REVIEW ARTICLE

Chromosomal DNA fingerprinting—a new method of species and strain identification applicable to microbial pathogens

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Introduction

DNA-probe technology has enormous potential in clinical diagnostic microbiology. It offers the possibility of faster and cheaper identification of fastidious pathogens, including the detection of strains bearing known virulence factors and genes conferring resistance to antibiotics, and allows the direct detection of infectious agents in clinical specimens and in contaminated foods. There is a wealth of information, about these and other applications in microbiology that has been reviewed at length (Highfield and Dougan, 1985; Edberg, 1986; Goldmann, 1987; Miotti, 1987; Tenover, 1988; Landegren *et al.*, 1988).

In the field of microbial identification, diagnostic probes are commercially available to detect various bacterial species including *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, enteropathogenic *Escherichia coli*, *Mycoplasma pneumoniae* and some *Salmonella* serotypes (Tenover, 1988). Another application, of increasing interest to epidemiologists investigating and controlling hospital and community outbreaks, is the use of probes in providing DNA fingerprints for identification. Because these DNA fingerprints are highly sensitive to minor genomic variations in nucleotide sequences, they offer a precise means of characterising species and of identifying individual strains of closely related bacteria when more traditional typing methods are unsuitable or unavailable.

The purpose of this review is to describe the uses of chromosomal-DNA fingerprints in clinical and public-health microbiology. The methodologies involved, their application to species and strain identification, and their potential for typing bacteria will be discussed.

Chromosomal digest analysis

For the past 25 years, analyses of genomic DNA

by base-composition (G + C mol%) estimation and DNA-DNA hybridisation have provided an invaluable basis for defining closely related groups of microbial strains. DNA-DNA relatedness of *c.* $\geq 70\%$ and a low level of sequence divergence are now generally regarded as the phylogenetic basis for defining species (Wayne *et al.*, 1987). However, DNA-DNA homology measurements between total genomes are not sensitive enough to detect the small variations within species revealed by epidemiological typing methods such as serotyping, bacteriophage typing, resistotyping and biotyping. The alternative, novel, molecular methods that are now becoming more widely used (Goldmann, 1987; Falkner, 1988) including plasmid and whole-cell protein electrophoretic profiling and chromosomal restriction-enzyme analysis (REA), the latter also referred to as bacterial restriction-endonuclease digest-analysis (BRENDAs).

BRENDAs provides a sensitive means of directly detecting minor genomic differences between micro-organisms. Restriction endonucleases specifically cleave DNA into different lengths, depending on the number and position of the individual recognition sequences, provided that they have not been modified in any way. A DNA polymorphism refers to the change in the size of a restriction fragment. If a change occurs in the sequence of the genome DNA—even a single nucleotide-base mutation—this can delete a site or create a new recognition site and result in the generation of a restriction fragment-length polymorphism (RFLP). Sequence changes may also arise as a result of insertions, deletions or inversions of DNA between sites. RFLPs are most specific and reliable when the relevant gene has been located, its sequence has been defined and a probe has been made that is complementary to the sequence. Even if a change does not specifically involve restriction-site sequences, it may still be detected if the relevant fragments contain a suitable target sequence. The significance of DNA polymorphisms is that they

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most often represent neutral mutations and do not cause any phenotype change.

The restriction enzyme-recognition sites consist, in most cases, of four or six nucleotides arranged in a specific order with diad symmetry. Because the genomes of bacterial species vary widely in their base compositions (26–72 mol% G + C), the distribution of fragment sizes produced by restriction-enzyme digestion can also be expected to vary considerably. The expected frequency a of restriction sites can be predicted from

