USs of *Haemophilus* or *Neisseria*, then they would have been detected even if present with a moderate degree of degeneracy. If such a sequence were to be non-continuous then we would also have expected to detect the component parts as long as they were themselves sufficiently conserved.

We were unaware of the papers on *Acinetobacter*. However, *Acinetobacter* is quite distinct from the other Gram-negative spp. with regard to DNA uptake and its very high transformability by plasmids, and therefore lies outside of the model upon which our search was predicated – being perhaps more similar in some respects to transformation processes seen in Gram-positive species.

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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://mecbad.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-

Table 1. PCR-based detection of mobile genetic elements in total community DNA

Based on Southern blot hybridization data. −, no hybridization; (+), weak hybridization; +, ++, +++, hybridization, strong hybridization and very strong hybridization, respectively.

Sample type	IncP-1α	IncP-1β	IncP-9	IncQ (*oriV*)	Tn*21*/Tn*501*-like *tnpR*	Tn*21*/Tn*501*-like *tnpA*	*repC**	Integron PCR*
Compost	+++	(+)	−	+++	+	−	−	+++
Sewage	++	++	−	−	+++	++	−	+++
Copper-treated rhizosphere	(+)	++	(+)	+++	(+)	++	(+)	+++
Untreated rhizosphere	−	−	−	+	−	+++	(+)	+++
Fish farm sediment	−	+	+	−	+++	+++	−	−
Waste water	−	−	(+)	(+)	+++	+++	−	+++
Farm soil	−	−	−	+++	+++	+++	(+)	+++
Potato rhizosphere	(+)	++	−	−	+	+++	+++	−
Linuron-degrading consortium from soil	+++	+++	−	−	−	++	+	(+)
Salt marsh	(+)	++	(+)	−	(+)	−	−	−
Uncontaminated sandy soil (A)	−	−	−	−	(+)	(+)	−	+
PCB-contaminated sandy soil (CD2)	−	+	(+)	−	−	+	−	+
Rape rhizosphere	−	−	−	−	−	−	++	+
Mouse gut	−	−	(+)	−	(+)	−	−	(+)
Chicken manure	−	−	(+)	−	+++	+++	−	+++
Cattle manure	−	−	(+)	+++	(+)	+	+	+++
Pig manure	−	−	−	+++	++	+	+	+++

*PCR products obtained.

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-1α 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), Tn*21*/Tn*501*-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 Tn*21*/Tn*501*-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-1α)- 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 for compost, fish farm sediment and PCB-contaminated soil. Hybridization with the IncP-1α-derived probe indicated a high prevalence of the IncP-1α-specific *trfA2* in compost, sewage and the consortium of linuron-degrading bacteria.

An interesting finding is the presence of both IncP-1β and IncQ (*oriV*) in the rhizosphere of the copper-treated soil, while these replicon-specific sequences were not detected in the rhizosphere of the untreated soil. A less striking but similar observation was seen with PCB-contaminated soil (CD2), where IncP-1β plasmid-specific sequences could be detected, whereas no signal was obtained from DNA of uncontaminated soil from the same site. IncP-9 plasmids were detected in the DNA from various environmental samples, but with the exception of the fish farm sediments, weak signals were observed. Strong hybridization signals, indicating a high abundance of both Tn*21*/Tn*501*-related resolvase and transposase genes, were detected in chicken manure, sewage, waste water and farm soil, while less intense bands of the expected size were found for potato rhizosphere, the bioreactor consortium (only *tnpA*), cattle and pig manure, PCB-contaminated soil (*tnpA* only), and rhizosphere from copper-treated and untreated soil (*tnpA* only for untreated soil). PCR amplification with primers flanking gene cassettes integrating into class I integrons resulted in products of varying size for most of the community DNAs. However, these results only indicate the presence of gene cassettes. Cloning and plating on selective media should allow us to detect whether these are antibiotic resistance gene cassettes. PCR products amplified using the *repC* primers were the expected size and surprisingly clean for amplifications from community DNA. The products from the eight positive *repC* amplifications were cloned. Positive clones were obtained from the potato (8/12) and oilseed rape (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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Teichoic acids in bacterial coaggregation

Intergeneric and intrageneric bacterial coaggregation are commonly observed phenomena, important in the growth of cultures and in the process of infection in disease (8). Intrageneric coaggregation requires divalent cations (Ca^{2+}) 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 Mg^{2+} to the teichoic acid (2). In the samples from which alanine had been removed by treatment with