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* or *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.

Aldefert Bart,1 Leonard C. Smeets2 and Johannes G. Kusters2,*

1Department of Medical Microbiology, Academic Medical Centre, University of Amsterdam, 1105AZ Amsterdam, The Netherlands
2Department of Medical Microbiology, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands

*For correspondence. Tel: +31 20 444 8294. Fax: +31 20 444 8318. e-mail: jg.kusters.mm@med.vu.nl.

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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

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**GUIDELINES**

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for the publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of *Microbiology* do not necessarily agree with the views expressed in *Microbiology* Comment.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.
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. 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-
and rhizosphere from copper-treated and untreated soil (trfA 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 oiled 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.

Kornelia Smalla,1,2,*, Ellen Krogerrekenklof,1 Holger Heuer,1 Winnie Dejonghe,2 Eva Top,2 Mark Osborn,3,4 Jessica Niewint,5 Christoph Tebbe,5 Michelle Barn,5 Mark Bailey,6 Alicia Greated,7 Christopher Thomas,7 Sarah Turner,8 Peter Young,8 Dora Nikolakopoulou,9 Amalia Karagouni,9 Anneke Wolters,10 Jan Dirk van Elsas,10 Karine Dronen,11 Ruth Sandaa,11 Sara Borin,12,13 Julia Brabhu,13 Elisabeth Grohmann13,14 and Patricia Sobeyc14,15

1 Biologische Bundesanstalt für Land- und \Forstwirtschaft, Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, Messweg 11–12, D-38104 Braunschweig, Germany
2 University of Ghent, Belgium2; GBF, Braunschweig, Germany3; University of Essex, UK4; FA, Braunschweig, Germany5; NERC-Institute of Virology and Environmental Microbiology, Oxford, UK6; University of Birmingham, UK7; University of York, UK8; University of Athens, Greece9; Ippo-Dlo, Wageningen, The Netherlands10; University of Bergen, Norway11; University of Milano, Italy12; Technische Universität Berlin, Germany13; School of Biology, Georgia, Atlanta, USA14

*For correspondence. Tel: +49 531 299 3814. Fax: +49 531 299 3013. e-mail: k.smalla@bba.de

**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). Intragenic 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 gordoni D1L (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...
firmly bound cation to be shared with other polymer chain. (b) Alanine ester residues partially the polyol phosphate chain of the teichoic acid with the Mg\(^{2+}\) ion, resulting in a less firmly bound Mg\(^{2+}\) ion compared to the remaining 42%. It was concluded 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 the 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.

James Baddiley

Hill Top Cottage, Hildersham, Cambridge CB1 6DA, UK
e-mail: james@baddiley.fsnet.co.uk