JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1999, p. 3083–3091 Vol. 37, No. 10
0095-1137/99/$04.00+0

MINIREVIEW

Amplified-Fragment Length Polymorphism Analysis: the State of an Art

P. H. M. SAVELKOUL,[1]* H. J. M. AARTS,[2] J. DE HAAS,[3] L. DIJKSHOORN,[4] B. DUIM,[5] M. OTSEN,[6] J. L. W. RADEMAKER,[7] L. SCHOULS,[8] AND J. A. LENSTRA[6]

Department of Medical Microbiology and Infection Control, University Hospital Vrije Universiteit, Amsterdam,[1] DLO-State Institute of Quality Control of Agricultural Products Centre De Born, Wageningen,[2] Novartis Seeds bv, Section Biotechnology, Enkhuizen,[3] Department of Medical Microbiology, Leiden University Medical Centre, Leiden,[4] Department of Bacteriology, DLO-Institute of Animal Science and Health,[5] Lelystad, Institute of Infectious Diseases and Immunology,[6] and Netherlands Culture Collection of Bacteria,[7] Utrecht University, Utrecht, and Research Laboratory for Infectious Diseases National Institute of Public Health and the Environment, Bilthoven,[8] The Netherlands

In the past decade, various methods have been developed for the identification and typing of prokaryotic and eukaryotic organisms at the DNA level. These methods differ in their taxonomic range, discriminatory power, reproducibility, and ease of interpretation and standardization (62, 67, 86, 87, 101, 106, 110, 116). The ideal genotyping method produces results that are invariable from laboratory to laboratory and allows unambiguous comparative analyses and the establishment of reliable databases.

One of the newest and most promising methods is amplified-fragment length polymorphism (AFLP) analysis (11, 118, 122), developed by Keygene BV, Wageningen, The Netherlands. This method combines universal applicability with high powers of discrimination and reproducibility (45). An increasing number of reports describe the use of AFLP analysis for plant and animal genetic mapping, medical diagnostics, phylogenetic studies, and microbial typing. This minireview describes the principles, advantages, and disadvantages of AFLP analysis and summarizes its applications in different fields.

PRINCIPLE OF AFLP

In the nomenclature of Vaneechoutte (110), AFLP analysis belongs to the category of selective restriction fragment amplification techniques, which are based on the ligation of adapters (i.e., linkers and indexers) to genomic restriction fragments followed by a PCR-based amplification with adapter-specific primers. For AFLP analysis (Fig. 1), only a small amount of purified genomic DNA is needed; this is digested with two restriction enzymes, one with an average cutting frequency (like *Eco*RI) and a second one with a higher cutting frequency (like *Mse*I or *Taq*I). Double-stranded oligonucleotide adapters are designed in such a way that the initial restriction site is not restored after ligation, which allows simultaneous restriction and ligation, while religated fragments are cleaved again. An aliquot is then subjected to two subsequent PCR amplifications under highly stringent conditions with adapter-specific primers that have at their 3′ ends an extension of one to three nucleotides running into the unknown chromosomal restriction fragment. An extension of one selective nucleotide amplifies 1 of 4 of the ligated fragments, whereas three selective nucleotides in both primers amplify 1 of 4,096 of the fragments. The PCR primer which spans the average-frequency restriction site is labeled. After polyacrylamide gel electrophoresis a highly informative pattern of 40 to 200 bands is obtained. The patterns obtained from different strains are polymorphic due to (i) mutations in the restriction sites, (ii) mutations in the sequences adjacent to the restriction sites and complementary to the selective primer extensions, and (iii) insertions or deletions within the amplified fragments.

Since the original publication by Vos et al. in 1995 (118) several enzyme combinations have been used, such as *Eco*RI, *Pst*I, *Hin*dIII, or *Apa*I combined with *Mse*I or *Taq*I. For animal genomes *Eco*RI and *Taq*I appear to be the most suitable (2). Alternative AFLP typing procedures are based on one enzyme with a single adapter and analysis by agarose gel electrophoresis (32, 105). A major improvement has been obtained by switching from radioactive to fluorescently labeled primers for detection of fragments in an automatic sequence apparatus (54). In addition, it has been shown that for small bacterial and fungal genomes a single PCR amplification with one and two selective nucleotides, respectively, on both primers is sufficient (25, 45, 46, 53, 54, 69, 84, 107).

