Application of nucleic acid mimics and spectral imaging to fluorescence *in situ* hybridization for the characterization of microbial communities

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Abstract

The application of nucleic acid mimics (NAMs) has improved the performance of FISH methods for the localization, detection and enumeration of microorganisms. However, an important limitation of FISH techniques is the low number of distinguishable targets. Recent advances in combinatorial labelling and fluorescence spectral imaging (CLASI-FISH) have allowed the simultaneous identification of several microorganisms in a single sample. In this work, we aimed to combine NAM-FISH and spectral imaging to develop/validate a new color-coded FISH methodology that allows a multiplexed and robust detection of microorganisms in complex communities. In a first stage, to implement/validate the method, we have selected 8 fluorophores with distinct spectral properties and 7 bacterial species. As a strong variation on the fluorescence intensities is found between species and between fluorophores, 8 versions of EUB LNA/2'OMe probe, each conjugated to one of 8 fluorophores were used to rank species/fluorophores by FISH. Then, mixed populations were used to evaluate the potential of the method for separating/quantifying the different species. Samples were analyzed by confocal laser scanning microscopy coupled with a spectral imaging detector and a linear unmixing algorithm was applied to identify the fluorophores present in each pixel of the images. Validation tests with different proportions of bacteria labelled with the respective fluorophore have shown the ability of the method to correctly distinguish the species. The method shows as a powerful tool for the characterization of environmental microbial consortia that might assist on the monitoring of bioremediation processes.

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