$$a = \left(\frac{g}{2}\right)^{r^1} \left(1 - \frac{g}{2}\right)^{r^2}$$

where g is the fractional G + C content of the genome DNA, r^1 is the number of G + C base pairs and r^2 the number of A + T base pairs in the recognition site (Nei and Li, 1979). The expected number of restriction sites is then given by frequency $\times$ genome size. The genome sizes of most prokaryotic DNAs fall in a tenfold range between 800 and 8000 kb with a typical genome being about 6×10^6 bases in length (Gillis and DeLey, 1975). Because of the large sizes involved, it is impossible to predict accurately to what extent a restriction enzyme will cut a particular DNA. However, calculations on the basis of base composition as a predictor provide some information about likely cutting frequencies. Thus, the probability of a G + C-rich site in a G + C-rich DNA is about 1 in 500 but only 1 in 80 000 in a G + C-poor DNA. The frequency of occurrence of particular sets of nucleotides is also a useful indicator. McClelland *et al.* (1987) showed that restriction endonucleases such as *Xba*I, *Avr*II, *Spe*I and *Nhe*I with CTAG in their recognition sequences cleave less than once every 10 000 bases. In contrast, CCG and CGG are the rarest trinucleotides in many genomes with base compositions of <45 mol% G + C, and so enzymes such as *Sma*I, *Rsr*II, *Nae*I and *Sac*II, which have those sets of bases in their recognition sequences, are rare cutters for low G + C-content DNAs.

The DNA fragments generated by restriction-enzyme digestion are separated according to size by electrophoresis in agarose gels to give a pattern of bands (fig. 1). Conventional electrophoretic techniques resolve fragments ranging in size from about 100 bases to 20 000 bases but recent developments by pulse-field electrophoresis enable much larger fragments (>9000 kb) to be separated (McClelland *et al.*, 1987; Sor, 1988). Restriction endonuclease-digest patterns have been used to distinguish between strains of various bacteria

Table I. Bacterial species of medical importance examined by restriction-endonuclease digest analysis to discriminate among strains

<i>Branhamella catarrhalis</i>	<i>Listeria monocytogenes</i>
<i>Brucella melitensis</i>	<i>Mycobacterium bovis</i>
<i>Campylobacter jejuni</i>	<i>M. tuberculosis</i>
<i>C. pylori</i>	<i>Mycoplasma pneumoniae</i>
<i>C. fetus</i>	<i>Neisseria meningitidis</i>
<i>Clostridium difficile</i>	<i>N. gonorrhoeae</i>
<i>Corynebacterium diphtheriae</i>	<i>Providencia stuartii</i>
<i>Cor. jeikeium</i>	<i>Pseudomonas aeruginosa</i> *
<i>Legionella pneumophila</i>	<i>Vibrio cholerae</i>
<i>Leptospira interrogans</i>	

* This species was examined by pulse-field electrophoresis (Grothues and Tummler, 1987) whereas all other species were tested by conventional electrophoresis techniques (for further details, see Owen, 1984).

(table I) including a number of genera with species of medical importance (e.g., *Campylobacter*, *Legionella* and *Mycobacterium*) as well as strains of other microbial groups such as *Candida*, *Chlamydia*, *Giardia* and cytomegalovirus. The usefulness of such patterns as diagnostic tools is limited by their complexity because they may comprise ≥ 50 bands of various sizes, depending on the cutting frequency of the restriction endonuclease used and the genome size of the organism. It is difficult, therefore, to identify minor, but possibly significant, RFLPs in complex multiband patterns of closely related strains. There is also the possibility, when strains contain plasmids, that significant differences in the chromosomal-band pattern are obscured by the background pattern resulting from complete or partial digestion of the plasmid DNA. The detection of strain differences in DNA fingerprints relies largely on direct visual comparisons but computer methods have been used to make more objective comparisons between strain fingerprints (Sorensen *et al.*, 1985; Ades *et al.*, 1988; Bruce *et al.*, 1988).

Chromosomal probe-hybridisation patterns

When the separated DNA fragments are transferred by capillary blotting on to nitrocellulose or nylon membranes (Southern, 1975), the location on the membrane of a restriction fragment containing a particular gene or nucleotide sequence can be determined by hybridisation with a specific labelled nucleic-acid probe. The resultant hybrid-band pattern provides a unique and reproducible fingerprint, described as the chromosomal probe pattern (Tompkins *et al.*, 1986). The DNA-hybridisation reaction consists of the **probe**, the **target DNA** and a **reporter molecule** on the probe. The key compo-

Fig. 1. Agarose-gel electrophoresis of ethidium bromide-stained *Eco*RI digest fragments of chromosomal DNA from strains of *Prov. rettgeri* (total-DNA restriction-digest patterns).

nent in Southern-blot hybridisation is the probe, which is a sequence of single-stranded nucleotides containing a reporter molecule that can search out and hybridise to stretches of the target DNA- or RNA-containing complementary sequences. The possibilities for probe design are almost infinite but, broadly, they fall into two categories. Firstly, there are short probes, which are usually chemically-synthesised oligomers of *c.* 20 nucleotides, long enough to represent statistically unique sequences in the genome but sufficiently short to be destabilised by an internal mismatch in a target-probe sequence hybrid. Secondly, there are probes comprising longer nucleotide sequences, which may be between 100 or several thousand bases long, and may comprise sequences representing a significant part of the genome including specific gene sequences.