DATA ANALYSIS

In DNA fingerprinting, the present-day challenge is to compile standardized patterns in a database for interlaboratory use and future reference. This requires an accurate measurement of fragment lengths. Analysis via molecular weight markers in adjacent lanes is straightforward and can be done automatically on digitized images. However, normalization on the basis of external standards has a limited accuracy and is not always adequate for comparisons of the complex AFLP patterns from different gels. A better correction for variation in migration rates and gel distortions is achieved by coelectrophoresis in each lane of both sample and marker fragments. One option is the use of invariant fragments with known lengths as internal markers. More elegant is the use of fluorescent labels with different emission spectra (FAM, ROX, JOE, TAMRA) for

* Corresponding author. Mailing address: Department of Medical Microbiology and Infection Control, University Hospital Vrije Universiteit, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands. Phone: 31 204440552. Fax: 31 204440473. E-mail: p.savelkoul@azvu.nl.

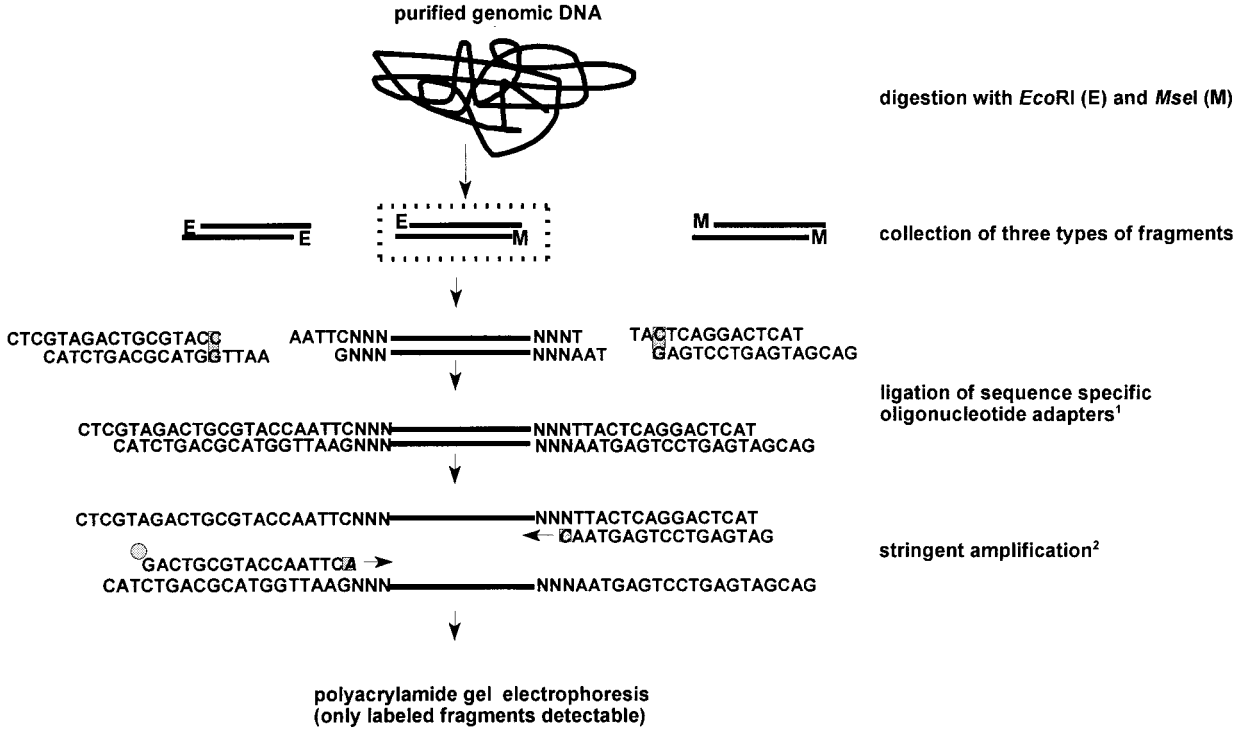

FIG. 1. Schematic representation of the AFLP analysis principle. 1, point mutations incorporated in the adapter sequences to prevent digestion after ligation are shaded. 2, one of the primers is labeled. In this representation both primers contain one selective nucleotide (shaded) in the unknown fragment.

analysis on a Perkin-Elmer ABI automated sequencer (25, 26, 32).

