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"!...Comment...?"

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Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

***Jon Saunders*, Editor-in-chief**

DNA uptake sequences in *Helicobacter pylori*

In a recent paper in *Microbiology*, Saunders *et al*. (4) claim to have demonstrated the absence of a DNA uptake sequence (US) for uptake of homospecific DNA during natural transformation in *Helicobacter pylori*. The method used was a frequent word analysis of the *Helicobacter pylori* genome for oligonucleotide words of lengths up to 12 bases. This analysis showed that the frequency of the most abundant words in *Helicobacter pylori* is lower than those of USs in *Haemophilus influenzae*. Also, the position of the most abundant words was not intergenic, in contrast to the USs of *Haemophilus influenzae* and *Neisseria* spp., which are mainly located after ORFs and possibly act as transcriptional terminators.

However, these data are insufficient to conclude that no US is present in *Helicobacter pylori*. The assumption that a putative *Helicobacter pylori* US is similar to those of *Haemophilus influenzae* and *Neisseria* spp., both conserved continuous 9–10 bp motifs, is not necessarily valid. In fact, *Helicobacter pylori* USs are unlikely to be structurally similar to those of *Haemophilus influenzae* and *Neisseria* spp. The analysis of Washio *et al*. (5) showed that *Helicobacter pylori* does not contain sequences that allow hairpin formation downstream of ORFs, suggesting that this organism uses different mechanisms to terminate transcription. Therefore, co-optation of hairpin-forming transcriptional terminators to a function in transformation, as proposed for *Haemophilus influenzae* and *Neisseria* spp., cannot have evolved in *Helicobacter pylori*. In our view, the absence of preferential uptake of homospecific DNA in *Helicobacter pylori* can only be demonstrated empirically.

Finally, Saunders and colleagues claim that *Helicobacter pylori* is the first Gram-negative species that lacks a sequence for homospecific DNA uptake. Two groups, however, have demonstrated independently that *Acinetobacter* does not discriminate between homologous and heterologous DNA in DNA uptake (1–3). This claim is therefore incorrect.

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Authors' reply

The conclusion that there was not an uptake sequence similar to that seen in *Haemophilus influenzae* or *Neisseria meningitidis* was founded upon an analysis of oligonucleotide frequencies and was independent of sequence location. A significant proportion of the USs of *Haemophilus influenzae* is located within the ORFs, so it would be inappropriate to rely upon intergenic location for the identification of such a sequence. Since many of the most frequent (although comparatively infrequent) words observed were potentially capable of hairpin formation, we investigated their context to be rigorous in our analysis, but the observation that they were not typically located was not the basis of our conclusions. Indeed, we suggested why, in the absence of a stem–loop terminator that could be co-opted for the purpose of transformation, an uptake sequence need not have structural similarity to those seen in *Haemophilus* or *Neisseria* spp. As stated in the Methods, we analysed the genome for all oligonucleotides up to 12 bases and we identified all abundant words up to and including this length, and indeed above, on the basis that they would include smaller component parts. Our search was therefore not constrained to find only words of a particular length. If any word were present at a similar or lesser frequency to the

▶ *GUIDELINES*

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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

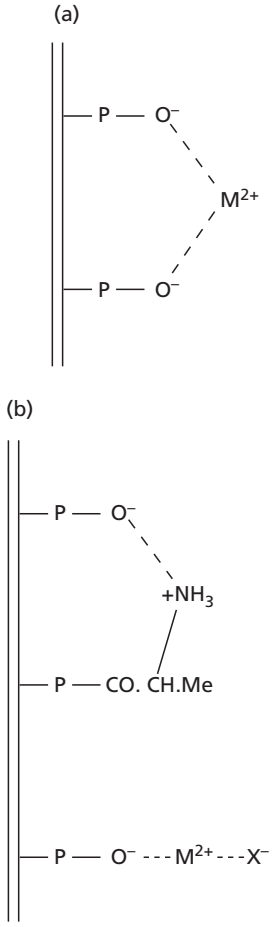

Fig. 1. (a) Teichoic acid without alanine. Mg^{2+} is bound firmly to phosphate groups within the polymer chain. (b) Alanine ester residues partially neutralize phosphate groups, allowing the less firmly bound cation to be shared with other anions, e.g. adhesin.

hydroxylamine solution the Mg^{2+} was strongly bound to phosphate groups within the chain of the teichoic acid, (Fig. 1a; M = Mg). When alanine ester groups were intact about 58% of the Mg^{2+} was less firmly bound than was the remaining 42%. It was concluded that the alanine amino groups compete with the Mg^{2+} for the anionic centres along the polyol phosphate chain of the teichoic acid (Fig. 1b; M = Mg, X = Cl). This result is consistent with the observation that wall teichoic acid binds less Mg^{2+} when alanine residues are intact than when they have been removed (6).

In the X-ray photoelectron spectroscopic work, Mg was chosen because of its abundance in nature and in the media used for growth of the organisms studied. In the coaggregation work, Ca was chosen because of its abundance in animal tissues. However, the same reasoning would apply in either study since, although these cations might not be identical in their affinity for lipoteichoic acid (7), they should be similar in their chemical behaviour. Moreover, it is reasonable to assume that lipoteichoic acid and wall teichoic acid would behave identically, since both are accessible at the cell surface and are structurally similar.

In Fig. 1(b) (M = Mg, X = Cl) the interaction between the alanine amino group and phosphate in the teichoic acid chain has resulted in the creation of less firmly bound Mg^{2+} associated through only one valency to a neighbouring phosphate group in the chain. In the samples used in X-ray photoelectron spectroscopy, the remaining valency on Mg^{2+} was assumed to be associated with Cl^- since this was the anion most likely to be available.

In the case of the teichoic acid lacking alanine residues (Fig. 1a; M = Mg) both valencies of each Mg^{2+} would be firmly associated with phosphate groups within the polymer chain and there would be no interaction with external anionic centres. When alanine ester residues are present, however, the less firmly associated Mg^{2+} is able to attract other anionic centres.

The lipoteichoic acid (or wall teichoic acid) in bacteria undergoing coaggregation would possess alanine ester residues and would be associated with Ca^{2+}. It would resemble Fig. 1b (M = Ca). However, instead of being associated with Cl^-, the Ca^{2+} would be shared between a phosphate in the teichoic acid chain and an anionic centre in the adhesin, as shown in Fig. 1b (M = Ca, X = adhesin).

There are many anionic centres in a teichoic acid chain and possibly also in adhesin, so Fig. 1b (M = Ca, X = adhesin) represents a simplified illustration of the complex. Nevertheless, it explains the requirements for coaggregation, namely the necessity for intact alanine ester groups on a teichoic acid and the presence of calcium ions. It is also clear why, in the case of mutant organisms lacking alanine, coaggregation does not occur, since the Ca^{2+} is so firmly bound to the teichoic acid chain that none is available to share with adhesin.

The role of teichoic acids in cell coaggregation is an example of the several functions of these major components in bacteria. Shortly after their discovery it was recognized that the predominantly anionic teichoic acids would have a powerful attraction towards cations, especially divalent cations, and their function in the cell would probably be related to this (1). Their cation affinity has been shown to play a major role in the enzyme systems of the cell membrane (5) and they have been implicated in a number of diverse properties, including control of cell shape, sensitivity towards antibiotics and activity of autolysins. It is possible that all, or at least several, of these activities are manifestations of cation affinity similar to that which now seems likely for the coaggregation described above, and the alanine ester residues might influence these in a similar manner.

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