Nucleic-acid probes are used in Southern-blot hybridisation analyses with the purpose of highlighting specific DNA restriction-site heterogeneities, which in turn can be used to detect strain differences both within and between species (fig. 2). The advantage of this technique is that it greatly reduces the number of bands in the fingerprint and so facilitates comparisons between strains. Its disadvantage is that it provides information about specific regions only within the genome. The various probes used for this purpose include cloned random

chromosomal sequences (Tompkins *et al.*, 1986), toxin-production genes (Pappenheimer and Murphy, 1983) and, most widely used of all, ribosomal (r)RNA sequences. The rRNA-based probes provide a widely applicable system to investigate the molecular epidemiology of diverse bacteria (Grimont and Grimont, 1986; Stull *et al.*, 1988) whereas other probes may be more limited in being species-specific or specific only for strains within a particular species.

The rationale for using rRNA genes in DNA fingerprinting has its basis in evolutionary history because such sequences are known to be highly conserved (Woese, 1987). A bacterial rRNA cistron has a total sequence of about 4600 bases comprising several subsequences, which include genes for 23S rRNA (~2500 bases), 16S rRNA (~1500 bases), 5S rRNA (~120 bases) and various intervening and flanking sequences. In eubacteria, the rRNA genes are a small (*c.* 0.1%) but highly conserved part of the genome and are found in clusters (rRNA operons) within which they are usually linked in the order 16S-23S-5S. Furthermore, the rRNA cistrons are present in multiple copies, which vary from species to species but mostly number 11 or less. In bacteria many of the rRNA-cistron sequences appear to have changed little during evolution and so DNA probes specific for these sequences can detect a wide range of bacteria containing similar sequences. Evidence for their conservation is provided by computed homologies between known sequences of rRNA molecules from different species. The partial 16S rRNA sequences of species in the genus *Campylobacter*, for example, are well documented (Thompson *et al.*, 1988) and phylogenies based on such data have been constructed. Homologies between 16S rRNA sequences of different *Campylobacter* species are in the range 85–95%, and there is a significant degree of homology of about 80% between them and *Escherichia coli*, and representatives of other gram-negative prokaryotes even though they are phylogenetically distant from *Campylobacter* (Thompson *et al.*, 1988). The fact that *E. coli* and *Pseudomonas aeruginosa* rRNA hybridise *in vitro* with rRNA cistrons of a wide range of gram-negative and gram-positive bacteria further supports the view that such sequences are highly conserved (Grimont and Grimont, 1986).

Applications of chromosomal DNA-probe fingerprinting

A number of microbial groups has been studied by means of chromosomal DNA-probe fingerprints

Fig. 2. Southern blot-hybridisation patterns of *P. aeruginosa* strains. A biotin-labelled ribosomal cDNA probe was hybridised by standard procedures (Owen *et al.*, 1988) to filter-bound chromosomal-DNA *Hind*III fragments. All strains had identical fingerprints except for those marked X and Z, which had single-band differences. Lane Y is an *Hind*III digest of bacteriophage λ DNA to provide molecular size markers. (Results kindly provided by Dr D. Pitcher).

with the purpose of distinguishing either between allied species or between strains within a species. In a broad-ranging study, Grimont and Grimont (1986) used a ^{32}P -labelled *E. coli* 16+23S rRNA probe to detect rRNA genes in *c.* 40 different species of gram-positive and gram-negative bacteria, and among the species of medical interest examined there were representatives of *Salmonella*, *Brucella*, *Serratia*, *Pseudomonas*, *Mycobacterium* and *Listeria*. They found that the probe reacted with portions of rRNA genes in the DNA of species that were phylogenetically remote from *E. coli*, and that each species could be characterised by one or several specific patterns of rDNA-restriction fragments. Available DNA-hybridisation data showed that identical patterns were highly correlated with insignificant divergence. Furthermore, different patterns within species corresponded to significant divergence as evaluated by thermal-stability studies of DNA-DNA hybrids. The species examined and the probes and restriction enzymes used are summarised in table II. The results obtained on the species of medical interest are considered in more detail below.