Digitized images in a standard graphical file may be obtained as scanned autoradiographs, as direct output of the Vistra (Texas-Red label; Amersham Pharmacia Biotech) or LI-COR (near-infrared cyanine dye; LI-COR, Lincoln, Nebr.) sequencers, or after conversion of raw data produced by the Amersham Pharmacia automated laser fluorescence sequencers (fluorescein isothiocyanate and Cy5 label) (54). These data can be imported in gel analysis software like GelCompar software (Applied Maths, Kortrijk, Belgium). ABI data are processed by the Perkin-Elmer GeneScan software, which carries out the normalization and fragment sizing for analysis by the dedicated ABI GenoTyper software. However, this program has no options for export of data to standard graphical formats or for cluster analysis, which is essential for epidemiological and phylogenetic analyses. A recent addition to the GelCompar software enables direct import of ABI as well as ALF data for an accurate normalization, background subtraction, and cluster analysis.

Two methods are used to compare fingerprinting patterns. With patterns of low complexity (<20 peaks and similar peak heights), band positions are assigned to peaks and similarity is calculated on the basis of band presence versus band absence. With complex patterns, such as those produced by AFLP analysis, it is more accurate to calculate the product-moment correlation coefficient (78, 99) of each pair of densitometic curves, which takes into account the whole of each curve without the assignment of bands. This method is insensitive to relative differences in concentrations. Both methods are available in the GelCompar package.

Proprietary software of Keygene BV converts AFLP patterns to digital genotypes and detects heterozygosities apparent from half-intense bands. Recently, a commercial version, Quantar, has been made available for dominant scoring. The CrossChecker freeware (123) offers automatic lane recognition, a convenient interactive conversion of complex gel patterns into 1/0 data matrices for cluster analysis, and codominant scoring. A discussion of these and other software packages that are useful for the analysis of AFLP data sets like ImageMaster, 1D Elite, Dendron, NT-SYS, and the Phylip and Felsenstein programs is beyond the scope of this review (30, 82, 90, 98).

COMPARISON OF AFLP ANALYSIS WITH OTHER TECHNIQUES

We compare the performance of AFLP analysis with those of other methods with respect to (i) reproducibility and robustness, (ii) discriminatory power, (iii) typeability, and (iv) operational aspects.

Reproducibility and robustness. Since relatively small amounts of DNA are digested and detection of AFLP fragments does not depend on hybridization, partial digestion and faint patterns, which are sources of irreproducibility with restriction fragment length polymorphism (RFLP) genotyping, can easily be avoided (110). Furthermore, the possibility of using stringent PCR annealing temperatures renders the AFLP analysis method more reproducible and robust than random amplified polymorphic DNA (RAPD) analysis (11). This was demonstrated in a recent between-laboratory comparative test by Jones et al. (48). The intragel-specific correlation levels were evaluated by Huys et al. (42) with *Aeromonas* spp. and were found to be as high as 95.0 to 98.5%. Similar results were reported by other groups (45–47, 53, 54, 118).

The main source of ambiguity in the comparison of AFLP patterns is a variation of peak heights, which is probably due to differences in PCR efficiency. As a consequence, the detection

