísticos sugirieron la división de las estaciones de muestreo en dos grupos, I y II, de acuerdo con el menor o mayor nivel de contaminación fecal registrado, respectivamente. En el grupo II se detectó una alta correlación entre los recuentos de colifagos y de *E. coli* (r: 0,73 p<0,01). Asimismo, en este grupo la abundancia de *E. coli* siempre superó a la de colifagos. En el grupo I la correlación fue estadísticamente significativa (r: 0,55 p < 0,05), pero de mediana magnitud, los recuentos de colifagos superaron a los de *E. coli*, y éstos fueron detectados aun en ausencia de la bacteria. En conclusión, los colifagos somáticos de *E. coli* ATCC 13706 serían una herramienta accesoria en el diagnóstico del nivel de contaminación fecal de aguas estuarinas, sobre todo en áreas donde ésta es baja.

Palabras clave: colifagos somáticos, contaminación fecal, aguas estuarinas

To ensure the microbiological quality of water that humans use for different purposes, constitutes a major challenge for those who work in environmental microbiology. Bacterial indicators (total and faecal coliforms, enterococci and *E. coli*) have historically been used to evaluate faecal pollution. However, some obstacles have appeared since the report of enteric disease cases caused by the use of water where bacterial indicators had not been detected. On the other hand, in the last years it has also been confirmed that these bacteria do not provide suitable information about the presence of enteric viruses in aquatic environments (3), particularly regarding their destiny in water and resistance to ecosystematic factors (7). The persistence of human enteric viruses in drinkable, irrigational or recreational waters is a serious risk for public health. Due to the fact that the direct search of enterovirus through cell culture is still expensive, time-consuming and not always reliable (8) and that specific detection of pathogenic viruses is not adapted to routine analysis (11), the use of faecal bacteriophages –mainly somatic coliphages, RNA F-specific phages and *Bacteroides fragilis* phages– have been proposed as alternative indicators of the presence of enteric viruses (4, 7).

The use of coliphages as indicators of faecal contamination is advantageous for their constant presence in water with this kind of pollution; also because they do not proliferate outside the host, they are easily enumerated by simple methods –with results in few hours– and principally because they offer more resistance and persistence than conventional indicators (2, 3, 9, 10).

The aim of this work was to evaluate the usefulness of *E. coli* ATCC 13706 somatic coliphages to detect faecal

pollution, mainly when the concentration of the traditional indicators cannot be quantified.

Six water sampling sites were established in Bahía Blanca estuary (Buenos Aires province) which is affected by the dumping of raw sewage from Bahía Blanca city (Figure 1b). Six campaigns were undertaken at the first five sites, and nine at the sixth, due to its easier access. Samples were taken by hand at 30 cm depth. While being transported to the laboratory they were maintained at 4° C and then analyzed within 6 h. In each sample *E. coli* was quantified on M-FC medium (membrane Fecal Coliform media) after membrane filtration according to standard methods (1). Somatic coliphages were quantified using the bacterial host *E. coli* ATCC 13706 according to standard methods (1, 13). *E. coli* ATCC 13706 (known as WG4), was selected due to its wide use as host strain for the detection of bacteriophages in aquatic environments (6), showing the highest efficiency for coliphage detection by plaque assays (5). After a logarithmic transformation, the results were statistically analyzed by Least Significant Difference (LSD) and the correlation coefficient (r) (12) was calculated.

In order to know the spatial distribution of *E. coli* and coliphages in Bahía Blanca estuary waters a two-way ANOVA without replies was applied. The sampling sites means were compared in pairs using LSD.

The statistical results with LSD suggested the division of the sampling sites into two groups, on the basis of their faecal contamination levels: Group I (low mean abundance ≤ 250 CFU or PFU/100 ml, sites 1, 5 and 6) and Group II (high mean abundance > 250 CFU or PFU/100 ml, sites 2, 3 and 4) (Figure 1a). This division coincided with the distance between the sampling sites and the wastewater discharge (site 4, Figure 1b).

The relationship between *E. coli* and coliphages within each group was analyzed using the correlation coefficient r. In Group I, this relationship was statistically significant ($r= 0.55$, $p < 0.05$). Coliphages counts always exceeded the *E. coli* ones and were detected even in the absence of these bacteria (Figure 2).

In Group II, a high correlation degree ($r= 0.73$, $p< 0.01$) was detected among coliphages and *E. coli* counts. In this Group, the bacteria always exceeded coliphage densities (Figure 3).

Based on these findings, it seems that *E. coli* ATCC 13706 somatic coliphages could become a promising tool to help in the diagnosis of faecal contamination level in estuarine waters, mostly in areas with low contamination. Furthermore, they appear to be capable of giving extra information, allowing to track pollution further (Figure 1).