DNA fingerprints of mycoplasmas and allied genera

This technique has been extensively applied to the mycoplasmas (class *Mollicutes*) and has pro-

vided a novel approach to the differentiation and identification of different species and strains. Several studies have described the use of plasmid pMC5 as a probe. This recombinant plasmid carries the 5S and 23S RNA genes and part of the 16S gene of one of the two rRNA operons of *Mycoplasma capricolum* (Amikam *et al.*, 1982; Glaser *et al.*, 1984). Due to the highly conserved nature of the prokaryotic rRNA genes, this plasmid hybridises with DNA-containing rRNA genes of any mycoplasma and so can be used in their detection and identification (Razin, 1985). When mycoplasmal DNA is cleaved with a restriction enzyme such as *Eco*RI, the hybridisation pattern consists of a relatively small number of bands because of the presence of only one or two copies of the rRNA genes in the mycoplasmal genome and the relatively few restriction sites within the operons. Because the rRNA operons in the various mycoplasmas may differ in restriction sites within the operons and in their flanking sequences, resulting hybridisation patterns are unique for the different species. Such RNA-probe hybridisation patterns revealed genotypic heterogeneity among *Acholeplasma laidlawii* and *Myco. hominis* strains but a remarkable degree of homogeneity among *Myco. pneumoniae* strains isolated from pneumonic patients during a 10-year period (Yogev *et al.*, 1988a). The genomic fingerprints of the *A. laidlawii* strains highlighted

differences that were not observed with the serological or biochemical tests used to differentiate these strains. The homogeneity of the *Myco. pneumoniae* strains was reflected also in their antigenic composition based on immunoblots and convalescent patient sera. Strain variation within *Myco. hominis* rRNA genes was also analysed with a cloned fragment of the rRNA-gene from *Mycoplasma* strain PG50 inserted in pMYC147 (Christiansen *et al.*, 1987a) and with a probe containing fragments of the genes for the α and γ subunits of the ATP synthase (*atp*) genes (Christiansen *et al.*, 1987b). Both probes revealed considerable heterogeneity within *Myco. hominis* although the subdivisions obtained by the different probes did not correspond. Another conserved-gene probe, namely the *E. coli tuf* gene coding for elongation-factor protein (EF-Tu), produced hybridisation patterns that confirmed the genotypic homogeneity of *Myco. pneumoniae* strains and the distinctions observed previously by Yogev and Razin (1986) among *Myco. gallisepticum* strains (Yogev *et al.*, 1988b). Other examples where these probes have proved useful include use in distinguishing the live vaccine F strain of *Myco. gallisepticum* from virulent field isolates of the avian pathogen and in detecting the F strain in areas where vaccination with the F strain took place (Yogev *et al.*, 1988a). The pMC5 rRNA gene probe was also tested as a means of detecting mycoplasmas in infected plants and insect material (Nur *et al.*, 1986). However, the probe reacted strongly with the rRNA-gene sequences of the plant chloroplasts and its use in detecting mycoplasmas in plants was hampered.

All the probes used in the above studies showed clear differences between the various species in their hybridisation-band patterns.

DNA fingerprints of gram-negative eubacteria

Providencia

A biotin-labelled cDNA synthesised by reverse transcriptase from a mixture of 16S and 23S rRNA from *Providencia stuartii* was used to identify rRNA cistrons in Southern blots of *Eco*RI and *Hind*III digests of *Providencia* total chromosomal DNA. The *Prov. stuartii* rDNA fingerprint was quite distinct from those of allied *Providencia* and *Proteus* species and 11 *Eco*RI-digest pattern-probe types were detected among the 26 strains of *Prov. stuartii* examined from hospitals in the UK (Owen *et al.*, 1988). It was possible to detect strain differences with this probe that were not apparent in the total-

digest patterns or in conventional test characters. This study demonstrated that biotin was an excellent probe-reporter molecule and a suitable alternative to the radiolabelled probes used in many other studies (table II). The pattern data were used also in a numerical analysis to provide a basis for estimating similarities between strains.