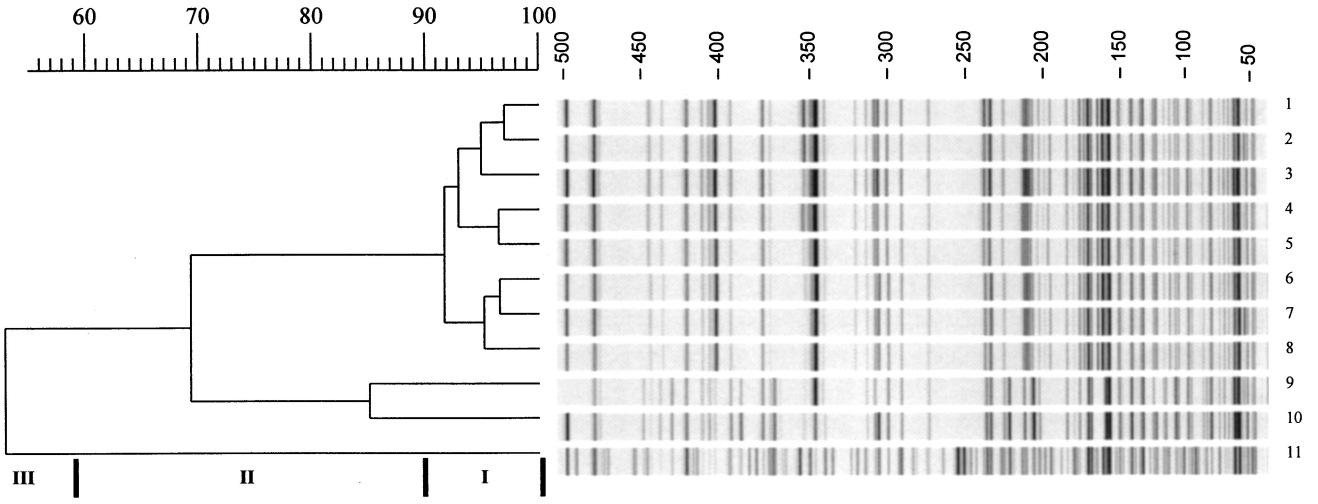

FIG. 2. Example of fluorescently labeled AFLP patterns and dendrogram for 11 different *Klebsiella* isolates. Patterns are the result of amplification of templates generated after restriction and ligation as shown in Fig. 1. The fragments were analyzed on an automated Vistra sequencer (Amersham-Pharmacia Biotech). The dendrogram was constructed with GelCompar (Applied Maths) software by using the Pearson correlation and cluster analysis by the unweighted pair group method using arithmetic averages. Percentages of similarity and molecular sizes (in base pairs) are shown above the dendrogram. Lanes 1 to 8, identical *Klebsiella pneumoniae* isolates; lanes 9 and 10, different *K. pneumoniae* strains; lane 11, a *Klebsiella oxytoca* strain. Within the AFLP patterns from *Klebsiella*, for instance, three windows of similarity may be applicable on the basis of the described experimental conditions: window I, 90 to 100% homology, identical strains; window II, 60 to 90% homology, different strains, same species (e.g., *Klebsiella pneumoniae*); window III, 40 to 60% homology, different species of the same genus; window IV, less than 40% homology, species from different genera.

of minor fragments may depend on the detection level and the amount of DNA loaded.

Discriminatory power. Genotyping methods differ in their power of discrimination, depending on the taxonomic level and category. In bacteriology, discrimination to the species level is mostly referred to as identification, while typing denotes differentiation to the strain level. AFLP analysis can be used both for identification and for typing. Figure 2 illustrates a typical AFLP analysis of *Klebsiella* with *Eco*RI and *Mse*I primers and one selective nucleotide on each primer. Typing and identification can be standardized by defining windows of similarity. For instance, patterns with 90 to 100% homology are considered to be derived from identical strains, patterns with 60 to 90% homology indicate different strains from the same species, while 40 to 60% homology is obtained with isolates of the same genus but of different species. Less than 40% homology denotes isolates from different genera. The same windows of discrimination were applicable to *Acinetobacter* and *Xanthomonas* (47, 84). AFLP analysis is not informative at the taxonomic level of the genus or family (47, 84), because at <40% similarity only a few bands are shared and unrelated species may become clustered. So for bacteria AFLP analysis seems to have the same taxonomic range as other fingerprinting techniques like RAPD analysis, pulsed-field gel electrophoresis (PFGE) and RFLP analysis, repetitive DNA sequence-based PCR (rep-PCR), and protein profiling (Fig. 3). AFLP combines several advantages of these different techniques, which in most cases results in the highest power of discrimination (23–25, 46, 53, 54, 79, 84, 105). Like RFLP analysis, RAPD analysis, PFGE, and rep-PCR, but unlike amplified ribosomal DNA restriction analysis (ARDRA), AFLPs correspond to mutations that are dispersed over the genome. Like PFGE, RFLP analysis, and ARDRA, variation by AFLP analysis is based on mutations in restriction sites or length variation of restriction fragments, but AFLP analysis also exploits the variation in the nucleotides that match the selective 3′ ends. Most important is that AFLP analysis displays more fragments than other fingerprinting techniques, sometimes with the exception of RFLP analysis (110).

For *Acinetobacter baumannii* RAPD analysis with five different primers was needed to obtain the same significance of clustering as AFLP analysis and DNA hybridization (54). Comparison of RAPD analysis, PFGE, and AFLP analysis for *Pseudomonas* (99) showed that AFLP analysis without selective nucleotides had the highest discriminatory power according to the criteria of Tenover et al. (102). However, PFGE was superior to AFLP analysis with one selective nucleotide on both primers. Another study of Desai et al. (23) demonstrated that AFLP analysis differentiated strains within the *Streptococcus pyogenes* group A better than PFGE did. For *Xanthomonas*

