CHARACTERIZATION OF HETEROTROPHIC BACTERIA ISOLATED

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Abstract
Research were carried out to evaluate the viable microbial communities structure in a two restricted areas of the Mediterranean Sea (Southern Tyrrhenian Sea and the northern side of Sicily Channel) in July 2005. Bacterial plate counts in Marine Agar medium were carried out in 202 water samples from 14 Stations of these poorly studied areas of the Mediterranean Sea and 61 bacterial strains were investigated. Isolates were tested by API 20NE system, BIOLOG GP microplates and by classical identification pathways and classified by molecular approach also.
Keywords : Bacteria, Tyrrhenian Sea, Sicilian Channel, Biodiversity.

In the framework of a multi-year investigation on distribution of the microbial communities in Mediterranean surface, intermediate and deep sea waters to compare bacteriological parameters with different water bodies [1-2] we had the opportunity to carry out researches on bacteria strains isolates from water samples taken during CIESM-SUB1 cruise (Fig. 1).

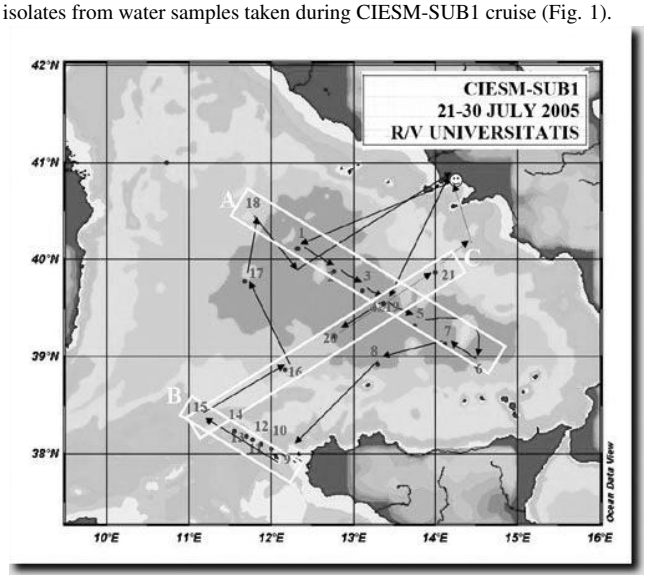

Fig. 1. Sampling area.

This multi-disciplinary oceanographic campaign, started on July 21th 2005 on board the R/V Universitatis, explored the deep waters of little-known areas in the southern Tyrrhenian Sea and the northern Sicily Channel to analyze physical and biological changes induced by the inflow of warmer, saltier waters from the eastern sub-basin.
At each station (St. 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 20 and 21) were collected sea water samples from surface down to 3596 m, according to the physical and chemical survey, by means of Niskin bottles fitted on CTD-Rosette sampling system.
Water samples were immediately sewed, by spread-plate technique, on Marine Agar plates and incubated at 20°C during 8 days; heterotrophic bacteria pure cultures on Marine Agar plates were obtained from colonies random selected, that were characterised in lab using morpho-physiological and taxonomic approaches.
The isolates were characterized using the following tests: gram staining, cell morphology, oxidase, catalase, motility, lipolytic activity on Tween 80, susceptibility to the vibriostatic O/129 (150μg), growth on TCBS medium via the classic methods. The biochemical characterization was carried out using API 20 NE for Gram negative bacterial strains and BIOLOG GP microplates.
Viable colonies on Marine Agar showed highest values (4,9 x 10^3CFU/ml) at 800-1200m layers. From 200 isolated strains, 61 grown on replicates were analysed. From obtained results 87,3% of isolates were gram-negative, 58,1% were rod-shaped, 95,8% were oxidase positive, 63,2% were catalase positive and 64,6% motile.
For molecular analysis [3], bacterial DNA was extracted and 16S rDNA amplified for sequencing to achieve phylogenetic affiliations. Taxonomic results showed presence of 68,9% of gamma- *Proteobacteria* (42,9% *Alteromonas*, 26,2% *Halomonas*, 9,5% *Pseudoalteromonas*, 9% *sea water bacterium*), 16,4 % of *alfa-Proteobacteria* (70% *Erythrobacter,*10% *Methylarcula,* 20% others), 8,2% of *Firmicutes* (60% *Bacillus,* 20% *Oceanobacillus,* 20% others *),* 4,9% of Actinobacteria (66,7% *Micrococcus*, 33,3% *Cellulosimicrobium*) and 1,6% of Bacteroidetes (100% *Salegentibacter*) (Fig. 2).

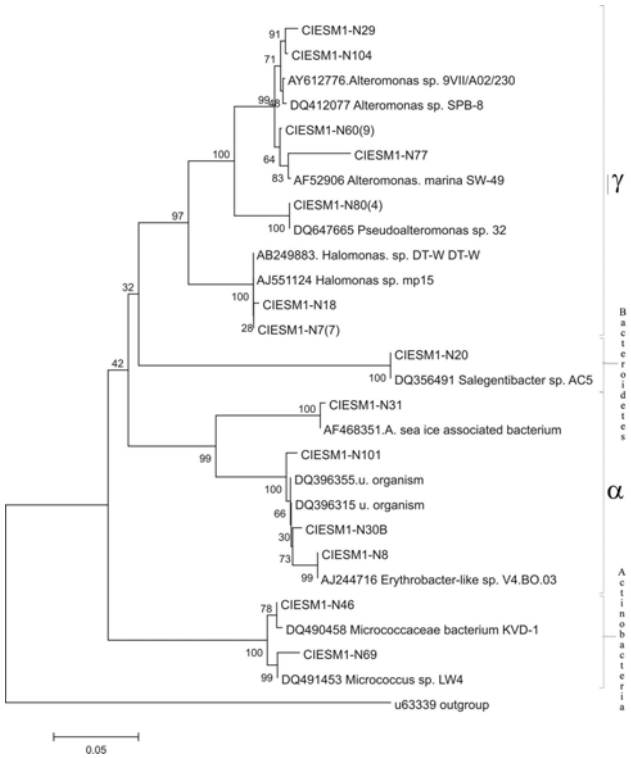

Fig. 2. Phylogenetic tree of isolates.

References

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