Pseudomonas

The genome of *P. aeruginosa* contains at least four sets of cistrons, each comprising the genes for 16S, 23S and 5S rRNA (Hartmann *et al.*, 1986). The distance between the 16S- and 23S-rRNA coding sequences was estimated to be between 100 and 700 bases and recognition sites for *Eco*RI, *Hind*III and *Bam*HI were identified within the rRNA sequences. In an epidemiological study of *P. aeruginosa* strains from patients with cystic fibrosis and a patient with endocarditis, strains were typed with a cloned 741 base-pair DNA probe derived from the region upstream of the exotoxin-A structural gene (Ogle *et al.*, 1987). More than 100 *P. aeruginosa* strains were distinguished by probing Southern blots of DNA digested with various enzymes including *Sa*II, *Bg*II and *Xho*I. Although only one hybrid fragment was detected in each strain blot, the target sequence proved to be a stable marker when tested under various in-vivo and in-vitro conditions. In many cases, typing of isolates with the probe confirmed the results obtained with serotyping, biotyping and antibiograms. The chromosomal-digest patterns supported the strain distinctions evident from the Southern-blot analysis, irrespective of the restriction enzyme used. The study also revealed that serial isolates (from individual patients), differing in morphology, serotype and antibiogram, were identical when compared in their probe-hybridisation patterns. Furthermore, isolates identical in serotyping, biotyping and antibiograms, but obtained from unrelated patients, were easily distinguished by their patterns.

Ribosomal-RNA genes were used as markers in a study of *P. cepacia* isolates from the sputum of patients with cystic fibrosis (Stull *et al.*, 1988). A *P. cepacia* probe was hybridised with *Eco*RI digests of DNA from eight strains of *P. cepacia* and polymorphisms based on patterns that comprised between four and nine distinct bands were detected in the majority. The study also showed that the use of *E. coli* rRNA as a probe yielded similar RFLP patterns to the *P. cepacia*-derived rRNA probe and provided further evidence that *E. coli* rRNA offers a widely applicable system to investigate the

Table II. Summary of microbial species investigated by chromosomal DNA-probe fingerprinting

Species	Probe(s)	Restriction endonuclease(s) used	Reference
Section 1. VARIOUS EUBACTERIA			
<i>Serratia</i> , <i>Salmonella</i> , <i>Citrobacter</i> , <i>Pseudomonas</i> , <i>Acinetobacter</i> , <i>Brucella</i> , <i>Listeria</i> , mycobacteria, <i>Bordetella</i> and others	16S + 23S rRNA from <i>E. coli</i> and <i>P. aeruginosa</i>	<i>EcoRI</i> , <i>BamHI</i> , <i>HindIII</i>	Grimont and Grimont (1986)
Section 2. MYCOPLASMAS			
<i>Acholeplasma laidlawii</i>	pMC5 containing 16 + 23S + part 16S rRNA gene of <i>Myco capricolum</i>	<i>EcoRI</i> , <i>PstI</i>	Yogev and Razin (1986), Yogev <i>et al.</i> (1988a)
<i>Myco. gallisepticum</i>	As above ptufA containing <i>E. coli</i> <i>tufA</i> gene and flanking sequences	<i>HindIII</i> <i>HindIII</i> , <i>EcoRI</i> , <i>BglII</i>	Yogev and Razin (1986) Yogev <i>et al.</i> (1988b)
<i>Myco. genitalium</i>	rRNA gene probe pMC5	<i>HindIII</i>	Yogev and Razin (1986)
<i>Myco. hominis</i> and other species	pMYC4—containing insert of rRNA gene of <i>Mycoplasma</i> PG50	<i>EcoRI</i>	Christiansen <i>et al.</i> (1987a)
<i>Myco. hominis</i>	pMYC405 containing part of <i>atp</i> operon of <i>Mycoplasma</i> PG50	<i>EcoRI</i> , <i>HindIII</i> <i>EcoRI</i> , <i>BglII</i>	Christiansen <i>et al.</i> (1987b)
<i>Myco. pneumoniae</i>	pMC5 containing 5S + 23S + part 16S rRNA genes of <i>Myco. capricolum</i>	<i>EcoRI</i> , <i>HindIII</i>	Yogev <i>et al.</i> (1988b)
Various <i>Mycoplasma</i> species	As above As above	<i>ClaI</i> <i>HindIII</i>	Yogev <i>et al.</i> (1988b) Yogev <i>et al.</i> (1988b)
Section 3. GRAM-NEGATIVE EUBACTERIA			
<i>Providencia and Proteus</i>			
<i>Providencia stuartii</i> , <i>Prov. alcalifaciens</i> , <i>Prov. rustigianii</i> , <i>Proteus vulgaris</i>	<i>Prov. stuartii</i> 16S + 23S rDNA	<i>EcoRI</i> , <i>HindIII</i>	Owen <i>et al.</i> (1988)
<i>Prot. vulgaris</i>	Synthetic oligonucleotide probe PV25 of selected 16S rRNA sequences	<i>EcoRI</i>	Haun and Gobel (1987)
<i>Pseudomonas</i>			
<i>Pseudomonas aeruginosa</i>	<i>E. coli</i> exotoxin-A gene and associated sequences <i>E. coli</i> 16S and 23S/5S rDNA	<i>SaII</i> , <i>XhoI</i> , <i>BglII</i> , <i>BamHI</i> <i>BamHI-EcoRI</i> , <i>BamHI-Hind III</i>	Ogle <i>et al.</i> (1987) Hartmann <i>et al.</i> (1986)
<i>P. cepacia</i>	<i>E. coli</i> and <i>P. cepacia</i> rRNA	<i>EcoRI</i>	Stull <i>et al.</i> (1988)
<i>Haemophilus</i>			
<i>Haemophilus influenzae</i>	<i>E. coli</i> and <i>H. influenzae</i> rRNA	<i>EcoRI</i>	Stull <i>et al.</i> (1988)
<i>H. influenzae</i> biovar aegyptius	<i>E. coli</i> rRNA	<i>EcoRI</i> , <i>EcoRV</i>	Irino <i>et al.</i> (1988)
<i>Salmonella</i>			
<i>Salmonella typhimurium</i> , <i>S. enteritidis</i> , <i>S. dublin</i>	Cosmid clones of <i>S. enteritidis</i> random genome sequences	<i>EcoRI</i> , <i>HindIII</i> , <i>PstI</i>	Tompkins <i>et al.</i> (1986)
<i>Legionella</i>			
<i>Legionella pneumophila</i> and other <i>Legionella</i> species	Cloned fragment containing 16S + 23S rRNA genes of <i>L. pneumophila</i>	<i>NciI</i>	Saunders <i>et al.</i> (1988)

