PHOTOBACTERIUM PHOSPHOREUM: UNIQUE REPRESENTATIVE OF THE LUMINOUS MICROBIAL COMMUNITY IN THE SOUTH-EASTERN IONIAN SEA?

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Abstract
Luminous bacteria were isolated from Mediterranean water samples taken from the surface to 3.300 m depth during oceanographic cruises in the Southern Ionian Sea and the Sicily Channel. Bacterial plate counts in SWC medium were carried out in 380 water samples from 16 Stations of this area of the Mediterranean Sea. Results ranged from 0 to 20 CFU/100ml. Generally, two peaks in abundance were detected: one in the upper 300 m of the water column, which mainly concerns the Sicily Channel; and a second between 500 and 1200 m, in the South-Eastern Ionian Sea. The physiological and molecular analysis show that all the isolates were *Photobacterium phosphoreum* differently from those found by others in different locations.
Keywords : Bacteria, Ionian Sea, Sicilian Channel, Biodiversity.

Luminous bacteria have been extensively studied and are well described phylogenetically and ecologically. Compared to the broad distribution and high abundance in the marine environment, only one luminous species has been isolated from fresh water and another has been isolated from soil. Luminous bacteria have been observed living in many ecological niches including planktonic, saprophytic, symbiotic, and parasitic niches. Some species inhabit more than one niche. Despite several studies describing the distribution and abundance of luminous bacteria, details regarding population dynamics, ecological function, and especially niche relationships remain poorly understood ([1]). *Photobacterium phosphoreum* has been well described relative to their light organ symbioses with several families of marine fish inhabiting cold and deep ocean waters. Free-living *P. phosphoreum* also has been isolated by direct plating of seawater. Aside from the free-living forms and symbioses formed with marine fish, *P. phosphoreum* has been described as living saprophytically and parasitically.
Isolation of luminous bacteria from 16 stations in the South-Eastern Ionian Sea and in the Sicily Channel was carried out, throughout the years 2004-2006 during two different oceanographic cruises.
Water samples were filtered through Millipore filters of 0.45 μm mean pore size, and filters were placed on SWC Agar and incubated in the dark at room temperature (ca. 20°C). Within 48 h after incubation, the presence of luminescence from colonies was checked visually in a dark room. The bioluminescent colony were purified and stored at -80 °C with 40% glycerol until analysis.
From ca. 200 luminous colonies randomly selected from the various plates, 89 grown on replicates were analysed for further morpho-physiological and taxonomic analysis. The isolates were characterized using the routine tests and by BIOLOG GN microplates and API 20 NE for Gram negative bacterial strains.
Viable counts on SWC varied from 0 to 20 luminous CFU/100ml and the population of bioluminescent bacteria constitued 0,007 (november 2004) to 0,011% (october 2006) of the total, viable, aerobic heterotrophic bacteria enumerated by the plate counts.
Luminous CFU were most abundant in two layers: between the surface and 300 m of the water column (mainly in the Sicily Channel) and with lower values respect the second layer between 500 and 1200 m, wich is present in the whole South-Eastern Ionian Sea.
Molecular analysis was performed with bacterial DNA extraction, 16S rDNA amplification and sequencing. The PCR amplification of 16S rRNA gene was done using the bacterial universal primers 27f and 1492r. The PCR products were digested with enzymes AluI and RsaI to develop an amplified ribosomal DNA restriction analysis (ARDRA) methods.
The physiological characterization of the isolated strains shows that there was only a species of bioluminescent bacteria, of the 89 isolates, *Photobacterium phosphoreum* (figure 1).
The marine bioluminescent bacteria ARDRA analysis of the 16S rRNA-gene, developed with two restriction enzymes, allowed discrimination of 3 clusters species of bioluminescent bacteria that during sequencing always gave the same result of the phenotypical analysis (figure 2).
The results of this research, in relation to previously studies, not suggest a seasonal variation of the luminous microbial community distribution in this area. The spatial distributionof luminous bacterial shows two peaks: one in the upper 300 m of the water column, which mainly interests the Sicily Channel; and a second between 500 and 1200 m, which is present in the Eastern area (Ionian Sea).
The species distribution observed in this study is different from data reported for the Mediterranean Sea and for the Atlantic Ocean([2], [3]). *Photobacterium phosphoreum* is prevalent in many different locations but is not the unique luminous bacterial species found, in our survey.

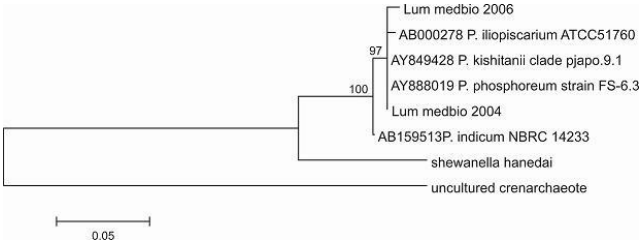

Fig. 1. Phylogenetic tree of luminous bacteria isolates in the two oceanographic cruises MEDBIO (2004 and 2006).

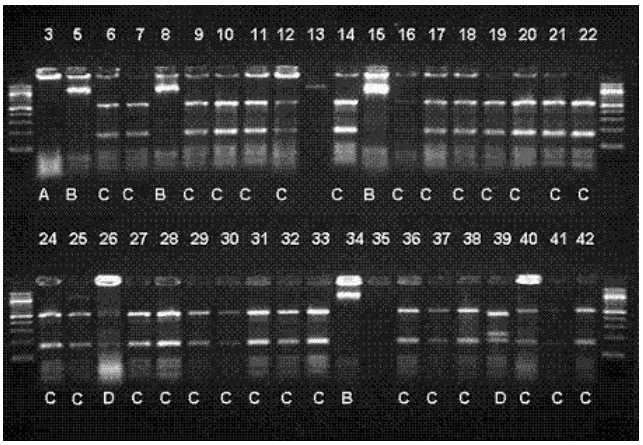

Fig. 2. Restriction patterns of the 16S rRNA gene (ARDRA method) amplicon from marine bioluminescent strains PCR-amplified 16S rRNA gene was digested with AluI and RsaI.

References

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