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Gold-FISH: A correlative approach to microscopic imaging of single microbial cells in environmental samples

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Fluorescence in situ hybridization (FISH) is routinely used for the phylogenetic identification, detection, and quantification of single microbial cells environmental microbiology. Oligonucleotide probes that match the 16S rRNA sequence of target organisms are generally applied and the resulting signals are visualized via fluorescence microscopy. Consequently, the detection of the microbial cells of interest is limited by the resolution and the sensitivity of light microscopy where objects smaller than 0.2 μm can hardly be represented. Visualizing microbial cells at magnifications beyond light microscopy, however, can provide information on the composition and potential complexity of microbial habitats - the actual sites of nutrient cycling in soil and sediments.

We present a recently developed technique that combines (1) the phylogenetic identification and detection of individual microorganisms by epifluorescence microscopy, with (2) the in situ localization of gold-labelled target cells on an ultrastructural level by SEM. Based on 16S rRNA targeted in situ hybridization combined with catalyzed reporter deposition, a streptavidin conjugate labeled with a fluorescent dye and nanogold particles is introduced into whole microbial cells. A two-step visualization process including an autometallographic enhancement of nanogold particles then allows for either fluorescence or electron microscopy, or a correlative application thereof.

We will present applications of the Gold-FISH protocol to samples of marine sediments, agricultural soils, and plant roots. The detection and enumeration of bacterial cells in soil and sediment samples was comparable to CARD-FISH applications via fluorescence microscopy. Examples of microbe-surface interaction analysis will be presented on the basis of bacteria colonizing the rhizoplane of rice roots.
In principle, Gold-FISH can be performed on any material to give a snapshot of microbe-surface interactions and provides a promising tool for the acquisition of correlative information on microorganisms within their respective habitats.