MICROBIAL DIVERSITY IN NORTHERN ADRIATIC SEA : PRELIMINARY OBSERVATIONS

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Marine bacteria often dominate the plankton biomass and are responsible for much of the cycling of organic matter. However, bacterial diversity is poorly understood because conventional identification methods neglect about 99% of the organisms (FUHRMAN *et al.*, 1992). Since 1993 a study of microbial diversity has been carried out in the Gulf of Trieste (Northern Adriatic Sea) using different enumeration and identification media. The experimental data obtained have been compared with epifluorescence estimation of bacterial number. A superficial water sample was collected aseptically in May 1993 in a station 300 m offshore in the Gulf of Trieste; 5 ml of the collected sample was filtered on 0,22 µm polycarbonate black membrane (Nucleopore) and stained with 4'6-diamidino-2-phenylindole (DAPI) (PORTER and FEIG, 1980) in order to detect autotrophic and heterotrophic microorgnaisms; the same amount was filtered on 0,22 µm polycarbonate black membrane (Nucleopore) for SEM observations which was carried out after fixing in OsO_4 freeze drying and gold coating. Serial dilutions of the same sample were inoculated (six replicates) by spreading on twelve different media : with or without natural or artificial sea water, with glucose, cellulose or chitin as carbon source (OKAZAKI and OKAMI, 1976; SCHNEIDER and RHEINHEIMER, 1991). In order to evaluate the antrophic contamination total coliforms were detected too. Results are reported on Table.

marine agar	PCA	PCA	PCA	PYA	PYA	PYS	CELL	CHI	MYS	GG	SC
DIFCO	0 NaCl	sw	asw	sw	asw						
200	300	25	50	10	20	20	10	10^3	0	0	0

Table : viable bacteria (CFU/ml) on different media-PCA (Plate count agar), PYA (Pectone yeast extract agar), PYS (Pectone yeast extract salt solution agar), CELL (Cellulose agar), CHI (Chitine agar), MYS (Maltose yeast extract agar), GG (Glycerol glycine agar), SC (starch caseine agar), sw (seawater), asw (artificiical seawater).

In spite of the absence of coliforms, terrigen contribution was evident, given the hight number of colonies developing on PCA without NaCl and considering that more than half are unable to grow on marine agar. On the other hand, the 75% marine agar growing strains develop in unsalted media. These observation suggest a dominance of bacteria unaffected by NaCl, rather than purely marine bacteria. The geographical position of the Gulf of Trieste with coastal areas densely populated and with inputs of important rivers, as Isonzo and Tinavo, may explain the situation observed (DEL NEGRO *et al.*, 1993). All colonies developed on Chitine agar are Actinomycetes belonging to the genus *Streptomyces*, a common component of the coastal environment (GOODFELLOW and WILLIAMS, 1983). Using epifluorescence technique 5,5 x 10^6 total cells/ml (5,8 x 10^4 autotrophic microorganisms/ml) were detected. Although a discrepance between epifluorescence and cultural counts may reflect the wellknown presence of unculturable mocroorganisms, the difference observed in this study is greater than previously reported results for the area considered (DOLZANI *et al.*, 1989). Nevertheless a longer incubation period (5-6 days) allows the development of several strains, on the higher dilution plates, belonging mainly to the genus *Rhodococcus* and to an unidentified spirillum. The SEM observation revealed a considerable amount of microorganisms of unusual shapes. Some of these have been reported previously as soil microorganisms (NIKITIN, 1973); others to the best of our knowledge, are unknown (Fig. 1 and 2). The cell wall of star shaped microorganism (Fig. 1) was studied by means of X ray microanalysis which identified the presence of silicone, a typical constituent of diatoms thecæ. In any case, the microorganism size may be consistent with a protistic nature.

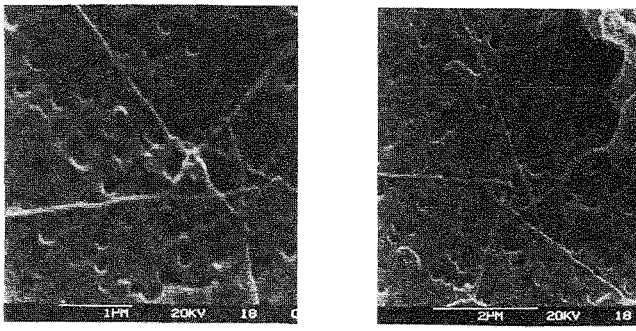

Fig.1 : Phæocystis trichocysts (SEM observation)

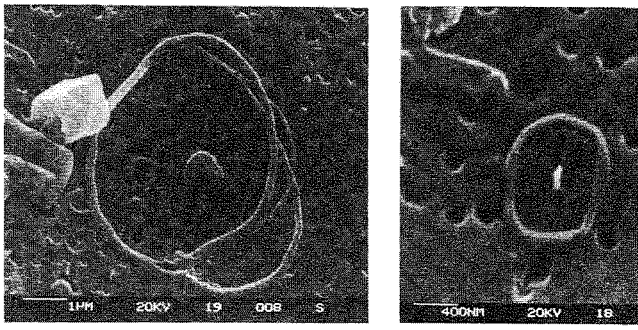

Fig.2 : Unusual shapes (SEM observation)

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