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Linking microbial community structure with function: fluorescence *in situ* hybridization-microautoradiography and isotope arrays

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The ecophysiology of microorganisms has been at the heart of microbial ecology since its early days, but only during the past decade have methods become available for cultivation-independent, direct identification of microorganisms in complex communities and for the simultaneous investigation of their activity and substrate uptake patterns. The combination of fluorescence *in situ* hybridization (FISH) and microautoradiography (MAR) is currently the most widely applied tool for revealing physiological properties of microorganisms in their natural environment with single-cell resolution. For example, this technique has been used in wastewater treatment and marine systems to describe the functional properties of newly discovered species, and to identify microorganisms responsible for key physiological processes. Recently, the scope of FISH-MAR was extended by rendering it quantitative and by combining it with microelectrode measurements or stable isotope probing. Isotope arrays have also been developed that exploit the parallel detection offered by DNA microarrays to measure incorporation of labelled substrate into the rRNA of many community members in a single experiment.

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Introduction

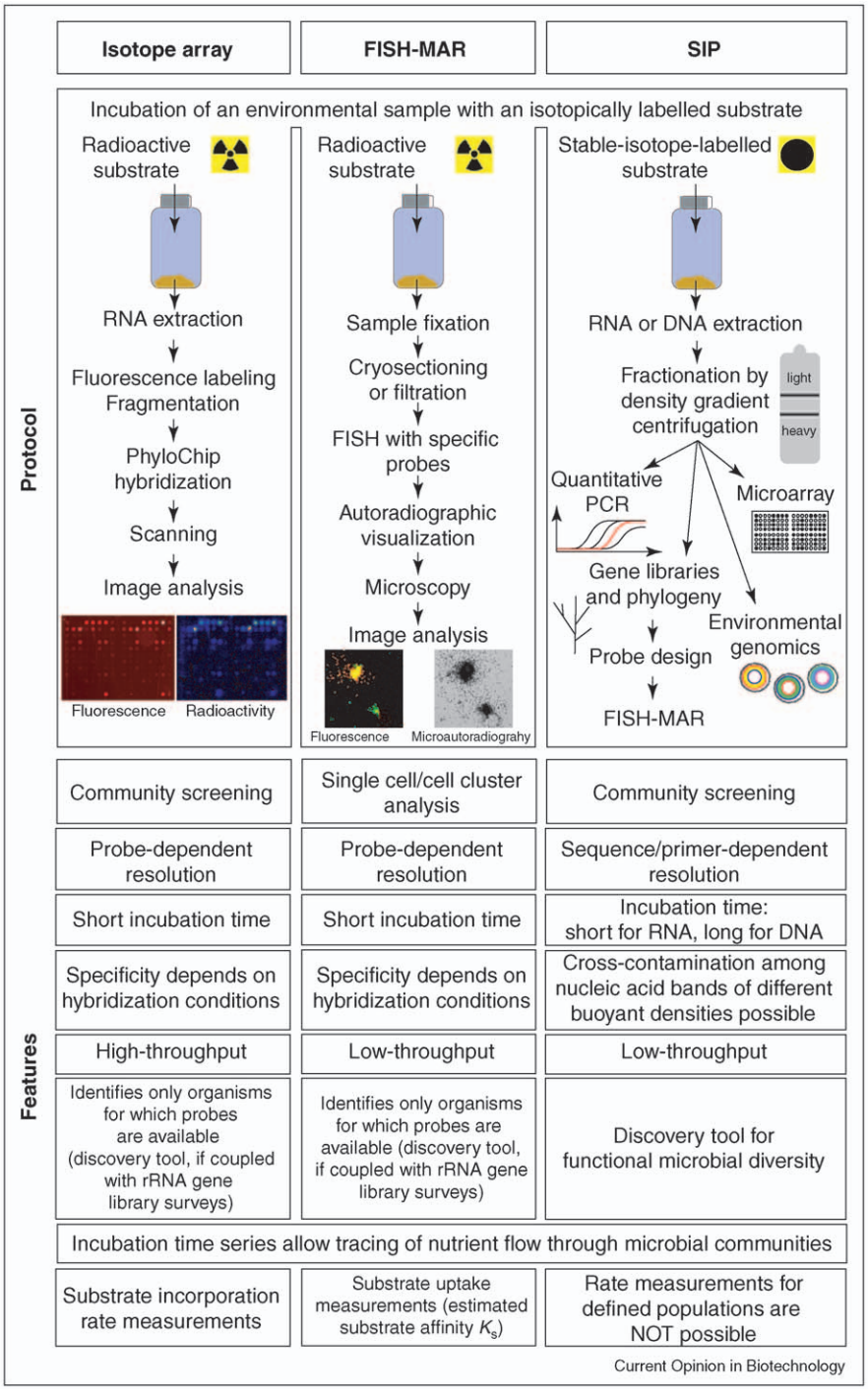
Since 1989, it has been possible to identify microorganisms *in situ* using fluorescently labelled ribosomal RNA-targeted oligonucleotide probes for fluorescence *in situ* hybridisation (FISH) [1]. Following this breakthrough, however, it was ten years before a method became available that also allowed specific functions to be assigned to the *in situ* detected microorganisms. In 1999, two research groups succeeded in combining FISH with microautoradiography (MAR) [2,3] and were thus able, after a short incubation of the environmental sample with radioactively labelled substrate, to observe under the microscope whether a probe-detected bacterium was capable of consuming the offered substrate under the incubation conditions applied (Figure 1). Such insights are of particular relevance for microbial ecology, as most microorganisms that thrive on our planet are not available as pure cultures and, even if the pure culture physiology of a particular bacterium is well known, it is still impossible to infer its ecophysiology as a