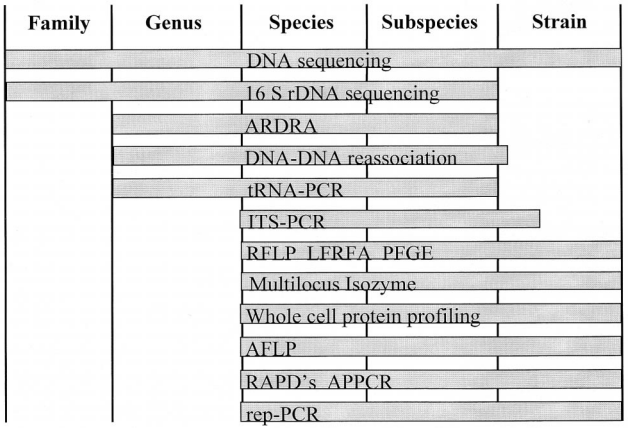

FIG. 3. Relative applicability of various fingerprinting and DNA techniques at different levels of taxonomic resolution. Reprinted from reference 83 with permission of the publishers. tRNA-PCR, tRNA intergenic spacer region PCR; ITS-PCR, 16S-23S rRNA intergenic spacer region PCR; LFRA, low-frequency restriction enzyme analysis; AP-PCR, arbitrarily primed PCR. The other abbreviations are defined in the text.

AFLP analysis even differentiated individual strains within the subdivisions of *Xanthomonas axonopodis* (45).

rep-PCR methods exploit the variation of insertion sites of interspersed repetitive elements. Universal for most gram-positive and -negative bacteria are ERIC, REP, and BOX elements (83, 115). A detailed comparison of DNA hybridization with AFLP analysis as well as rep-PCR genomic fingerprints of xanthomonads showed that the clustering based on DNA hybridization was reproduced by AFLP analysis, while the ERIC PCR revealed more strain variation within the clusters. Moreover, the clear correlation between genomic DNA-DNA hybridization and similarity coefficients of fingerprints of AFLP analysis and rep-PCR did not depend on the statistical model, classification scheme, or scoring method (84). This suggests that AFLP analysis and other fingerprinting methods can function as core techniques in a polyphasic taxonomy system, complementing methods based on DNA-DNA homology.

More species-specific repeats are the *Mycobacterium* IS*6110* transposon (111) and the *Mycobacterium* direct repeats (51). With mycobacteria it was found that spoligotyping, which exploits the variability of a mycobacterial repeat structure, may have a higher power of discrimination at the strain level than IS*6110* typing, which depends on the number of insertion sequence elements (51).

In plants, AFLP analysis has been found to be more informative than RAPD analysis (11, 22, 62, 80, 92), RFLP analysis (38, 60, 80, 92) and simple sequence repeat analysis (80, 92) but as informative as repeat-based fingerprinting (27, 95). In animals and humans microsatellite-based genotyping is probably more useful for linkage analysis, parentage testing, and forensic identification, but AFLP analysis seems more suitable at the subspecies and zoological family levels (16). However, scoring of the presence or absence of an AFLP band of dizygotic organisms yields dominant markers, and accurate quantitation of band intensities and special software are needed to discriminate homozygotic and heterozygotic signals for codominant scoring.

Typeability. A unique feature of AFLP analysis is that it can be adapted to the DNA of any organism via the use of selective nucleotides. Isolation of typeable DNA from microorganisms that produce endonucleases, like *Clostridium*, may present some problems that hamper every DNA-based typing method. A basic limitation of AFLP analysis (and other genomic typing procedures) is that the organism to be typed must be isolated, since DNA from other sources disturbs the AFLP pattern. For example, typing of bacteria in tissue, stool, or soil samples by the direct use of such samples may be feasible with specific PCR or hybridization assays but not with AFLP analysis.

Operational aspects. Since AFLP analysis is a PCR-based assay, only a small amount of DNA is required, e.g., 10 to 100 ng from one to three bacterial colonies or 0.1 to 1 μg of eukaryotic DNA from 50 μl of blood. This DNA must be pure and double stranded, but its exact concentration seems to be less critical than it is for RAPD analysis.

Oligonucleotide adapters and primers can be custom synthesized. Commercial kits for AFLP typing of plants and microorganisms (Perkin-Elmer Biosystems, Foster City, Calif.) are also available. The subsequent digestions, ligations, amplifications, and analysis on a polyacrylamide gel are relatively time-consuming, but with fluorescent primers and analysis on an automatic sequencer, AFLP analysis can be performed within 24 h.

Analysis on an automated sequencer with software to analyze the informative but complex banding patterns allows an automatic compilation of a database and comparison of the patterns to reference patterns. The reproducibility is high enough to compare patterns to patterns of later or previous isolates or even to patterns obtained from other laboratories.