Species	Probe(s)	Restriction endonuclease(s) used	Reference
Section 3. GRAM-NEGATIVE EUBACTERIA (cont.)			
<i>Campylobacter</i>			
<i>Campylobacter jejuni</i> and other species	17-nucleotide synthetic probe complementary to a unique sequence in 16S rRNA of <i>Campylobacter</i>	<i>EcoRV</i> , <i>RsaI</i>	Romaniuk and Trust (1987)
<i>Xanthomonas</i>			
<i>Xanthomonas campestris</i> pathotypes	Cosmid clones of <i>X. campestris</i> random genome sequences	<i>EcoRI</i>	Lazo <i>et al.</i> (1987)
Section 4. GRAM-POSITIVE EUBACTERIA			
<i>Bacillus</i>			
<i>Bacillus</i> species	Various cloned rDNA probes	<i>EcoRI</i>	Gottlieb and Rudner (1985)
<i>Corynebacterium</i>			
<i>Corynebacterium diphtheriae</i>	corynephage DNA	<i>EcoRI</i>	Pappenheimer and Murphy (1983)
	<i>tox</i> gene probe	<i>BamHI</i>	Groman <i>et al.</i> (1983)
<i>Mycobacterium</i>			
<i>Mycobacterium avium</i> , <i>M. intracellulare</i> , <i>M. paratuberculosis</i> , <i>M. lepraemurium</i> , <i>M. paratuberculosis</i> , <i>M. avium</i> , <i>M. kansasii</i> , <i>M. phlei</i> , and C-D isolated	recombinant clones in vector pGEM-1 of DNA of <i>M. paratuberculosis</i> strain BEN (8 different probes) As above	<i>PvuII</i> <i>PvuII</i>	McFadden <i>et al.</i> (1987b) McFadden <i>et al.</i> (1987a)
<i>Mycobacterium</i> strains <i>M. tuberculosis</i> , <i>M. phlei</i> , <i>M. intracellulare</i> , and <i>M. smegmatis</i>	16S rRNA, 16S + 23S rRNA and 23S + 5S rRNA probes of the <i>E. coli</i> rrnB operon	<i>PstI</i>	Bercovier <i>et al.</i> (1986)
Section 5. OTHER MICROBIAL GROUPS			
<i>Candida</i>			
<i>Candida albicans</i> and other species	plasmid containing <i>Saccharomyces cerevisiae</i> rDNA (17S + 25S rRNA genes)	<i>EcoRI</i>	Magee <i>et al.</i> (1987)
<i>Cyanobacteria</i>			
Cyanobacteria including species of <i>Anacystis</i> , <i>Anabaena</i> and <i>Nostoc</i>	rRNA	<i>HindIII</i>	Nichols <i>et al.</i> (1982)

molecular epidemiology of genetically diverse bacteria.

