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PCR-based detection of mobile genetic elements in total community DNA

Mobile genetic elements (MGEs) endow their host bacteria with genetic variability and flexibility in response to environmental stress. MGEs are an important part of bacterial diversity (8). Of the MGEs, plasmids represent perhaps the most important reservoir for both gene transfer and capture. Although we have a detailed knowledge of many plasmids, the majority of these have been isolated from clinically important bacteria and have been responsible for the rapid spread of antibiotic resistance and pathogenicity determinants. Consequently, our knowledge of the prevalence and diversity of plasmids in bacteria from non-clinical environments is very limited. Systematic studies on the incidence and abundance of plasmids and other MGEs in different environmental niches have not yet been performed. Lack of information on the distribution of MGEs in natural environments is also due to the fact that only a minor proportion of bacteria are accessible to traditional cultivation techniques. Recently, methods for extraction of nucleic acids directly from environmental samples have been developed to allow studies of bacterial communities independently of cultivation. Coupled with the progress in development of molecular tools, this now offers a powerful new dimension in our ability to investigate the prevalence and diversity of MGEs in environmental bacteria. The application of MGE-specific primers to total community DNA can greatly facilitate the screening of different environments for the presence of specific plasmids (1). This approach allows the detection of MGEs independently of the culturability of their hosts, from the presence and expression of selectable markers and from their ability to transfer to, and replicate in, a new recipient. The main advantage of this approach is that large sample numbers can be analysed, making extensive screening programs for a variety of environments more realistic.

To explore the power of this technique we have applied PCR to study the prevalence of a range of MGEs in total DNA from different environments during the first workshop of the EU-funded Concerted Action entitled ‘Mobile Genetic Elements’ Contribution to Bacterial Adaptability and Diversity’ (MECBAD; http://meceb.bba.de) (8) in Braunschweig, 1–6 June 1999. This workshop, organized by Mark Osborn, Christoph Tebbe and Kornelia Smalla, aimed to provide a theoretical and practical introduction to the study of the prevalence of mobile genetic elements in different environments.

Most of the participants provided community DNA from their own research projects, thus allowing us to analyse a total of 17 types of DNA directly extracted from: pig, chicken and cattle manure; PCB-contaminated, uncontaminated and farm soil samples; oil seed rape, potato and copper-treated and untreated rhizosphere samples; coastal salt marsh; fish farm sediment; sewage; compost; a waste water sample; and a linuron-degrading consortium, isolated from linuron-treated soil in Belgium (see Table 1). Amplification of 16S rRNA genes from each environmental DNA sample, with subsequent analysis by denatur-
ing gradient gel electrophoresis (DGGE; 3) or single-strand-conformation polymorphism (SSCP; 6), was used to ensure that the DNA was of a sufficient purity (e.g. free from inhibitory compounds such as humic acids) for use in the subsequent PCR assays for MGE detection. Several primer systems designed on the basis of published and unpublished sequences were applied to detect IncQ (oriV) (1), IncP-1 and IncP-1β (trfA2) (1, 7), IncP-9 (rep) (2), rhizobial plasmid repC replication regions (K. M. Palmer, S. L. Turner & J. P. W. Young, unpublished data), Tn21/Tn501-type resolvase (tnpR) (5) and transposase (tnpA) genes (5), and gene cassettes integrated in class I integrons (4). PCR allows the specific amplification of the DNA sequence spanned by the primers. PCR products were analysed by agarose gel electrophoresis and Southern blot hybridization with PCR-generated digoxigenin-labelled probes to increase the sensitivity of detection for IncQ, IncP-1, IncP-9, and Tn21/Tn501-like sequences. PCR products obtained with the integron and the repC primer were cloned and sequenced. Strong hybridization signals were obtained for IncQ (oriV) in community DNA extracts from cattle and pig manure, in the rhizosphere of copper-treated soil, and in compost and farm soil. Southern hybridization of trfA2 PCR products was performed with both an RP4 (IncP-12)- and an R751 (IncP-1β)-generated probe. While strong hybridization was observed with the IncP-1β-derived probe for PCR products amplified from DNA extracted from the potato rhizosphere, the linuron-degrading consortium, coastal salt marsh, sewage and copper-treated soil, less intense hybridization was observed with the IncP-1 repC amplification was cloned. Positive clones were obtained from the potato (8/12) and oiled sewage (2/12) rhizospheres and the untreated rhizosphere (1/12). All were previously unknown repC sequence types, indicating previously unknown diversity in soil populations.

Thus the workshop demonstrated that by using PCR-based detection it is feasible to analyse a large number of samples and to provide data on the prevalence of MGEs. PCR screening of total community DNA allows the identification of environments that contain bacteria with a high incidence of MGEs. Where proper controls exist, the apparent correlation in specific cases between environmental conditions and prevalence of MGEs suggests that these elements may fuel responses to selective pressure. These studies thus provide the basis for further work to determine whether such promiscuous elements carry hot spots for insertion and whether they carry genes for phenotypic markers that are being selected in these environments.

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**Guests, not members of the MECBAD consortium.


Teichoic acids in bacterial coaggregation

Intergeneric and intragenic bacterial coaggregation are commonly observed phenomena, important in the growth of cultures and in the process of infection in disease (8). Intergeneric coaggregation requires divalent cations (Ca2+) and a protein, adhesin, associated with the cell surface (3). Insertional inactivation of genes responsible for the d-alanylation of lipoteichoic acid in Streptococcus gordonii DL1 (Challis) has shown that the alanine ester residues are essential for cell aggregation (4). It was concluded that they play a role in providing binding sites for the putative 100 kDa adhesin and the correct presentation or orientation of this protein for coaggregation.

In an earlier study on the teichoic acid in the cell walls of Lactobacillus plantarum ATCC 10241 and Bacillus licheniformis ATCC 9945, using X-ray photoelectron spectroscopy, it was shown that there are two different forms of binding of Mg2+ to the teichoic acid (2). In the samples from which alanine had been removed by treatment with