Microautoradiography combined to fluorescence in situ hybridization as a way to interrogate single cells about their phylogeny and activity: a review of its use in marine microbial ecology, of its advantages and limitations, and some forecast

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Abstract

Microautoradiography (MAR) is one of the earliest single-cell methods; the first reports of its use in aquatic microbial ecology were published in 1959, and Thomas Brock used it to quantify the growth rate of a conspicuous freshwater bacterium as early as 1967. It was used in marine samples already in 1976. In this technique, microbial assemblages are incubated with a radiolabeled substrate and cells are then placed in contact with an autoradiographic emulsion, and subsequent exposure of the emulsion to the radioactive emissions produces silver grain deposits around the cells that are radioactive. Aminoacids, acetate, glucose, along with leucine and thymidine were employed in the initial studies, but recent studies have expanded to other substrates such as chitin, NAG, DMSP and even 14CO2-bicarbonate. In these new studies, the activity probing has been combined to fluorescent in situ hybridization (FISH) or CARD-FISH creating the technique called MAR-FISH, STAR-FISH, or MICRO-FISH. I will summarize the technical details of the technique, with special detail on the current limitations. I will also show some avenues for getting to understand better marine microbial communities with the use of the technique, e.g. by using well-characterized molecules such as organic phosphorus, or less-well characterized substrates such as the phytoplankton-excreted dissolved organic carbon. I will also try to show how the method can be used not only to describe the patterns of activity across microbial groups or species, but also as a very sensitive way of testing the effects of pollutants or other chemical substances on the activity of different bacterial groups. Finally, I will also analyze most of the published to-date results to illustrate how useful has been the technique to link microbial diversity with the marine plankton ecosystem functioning.

Keywords: Bacteria, marine, microautoradiography, FISH, MAR, FISH, ecology

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