Haemophilus

The binding patterns obtained from probing whole-cell DNA with *E. coli* rRNA were used to test the genetic relatedness of 10 non-typable isolates of *H. influenzae* (Stull *et al.*, 1988). The banding patterns, comprising up to eight or nine bands, were heterogeneous except for those from three epidemiologically related strains from the

trachea and blood of an infant and from the mother's cervix, all of which had identical patterns.

The rRNA gene-restriction patterns of 92 strains of *H. influenzae* biovar *aegyptius* that were associated with conjunctivitis or Brazilian purpuric fever (BPF) were studied by the use of *E. coli* 16 + 23S rRNA as a probe (Irino *et al.*, 1988). All strains were classified in one of 15 patterns, one of which was associated with most of the cases of BPF, although all patterns were seen among the conjunctival isolates. Most strains showed 6–8 strong bands and 1–5 weak bands. The efficiency of rRNA gene-

restriction patterns as an epidemiological tool compared with clinical history, plasmid-restriction fingerprinting, protein electrophoresis and multi-locus-enzyme electrophoresis was demonstrated in a companion study (Brenner *et al.*, 1988) which showed that strains ascribed to two different RFLP patterns were not separated by any of the other methods.

Salmonella

Sequences of cloned chromosomal DNA were used as a probe to highlight restriction-site heterogeneity and to differentiate between strains of three *Salmonella* serotypes (Tompkins *et al.*, 1986). Analysis of *Hind*III-digested DNA from isolates of *S. typhimurium* from 11 outbreaks by probing with cosmid-containing random chromosomal inserts from *S. enteritidis*, revealed six unique fingerprint patterns. Patterns containing about eight bands were reported for *S. dublin* and six different fingerprint patterns were observed in the 24 isolates of this serotype. The fingerprinting method detected differences in three strains that could not be demonstrated by other means. The probes clearly distinguished between the three *Salmonella* serotypes and all isolates considered to be epidemiologically and phenotypically distinct were grouped. Several isolates that were not related epidemiologically and had unique phenotypes (including plasmid profiles and phage types) nevertheless had similar chromosomal-probe fingerprint patterns. It was concluded that isolates giving homogeneous patterns were derived from a single clone, which had been disseminated to several geographical locations over a number of years. In some cases plasmids appeared to have been acquired by some clones after spread, so providing evidence of more recent evolution. These results suggested that *S. enteritidis* contained highly conserved sequences because isolates recovered over a 30-year period from widely separated locations showed few variations.

Legionella

DNA probes have proved particularly useful in distinguishing *L. pneumophila* from allied species (Grimont *et al.*, 1985), and in strain characterisation (Saunders *et al.*, 1988). The latter workers used a cloned *Eco*RI DNA fragment from *L. pneumophila*, which included 16S and 23S rRNA genes, as a probe to identify members of the genus *Legionella* and they found that the type strains of 28 species gave different patterns in every case. Also the

variation between and within strains of different recognised serogroups within *L. pneumophila* was assessed and variable patterns of at least 10 bands were detected in *Nci*I DNA digests.

Campylobacter

*Eco*RV or *Rsa*I digests of chromosomal DNA from strains of *C. jejuni*, *C. fetus*, *C. coli* and *C. laridis* were probed with a 17 nucleotide-long probe complementary to a unique sequence present in the 5' domain of the 16S ribosomal RNA of *Campylobacter* (Romaniuk and Trust, 1987). Six distinct hybridisation patterns were obtained with differences between species as well as between individual members of the same species. It was suggested that DNA fingerprinting with this probe was a potentially useful identification method in epidemiological studies of *Campylobacter* infections.

DNA fingerprints of mycobacteria

The rRNA genes of a number of *Mycobacterium* species have been studied principally with the aim of distinguishing between species. Cox and Katoch (1986) found that the distribution of *Bam*HI and *Eco*RI sites within the rRNA genes and flanking sequences of *M. phlei* and *M. smegmatis* were characteristic for each of these species. Hybridisation with labelled rRNA from those species revealed between three (*Eco*RI) and five bands (*Bam*HI) containing homologous sequences. It was concluded that the sequences flanking the rRNA genes were not identical and that the sequences of the rRNA genes themselves were significantly different in the two species.

