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BOOK OF ABSTRACTS

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OR

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.

OR

IMPROVEMENT OF IN SITU HYBRIDIZATION EFFICIENCY OF RRNA-TARGETED PROBES USING LOCKED NUCLEIC ACID

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Poor fluorescence signals due to low probe hybridization efficiency are problematic in rRNA-targeted in situ hybridization. There are two major factors affecting the hybridization efficiency, probe accessibility and affinity to the targeted rRNA. This study focuses on the affinity and here we show remarkable improvement in the in situ hybridization efficiency by the application of locked nucleic acid (LNA)-incorporated oligodeoxynucleotide probes (LNA/DNA probes). Fluorescently labeled LNA/DNA probes exhibited strong fluorescence signals equal to or more than the signals from Eub338, although DNA probes with the same sequences showed weak signals. Dissociation profiles of the probes used in this study revealed that the dissociation temperature (or formamide concentration) was directly related to the number of LNA substitutions and the fluorescence intensity. These results suggest that LNA/DNA probes will be useful for improving hybridization efficiency.

OR

IDENTIFICATION OF FAST GROWTH SPECIALISTS, SLOW GROWTH SPECIALISTS, AND STEADY RESPONDERS IN BIPHENYL-FED SOIL MICROBIAL COMMUNITIES

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Background and Aims: Very little is known about influence of pollutant exposure history on community structure and population dynamics among biodegraders in soil. In this study, we attempted to identify fast growth specialists, slow growth specialists, and steady responders among biphenyl-utilizing populations in soil. Methods: In microcosms, soil was fed with 13C-labeled biphenyl, and stable isotope probing (SIP) was conducted. For early time experiment soil DNA samples were taken on day 10. For late time experiment on day 28. A standard CsCl gradient method was used to isolate heavy DNA portions from soil DNA. For bacterial community structure and population dynamics, 16S rDNA were amplified, cloned and sequenced. Results: The length of biphenyl incubation period did not affect the degree of diversity of biphenyl-utilizing populations but changed their community structures and population dynamics. In the early time SIP experiment, β-Proteobacteria was the predominant biphenyl-utilizing group (44%) while Actinobacteria was the predominant phylogenic group in the soil microbial community. In the late time experiment, meanwhile, α- and β-Proteobacteria groups were equally dominant among the biphenyl utilizing populations (31% and 33%, respectively). The further analysis revealed that the α- and β-Proteobacteria groups included a wide range of biphenyl-growth responders (fast responders, slow responders, and steady responders). However, Actinobacteria group included only steady responders. Conclusions: These findings provide an insight into the effect of pollutant exposure history on community structure and population dynamics among polychlorinated biphenyl degraders in soil.

OR

ALTERATION OF SOIL MICROBIAL COMMUNITIES ASSOCIATED WITH BT GENETICALLY MODIFIED TREES

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Application of plant genetic manipulations to agriculture and forestry in the aim of alleviating insect damages through Bt transformation could lead to a reduction of pesticides being released into the environment. However, many groups have come forward with valid and important questions relating to potentially adverse effects this technology might have. So far, the few studies that have attempted to measure these undesired effects, such as impact on soil microbial populations, did not demonstrate significant changes. Clearly, the lack of sufficient reliable data resulting from environmental impact studies makes the possible effects of transgenic DNA insertion into a plant's genome to yet be fully evaluated. In this study, soil samples collected from the rhizosphere of the first Bt-transformed trees grown in Canadian soil were analyzed as part of an ecological impact assessment project. Using a robust culture-independent Amplified Ribosomal DNA Restriction Analysis approach coupled with sequencing, the rhizosphere-inhabiting microbial communities of white spruce genetically modified by the biolistic insertion of the CryIa (b)/GUS/nptII genes were compared with the microbial communities associated with its non-genetically modified counterparts and with trees in which only the reporter genes GUS/*npt*II have been inserted. The 1728 rhizosphere bacterial clones analyzed indicated a statistically significant difference between the microbial communities inhabiting the rhizospheres of trees carrying the B*t* encoding gene, reporter genes only, and control trees. Clear rhizosphere microbial community alterations due to tree genetic modification has to our knowledge never been demonstrated before and opens the door to interesting questions related to B*t* genetic transformation.