Moreover, it must be considered that the detection methods are fast (results in 6 h) and cheap. Finally, their search might give an idea of the human pathogenic virus status in marine waters, since –according to Skraber et al (11)– a relationship seems to exist between the concentration of these indicators and viral contamination in water.

However, additional data on coliphage counts around the country are required before serious consideration can be given to their acceptance as indicators of faecal pollution in estuarine waters.

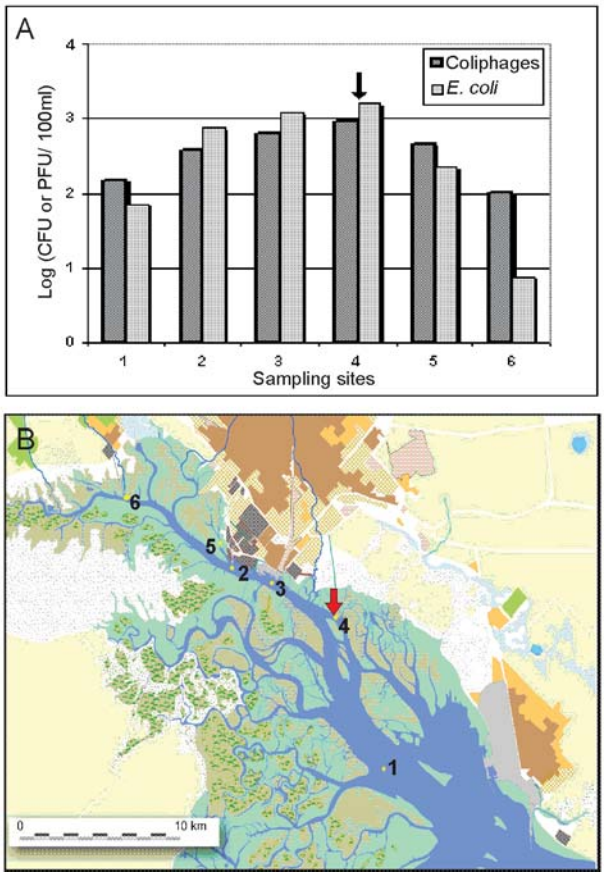

Figure 1. a) Mean abundance of the indicators used, for each one of the sample stations in Bahía Blanca estuary. **b)** The numbers indicate sampling sites and the arrow, sewage input of Bahía Blanca City.

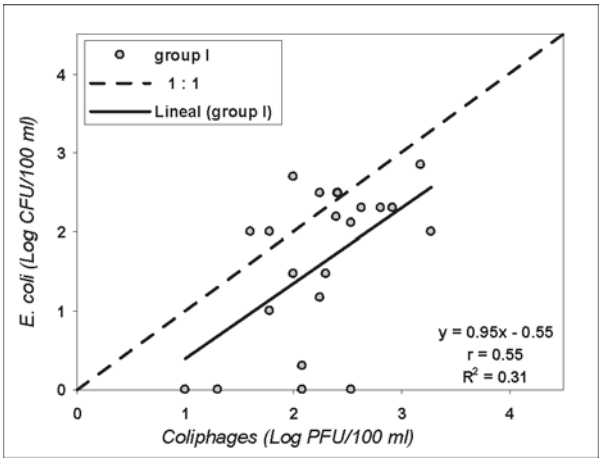

Figure 2. Correlation between *E. coli* and coliphages in sampling sites with low level of faecal pollution (1, 5 and 6) group I. n= 21.

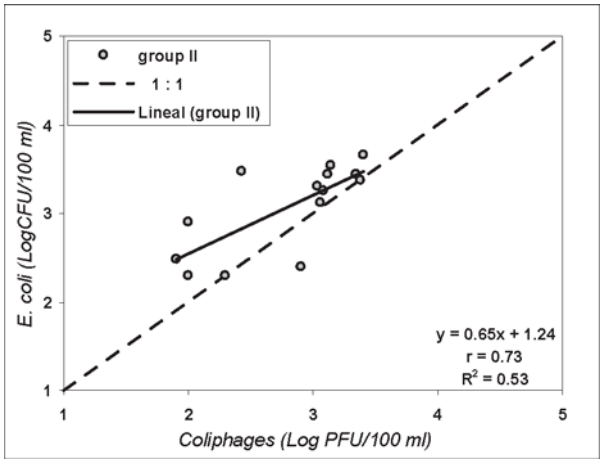

Figure 3. Correlation between *E. coli* and coliphages in sampling sites with high level of faecal pollution (2, 3 and 4) group II, n= 18.

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