Characterization of survival stages of enteric bacteria in natural aquatic ecosystems

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Differentiation between culturable and non-culturable (somnicells) enteric bacteria in natural waters have attracted very much attention in last years (Barcina *et al*, 1989; Roszak & Colwell, 1987). Until now, that classification have been studied only for enteric bacteria inoculated in aquatic ecosystems in the absence of natural microbiota (Barcina *et al*, 1989; Roszak & Colwell, 1987), and numbers of CFU (colony-forming-units) on selective culture media were the single parameter estimated when natural microbiota were present (Mc Cambridge & Mc Meekin, 1981; Rhodes & Kator, 1988).



🖀 Culturable cells 🛛 Non-culturable cells (somnicells) 🗖 Death cells

FIGURE 1. Characterization of survival stages of *Escherichia coli* and *Enterococcus* faecalis in freshwater and marine ecosystems when natural microbiota were absent (A) and present (B).

This study was undertaken to differentiate between culturable and non-culturable cells of enteric bacteria inoculated in natural aquatic systems both in the presence and in the absence of natural microbiota. To reach this objective, enteric bacteria were stained with rhodamine isotiocyanate (Landry *et al.* 1987). So, these labelled cells maintained their culturability. Similar counts of CFU were obtained with both the evolution of stained and non-stained enteric bacteria in the water samples. Fractions of culturable, non-culturable and death enteric bacteria were determined throughout the experiences (Figure 1). From these results, the real effect of natural protozoa on the elimination of inoculated enteric bacteria can be estimated from the difference between direct counts of rhodamine stained bacteria in the absence and presence of natural microbiota. An important fraction of somnicells were detected in the presence of natural microbiota (Figure 1) and that lead us to deduce the inadequacy of plate counts on selective culture media to estimate numbers of enteric bacteria, which remain in natural waters.

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Microbiological evaluation of the water quality in a Mussel culture area (Sardinia)

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About 500,000 m^2 of the inner part of the Olbia Gulf is occupied by mussel culture nurseries (Fig.1). The partially treated or untreated domestic and industrial wastes flowing into the area have contributed to lower its already precarious hygienic and sanitary standards (Contu et al., 1988). The harbour of Olbia is also located in this area.

The aim of the present study is to investigate whether the vicinity of an area exposed to high faecal contamination can affect the microbiological quality of the water in the mussel nurseries. The geomorphology of the Gulf could enhance the effect both of the sea currents and of the metereological conditions in transporting contaminants in the direction of the nurseries. This is hazardous to the public health and at the same time calls for a check on the suitability of the nurseries. Seven sampling stations have been located in the Gulf. In order to evaluate the entity of the transport of contaminants in depth, the samples were taken at - 0.5 m and \sim 4 m at each station. Nylon nets containing mussels that had been kept for 48 hours in clean seawater, were placed in each station at the two depths 2 weeks before each sampling. The following indicators of faecal pollution were considered: Total Coliform, Faecal Coliform , Salmonella and Escherichia coli phages in the mussels; Total Coliform, Faecal Coliform and Enterococci in the water. Both mussel and water samples were collected monthly for 14 months starting from May 1986. Moreover the most important streams and effluents from wastewater treatment plants flowing in the Gulf were monitored. The water was analysed according to the Standard Methods for Water and Wastewater (1985), and the mussels according to the Gazzetta Ufficiale DM 24/7/1978.

The bacteriological analyses of the seawater have shown widespread faecal contamination of the whole area throughout the period of observation. The period between May 1986 and February 1987 showed the highest values of indicators of faecal pollution, which was referred to the whole water body. Contamination was highest in the innermost part of the Gulf and in the surface layers, and showed a tendency to decrease in direction of the open sea.

The same trend was confirmed in the mussels , at the surface and in depth.

The results ooint out: 1) a hazard of bacteriological pollution due to the discharge of organic substances and nutrients near the nurseries 2) the usefulness of the analyses carried out in depth 3) that the mussels can be used to show presence of faecal pollution indicators and their transport.



Figure 1. The inner part of the Olbia Gulf (Sardinia)

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