IMMUNOFLUORESCENT PROBES TO STUDY THE DIVERSITY OF MARINE PICOPHYTOPLANKTON COMMUNITIES OF THE MEDITERRANEAN SEA

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Cultural methods may underestimate the abundance of bacteria present in natural samples. Direct counts by epifluorescence microscope utilizing specific DNA probes (fluorochrome as DAPI and AO) are more useful methods to enumerate the marine bacterial populations. The numbers obtained by direct microscopic method exceed plate counts by 2 to 3 orders of magnitude. But this method is not specific since most marine bacterial cells appear similar under epifluorescence microscope.The indirect immunofluorescence (IIF) may be a specific approach to know the species composition of marine microbial communities. We used IIF technique to demonstrate the presence of fecal indicators as *E.coli* (ZACCONE *et al.*, in press) and *Salmonella strains* (MAUGERI *et al.*, 1992a).

The presence of the picophytoplankton in sea water samples - the autofluorescent cells of picoplankton - has been recognized by the epifluorescence microscope. It represents an important fraction of plankton even if its quantity and quality are highly variable. In many regions of the oceans prokaryotic cells (cyanobacteria) outnumber the eukaryotic cells by approximately an order of magnitude. Cyanobacteria at epifluoresecence appear yellow orange, eukaryotic cells red (MAUGERI *et al.*,1992b); *Synechococcus* is the genus more studied fluorescence microscope appear yellow-orange, eukaryotic cells red; the species of this genus can be distinguished by the different prevailing pigment. Different clones contain, phycoerythrin (PE) and phycocyanin (PC) dominant pigments. Autofluorescence has been used successfully to identify and enumerate PE-containing strains. By this method the PC-containing cyanobacteria are not distinguishable. Specific sera labelled with fluorescein isothiocyanate has been proposed to identificate and enumerate marine chroococcoid cyanobacteria (CAMPBELL *et al.*,1983; CAMPBELL,1987). Results of a preliminary survey of the distribution and population density of picophytoplankton in the coastal waters surrounding Messina were presented in a previous paper (MAUGERI *et al.*,1992b).

The fluorochrome DAPI has been used to demonstrate the total number of picoplanktonic cells, the total number of autofluorescing cyanobacteria and two prepared antisera against the cyanobacterial strains from Culture Collection of Marine Phytoplankton, *(Synechococcus* sp. WH 7803,CCMP 1334 and *Synechococcus bacillaris* WH 5701,CCMP 1333, PC-dominant strain and that lacks PE) and the specific abundance of cyanobacterial strains in the surface water samples collected in September 1993 from 18 stations in the Ionian and Tyrrhenian Seas. The used procedures were as described by MAUGERI *et al.* (1992b) and CAMPBELL *et al.* (1983). In the coastal waters of Messina, where urban and industrial wastes are usually dumped, picophytoplanktonic cells ranged between $3.7x10^2$ cells/ml and $2.0\ x10^3$ cells/ml. The abundance of PE-containing (WH 7803) ranged from 4.1x10 cells/ml (stations a, j and m) to $1.3x10^3$ cells/ml(st c), whereas the PC-containing *S. bacillaris* ranged from 1.4x10 (st c and e) to $6.7x10^2$ cells/ml (st b) in the examined samples. Fig. 1 shows the abundance of cyanobacterial cells. Fig. 2 shows the concentration of two cyanobacterial serogroups tested by IIF. The IIF showed only a few cross reactions in *Synechococcus* genera (ZACCONE *et al.*, 1994). These are the first existing data on the distribution of specific serogroups of picophytoplanktonic cyanobacteria in the Mediterranean sea. These data show a correlation with water temperature and emphasize the seasonal studies to define factors that could control or influence the serogroup distribution. The low numbers of strains specific by IFF demonstrate that this method is of limited use in quantifying functional groups of microorganisms but that it provides specific information on the diversity of natural populations and their relation to culturable strains of bacteria and cyanobacteria.

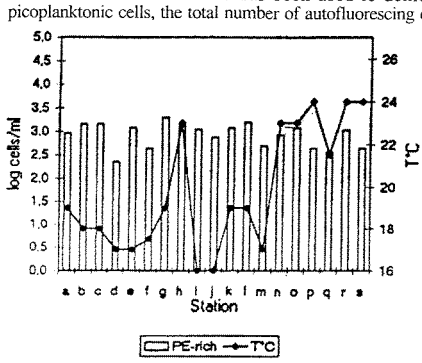

Fig 1.

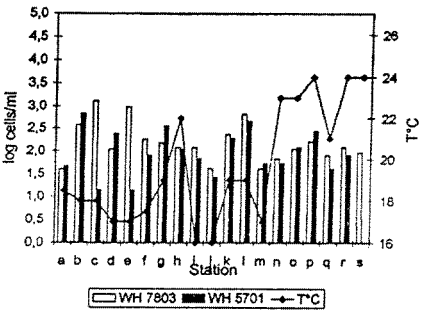

Fig 2.

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