Comparative survival studies of several bacteriophage groups in littoral seawater

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The amount of domestic sewage discharged into marine environment is increasing daily. These discharges produce physico-chemical and ecological disturbances, the importance and extent of which depend on several factors, such as: intensity and frequency of the discharges; dilution capability of the receiving marine ecosystem; and self-depurating processes in the sea. However, the most serious consequence derived from sewage discharges is related to the health-hazard associated with the recreational use of the contaminated-marine seawaters. There is considerable evidence suggesting the short-lived viability of most enteric pathogens outside the intestinal tract, except for the enteric viruses.

Several authors have suggested the use of bacteriophages as enteric viral pollution indicators, because of their similar behaviour and survival in the aquatic environment (SIMKOVA & CERVENKA, 1981; STETLER, 1984; BORREGO et al.,1990). However, the great variety of bacteriophage groups is an important shortcoming to the general use of the phages as viral indicators. Therefore, a comparative study on the survival capabilities of different bacteriophage groups is needed to establish their real validity as viral indicators.

Five bacteriophage groups (coliphages C, coliphages K12, F-specific coliphages, somatic *Salmonella* phages and phages active against *Bacteroides fragilis*) were comparatively studied on the basis of their survival and dispersion in littoral seawater. In all the cases, the double agar layer technique was performed for the phage assays, using as host bacteria the following: *Escherichia coli* C (ATCC 13706) for coliphages C; *E. coli* K12 Hfr (PC0008) for coliphages K12; *Salmonella typhimurium* WG49 for F-specific phages; *S. typhimurium* WG45 for somatic *Salmonella* phages and *B. fragilis* HSP40 for *Bacteroides* phages. Phage methodologies used in this study were those described by CORNAX *et al.* (1991).

Survival studies were conducted applying the techniques described by BORREGO *et al.* (1990) and CORNAX *et al.* (1991), and the time of residence or distance of dispersion were calculated according to the expression :

$$C = Co \times 10^{-x/x90}$$

X being the time or distance, and X90 the characteristic value of time or distance in which is achieved the 90% of disappearance.

Comparative survival characteristics of the different phage groups, measured both in time of residence and distance of dispersion in the seawater are given in Table 1.

All the bacteriophage groups showed higher survival capabilities than coliforms and generally a higher dispersion too. Inactivation rates of bacteriophages in the marine environment were similar to those of fecal streptococci. However, in laboratory conditions the bacteria are affected at high degree by seawater. This finding may be explained by the fact that in laboratory conditions the effects of adsorption, precipitation and sunlight are omitted, being these the main factors involved to the virucidal effects of seawater (BITTON, 1980; KAPUSCINSKI & MITCHELL, 1983). On the contrary, F-specilc phages possessed the lowest survival on laboratory conditions (5.7 h), which is in contradiction with the results obtained by several authors (AYRES, 1977; YATES *et al.*, 1985; HAVELAAR, 1987).

In conclusion, on the basis of the higher survival in marine environment, coliphages seem to be appropriate indicators of both, virus and bacterial pollution of this ecosystem.

Table 1. Comparative survival capabilities of different phage groups and indicator microorganisms in seawater.

	D90	T90 (h)		
	(M)	Estuary	Open sea	Lab. Conditions
Total coliforms	215	0.73	0.26	3.7
Fecal coliforms	241	0.83	0.24	3.6
Fecal streptococci	346	0.69	0.34	8.0
Coliphages C	293	0.79	0.33	>15
Coliphages K12	284	NT	0.38	>15
F-specific phages	322	0.84	0.33	5.7
Salmonella somatic phages	206	0.70	NT	9.6
Bacteroides phages	170	0.67	NT	7.3

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