In studies of the rRNA genes of *M. tuberculosis*, *M. intracellulare* and other species (Bercovier *et al.*, 1986) different regions of the *E. coli* *rrnB* operon were used as gene probes. It was concluded from the resulting hybridisation patterns that the slow-growing species (*M. tuberculosis* and *M. intracellulare*) appeared to possess only one rRNA operon whereas the faster-growing species (*M. phlei* and *M. smegmatis*) had two rRNA operons. Similar results were reported by Suzuki *et al.* (1987) who found that *M. bovis* strain BCG carried at most two copies of rRNA genes. The genes were arranged in the order 16S, 23S and 5S rRNA but no tRNA gene was detected in the spacer region between the 16S and 23S rRNA genes. Cloned DNA from an unclassified *Mycobacterium* isolated for a patient with Crohn's disease (CD) was used as a probe to identify RFLPs in DNA samples from various

species of *Mycobacterium* and to examine their relationships (McFadden *et al.*, 1987a). It was found that three CD-isolated strains were indistinguishable from each other and from the type strain of *M. paratuberculosis*. Furthermore, many RFLPs were found that distinguished between *M. paratuberculosis*, representatives of the *M. avium* complex (serotypes 2 and 5), *M. kansasii* and *M. phlei*. The same DNA probes identified RFLPs providing evidence that the *M. avium*-complex could be divided into *M. avium* and *M. intracellulare* (McFadden *et al.*, 1987b). These data were also used as the basis of a phylogenetic analysis of the *M. avium*-complex.

DNA fingerprints of other microbial genera

Other microbial groups not discussed above but examined by Southern-blot hybridisation analysis to distinguish both between different species and between strains within species include various cyanobacteria (Nichols *et al.*, 1982), pathotypes of *Xanthomonas campestris* (Lazo *et al.*, 1987), *Bacillus* species (Gottlieb and Rudner, 1985), *Corynebacterium diphtheriae* (Groman *et al.*, 1983), and *Candida albicans* and associated species (Magee *et al.*, 1987). Further details on the probes used are listed in table II.

Comments and conclusions

The substantial set of data now available on probe-hybridisation patterns of chromosomal-DNA digests demonstrates that they provide a valuable new method of distinguishing among related species and, most significantly, of detecting variation among strains within species. The principal advantages and disadvantages of the method are summarized in table III. The most important advantage is the possibility that it offers a universally applicable method of identifying, and possibly of typing, any micro-organism. The approach is facilitated by the use of broad-spectrum probes, such as those based on rRNA genes, but other more specialised probes may be needed for increased discrimination or specificity within particular spe-

Table III. Advantages and disadvantages of DNA fingerprinting by probe hybridisation

Advantages

- Applicable to any micro-organism
- Reproducible
- Wide range of restriction enzymes available
- Commercially available rRNA can be used as a broad-spectrum probe
- Biotin is an excellent reporter molecular in probes
- Highly sensitive to minor genome variations
- Patterns are relatively simple (≤ 10 bands)
- Pattern matching can be computerized*

Disadvantages

- Methodologies relatively time consuming
- Loci detected by probe may not be representative of genome

* Commercially available software enables band detection, quantification and automatic pattern-matching (RFLP Interpretation Software, BioImage, Ann Arbor, MI, USA).

cies. Because the patterns are amenable to computer analysis, it should be possible in the future to establish data bases of profiles for identification purposes.

DNA fingerprinting is relatively laborious at present and, hence, unsuitable for routine use in the clinical laboratory. However, it has an important place in a reference laboratory if used in conjunction with existing methods of epidemiological typing, particularly in the investigation of strains that are untypable by other methods. Research is needed to evaluate fully DNA fingerprinting as a typing method, particularly in establishing its discriminatory power, although there is evidence that it is superior to some of the other methods currently used as shown for instance by the studies on *A. laidlawii* (Yogev *et al.*, 1988a), *C. albicans* (Hunter and Gaston, 1988), *H. influenzae* (Brenner *et al.*, 1988), *P. aeruginosa* (Ogle *et al.*, 1987). Finally, it is important that the probe-hybridisation patterns should be interpreted in conjunction with the total chromosomal-digest patterns because in some circumstances the latter may provide a more sensitive measure of variation between closely related strains at the sub- and infrasubspecific level.

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