ENHANCED PERFORMANCE OF PEPTIDE NUCLEIC PROBES FOR FLUORESCENCE IN SITU HYBRIDISATION DETECTION OF MICROORGANISMS IN THE ENVIRONMENT

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Background and Aims: Fluorescence in situ hybridisation (FISH) has dramatically changed our understanding of microbial ecology. Traditionally, fluorophore-labelled DNA probes have been used to detect specific or more generic sites on rRNA. However, DNA probes have a number of disadvantages which limit their use on complex environmental samples. The aim here was to develop peptide nucleic acid probes and demonstrate their superiority for FISH detection of pathogens in planktonic and biofilm samples. Methods: PNA probes specific to *Legionella pneumophila, Escherichia coli, Helicobacter pylori* and *Mycobacterium avium* were labelled with various fluorophores and their specificity confirmed against a wide range of microbial species. Planktonic and biofilm samples were obtained from water supplies and environmental sediments and labelled samples were examined using epifluorescence microscopy. Results: A comparison of PNA probes with equivalent DNA probes showed their greater specificity and sensitivity to detect target pathogens when incubated at various temperatures and formamide stringency. PNA probes successfully detected pathogens in unspiked and spiked planktonic and biofilm samples from water and sediment samples, with no non-specific binding observed. Even highly corroded surfaces could be observed using appropriate in situ microscopy techniques, and demonstrated preferred biofilm locations for particular species corresponding to their known physiology. Conclusions: DNA probes require stringent hybridisation conditions, specific to each individual probe, compromising labelling efficiency for multiplex reactions; hybridisation protocols also exacerbate sample autofluorescence. The improved physico-chemistry of PNA probes facilitates their use in duplex and multiplex assays, demonstrating their greater versatility and reliability for FISH analysis of ecosystems.