

How Deep is our Current Knowledge of Microbial Metabolism in the Mediterranean Sea?

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Exploring microbes' world is always a variety of exciting topics. Since the concept of "microbial loop" was first formulated [1], a plethora of studies have tried to look inside the "black box" of the microscopic world of microorganisms and to investigate the wide diversity of its members (virus, bacteria, Archaea, protists) [2]. Addressing our attention to microbes inhabiting aquatic ecosystems, seminal work has been performed in the last decades by many aquatic microbial ecologists (i.e. Professors Azam, Hoppe, Chrost, Danovaro, Genovese, Herndl, Gasol, Lebaron and Velimirov, and their co-workers, to cite some of them only); in their papers, the central role of microorganisms as decomposers, mineralizers and producers of organic matter has been fully recognized. Microorganisms affect the chemistry and productivity of aquatic environments thanks to their by their metabolic activities, both productive and degradative; they are the major actors in the biogeochemical fluxes of elements but the mechanisms by which these reactions proceed, there in situ transformation rates and quantitative significance to element cycling are still poorly understood [3].

In spite of the proliferation of scientific literature focusing on the distribution and activity of microbes in the different basins of the Mediterranean Sea, to date the implications of microbial metabolism in the functioning of this important ecosystem are still poorly unknown. Due to its characteristics of a semi-closed basin, with short residence times of water masses and the occurrence of deep water formation phenomena, the Mediterranean Sea is an oligotrophic basin very vulnerable to environmental changes and therefore it is considered as an ecosystem particularly suitable for biogeochemical studies [4-6]; a complex microbial food web exists in this basin, through which organic carbon and nutrients are efficiently remineralized and recycled and only small amounts of energy are transferred to the highest trophic levels [7].

An important help/support to the definition of the "in situ" biogeochemical role of microbes in different ecosystems comes from the development in chemical compounds such as fluorogenic analogues to proteins, carbohydrates and organic phosphorus esters, which make possible to estimate the "in situ" decomposition rates of organic polymers [8]. Through the use of these compounds, light has been shed on the significance of the prokaryotic activities in organic matter turnover and the biogeochemical implications of microbially-mediated hydrolytic processes, even in deep environments [9-16].

Changes in the prokaryotic metabolism in response to environmental changes, among which variations in the thermohaline properties occurring in the last decades such as the Eastern Mediterranean Transient [17,18], and the evolution of this ecosystem toward autotrophy or heterotrophy have been explored in a few studies only [11,14,16]. Results of recent studies have shown enzyme activity measurements to be powerful sentinels of environmental changes [12,13]; the response of microorganisms to changing environments has recently been reviewed through the analysis of the main factors modulating the patterns of metabolic activity [19]. Simultaneous

measurements of environmental parameters like temperature, nutrients, trophic variables, abundance of the main biotic components, should be taken since enzyme activity is the expression of the interaction of microbes with their environment.

In the framework of the next coming European FP PERSEUS program, information will be gained on the efficiency of Carbon transfer through the microbial compartment in some sites selected for their biogeochemical interest, being sites of dense water formation; some questions that will be addressed during the program will be: "How environmental and biological forcings influence the ecosystem dynamics and C cycling?", "What is the role of enzyme activities in nutrient regeneration?" "Will there be any feedback between altered marine trophic structure, and the efficiency of the biological pump?" Answers to these questions are crucial to improve understanding of the fundamental control mechanisms of the carbon cycle and thus to enable the prediction of the ecosystem's responses to human impacts like eutrophication, increasing atmospheric CO₂ concentrations and consequences of climate change, e.g. changing temperatures and wind fields.

Integration of the microscale processes, like those microbially mediated, in the overall functioning of the Mediterranean Sea is recognized as an important challenge of future investigations. Azam [2] first recognized failures in the ability to predict the influence of the microbial loop on ecosystem behaviour and that these make difficult to make decisions on various environmental and economic issues such as conservation of marine diversity, fishery management and ocean pollution. But an important question that needs an urgent answer is: are current methods adequate to describe the functional diversity of microbial communities and to what extent? Important contributions to knowledge of microbial metabolism at sea can come from the combination of recent technologies which address both biological diversity and environmental processes occurring at microscale; also new automatic systems may allow to map the distribution of microbial enzyme activity rates [20].

Studies on the functional role of microbes in aquatic environments should relate microbial diversity to their physiological properties; most analyses of community composition based on rRNA sequences do

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not provide information on the degradative capabilities of uncultured organisms. A first study [21] relating the structure of bacterial communities as obtained from Fluorescent in Situ Hybridation with the measurement of enzymatic rates for the most frequently occurring phylogenetic groups (alpha- and gamma-Proteobacteria, CFB) has suggested that an integrated structure-functional approach might help to elucidate not only the genetic potential for extracellular enzyme production but also the biochemical expression of functional genes. This combined approach could help to elucidate the complex regulatory mechanisms on the community level, which are still poorly understood. There is also an urgent need to investigate the diversity of ectoenzymes as a way to discover the diversity in the bacterioplankton metabolism in the marine environment [22]. Also flow cytometry using multiple probes specific for several targets (i.e. membrane integrity, respiratory activity, membrane potential and so on) could provide interesting information on the physiological functions of bacteria at single-cell level allowing also multiparameter data acquisition, high-speed analysis and cell sorting [23].

In the light of previous considerations, how deep is then current knowledge of microbial metabolism? We can state that current knowledge is still far to be complete, and that further research in this field is needed to improve knowledge of the ecological role of microorganisms in the ecosystem processes [24]. Long-term studies could allow to perform Carbon budget in the different Mediterranean ecoregions and to assess its biogeochemical evolution in relation to recent global changes.

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