

Comparative survival of fecal indicators in seawater

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Introduction

The high inactivation rates in the marine environment of total and fecal coliforms are an important shortcoming for the use of these microorganisms as indicator of remote and viral pollution (1). Other microorganisms such as fecal streptococci and several groups of bacteriophages (Coliphages, F-specific phages and *Bacteroides fragilis* phages) have been proposed as alternative fecal indicators because of their higher survival capacity in sea water.

The objective of this study was to evaluate the effect of marine water upon the stability of several indicators microorganisms under laboratory conditions.

Material and Methods

To study the comparative survival of bacteria and bacteriophages an Erlenmeyer flask with 900 ml of unpolluted seawater was inoculated with 100 ml of sewage. The mixture was incubated in the dark at 18°C for up to 15 days. Bacterial and bacteriophages survivors were enumerated at time 0 and subsequent days (3, 7, 10 and 15 days) using the following growth media and assay techniques.

Bacterial counts:

Total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) were enumerated by spread technique using m-Endo agar (Difco Lab. Detroit Mich.), m-FC agar (DIFCO) and m-Enterococcus agar (Difco) plates. When there was a low concentration of bacteria, membrane filtration procedure (2) was used for the analysis.

Phages count:

Specific phages of the strains *Escherichia coli* C (CP) (ATCC 13706) and *E. coli* K12 Hfr (K12P) (PC0008) were enumerated by the double agar layer method (3). Selective counts of F-specific bacteriophages (FSP) were obtained using *Salmonella typhimurium* WG49 as bacterial host (4). To evaluate the possible interference by somatic *Salmonella* phages (SSP) parallel counts were also made using F⁻ strain (*S. typhimurium* WG45). Phages active against *Bacteroides fragilis* HSP 40 (BFP) were enumerated by the soft agar overlay method (5) using the samples decontaminated by membrane filtration (0.45 µm filters previously treated with 3% beef extract at pH 9.5).

Results and Discussion

Figure 1 (A and B) shows the effect of the marine water on the survival of the microorganisms. Fecal and total coliforms showed the highest rate of inactivation in seawater whilst the die-away rate of fecal streptococci was more closely paralleled that of bacteriophages, excepting coliphages that did not present a significant inactivation after the sampled period.

F-specific and *B. fragilis* bacteriophages were the groups of phages that showed the least stability in seawater. Low survival of some members of F-specific phages as f2 have been described by others authors (6).

Because of the lower persistence of coliform bacteria in marine water these microorganisms can be useful indicator of enteric bacterial pathogens but not of virology pollution. Somatic coliphages can be more appropriated indicator microorganisms.

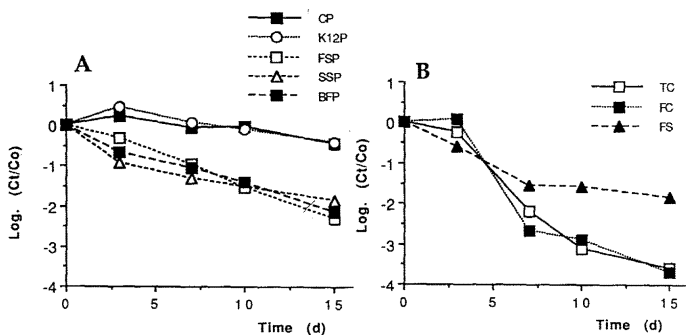


Figure 1 (A and B) : Microbial inactivation in seawater.

References

- FATTAL, B.; R.J. VALS; E. KATZENELSON & H.I. SHUVAL, (1983). *Water Res.* **17**:397-402.
- APHA, AWWA & WPCF (1985). *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.
- HAVELAAR, A.H. & W.M. HOGEBOM. (1983). *Antonie Van Leeuwenhoek J. Microbiol.* **49**:387-397
- HAVELAAR, A.H. & W.M. HOGEBOM (1984). *J. Appl. Bact.* **56**:439-447.
- TARTERA, C & J. JOFRE (1987). *Appl. Envir. Microbiol.* **53**: 1632-1637
- BITTON, G; S.R. FARRAH; R.H. RUSKIN; J. BUTNER & Y.J. CHOU (1983). *Ground Water* **21**:405-410