member of a microbial community. Consequently, it is not surprising that FISH-MAR is now widely applied. However, this technique has two major limitations. Firstly, no more than seven bacterial populations can be specifically detected in a single FISH experiment, owing to the limited number of different fluorophores that can be applied simultaneously [4]. Keeping in mind that natural microbial communities can comprise thousands of species [5], then compiling a comprehensive list of those microorganisms that consume a specific substrate in the system of interest can quickly become very cumbersome, or even impossible. Secondly, not all environmental samples are well-suited for FISH analysis. For example, only a minor fraction of the resident bacteria will be detectable by FISH in bulk soil and thus most of these soil bacteria cannot be characterized by FISH-MAR. The so-called isotope array overcomes both problems by using rRNA-targeted DNA microarrays to measure incorporation of radioactively labelled substrate into the rRNA of the target organisms (Figure 1) [6••]. In principle, thousands of probes can be applied simultaneously in this approach, which should be applicable to any sample from which rRNA of sufficient quality and quantity can be purified.

In this review, we will describe new developments of the FISH-MAR approach, discuss the principle of the isotope array approach, and give examples of how these techniques have been used to reveal new and exciting insights into the ecophysiology of uncultured microorganisms.

FISH-MAR: features, new developments and applications

FISH-MAR has, with today's instruments and depending on the radiotracer used, a resolution of 0.5–2 μm and is thus a single-cell tool [7] (see also Update). However, in

Figure 1



Overview of the protocol and selected features of the isotope array (left), FISH-MAR (middle) and SIP approaches (right).

biofilms or other dense cell aggregates cryosectioning of the biomass, or efficient cell dispersal, is required before FISH and autoradiography to enable silver grains, which indicate the assimilation of the radioactive substrate, to be assigned to individual cells. For interpretation of FISH-MAR data, it is important to keep in mind that, in contrast to stand-alone MAR, this combined method does not measure total uptake of the radiolabelled substrates but only assimilation into macromolecules. Unincorporated labelled compounds are not retained inside the paraformaldehyde- or ethanol-fixed cells. Nevertheless, FISH-MAR is very sensitive compared with DNA- or