A quantification of the signal is required for discrimination of homo- and heterozygotes and for the comparison of expression patterns on cDNA (5, 35, 49, 66). This can be accomplished by phosphorimaging of radioactive gels, with capillary-based automatic sequencing machines, and with the LI-COR slab gel apparatus but not with other slab gel machines or by the scanning of X-ray films.

APPLICATIONS IN PLANT AND ANIMAL GENETICS

We will briefly review applications of AFLP analysis that have proven to be useful in fields other than microbiology.

Plant molecular genetics. (i) Phylogeny and diversity. In plants, AFLP analysis is a multilocus PCR technology that generates as many as 150 locus-specific bands, a high percentage of which can be polymorphic. Estimates of genetic distances based on differences in AFLP patterns are informative about genetic diversity (33, 80, 94, 95, 104), phylogeny (38, 95), and the geographic origins of genotypes and gene pools of plants (8, 36, 77).

(ii) Breeding. It has been verified that despite some apparent clustering (85, 109) AFLP markers (i.e., discriminatory fragments by AFLP analysis) cover all chromosomes and are inherited in a Mendelian way (3), which is a prerequisite for molecular marker applications in breeding analysis. The AFLP technology has four major applications in marker-assisted breeding (13).

(a) Variety identification. F_1 hybrids are the result of a cross between a female and male parental homozygous breeding line and often have agronomic performance superior to those of the parental homozygous lines. However, self-pollination in the female line and pollen from other male lines may interfere with the production of F_1 seeds. AFLP analysis allows the identification of the contaminating variety (65).

(b) Germplasm management. AFLP profiles of breeding lines and F_1 hybrids were compiled in a database (61). The information accumulated in this database allows the prediction of the agronomic performance of F_1 hybrids on the basis of their relative genetic distance.

(c) Indirect selection of agronomically important properties (traits). For monogenic traits, AFLP analysis needs a large collection of primer pairs to yield markers closely linked to these traits for diagnostic use (64). This technique allowed the localization of genes that confer resistance to viruses, nematodes, fungi, or bacteria (6, 7, 14, 17, 50) and positional cloning of the relevant genes (103). For the localization of polygenic quantitative traits AFLP analysis allows the fast and efficient construction of dense genetic maps (20, 81). Genotyping of large segregating populations (70, 75, 117) can lead to the localization of one or more quantitative trait loci.

(d) Backcross breeding. To transfer inherited traits into elite parental genotypes a donor parent carrying a trait of interest is crossed to an elite line without this trait. The resulting F_1 line is crossed back to the elite line during selection of individual plants with the desired trait. After about six generations, the average contribution of the donor parent has been reduced to an average of 1%, but this percentage is variable. AFLP analysis can then be used to select backcross offspring with the lowest percentage of donor-specific alleles or offspring with crossovers near the trait to minimize negative linkage drag (114).

Animal genetics. The availability of dense microsatellite maps, which already allowed the localization of numerous genetic diseases and traits, has delayed the application of AFLP

analysis to linkage analysis for map-rich species (humans, mice, and domestic animals). AFLP analysis, however, made it possible to map a blood pressure quantitative trait locus in the rat after integration of AFLP markers in the microsatellite map (73). A dense AFLP genetic map of rabbits for which no microsatellite map was available has been constructed in a relatively short time period (113). AFLP analysis is especially useful for estimation of the divergence of nuclear genomes of related species, as demonstrated for wild and domestic cattle species (16). For this application, AFLP analysis is more sensitive than the comparison of gene sequences and is, unlike by comparison of mitochondrial sequences, not disturbed by anomalous inheritance of the maternal lineage. Within species, AFLP analysis yields a direct estimation of genetic diversity among and within domestic breeds as an alternative to the tedious measurement of microsatellite allele frequencies (2).

APPLICATIONS IN MICROBIOLOGY

Below we review examples of the application of AFLP analysis for microbial identification and strain typing.

Lower eukaryotes. (i) Parasites. AFLP markers have been used to analyze populations of *Haemonchus contortus*. AFLP analysis revealed a remarkably high degree of genetic diversity within non-inbred *Haemonchus* populations; this diversity was hardly reduced by drug selection but was clearly decreased by inbreeding (74). AFLP combinations also proved to be successful in the genetic analysis of populations of the potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* (31).

(ii) Fungi. A discrimination of intraspecific as well as interspecific genetic variation of *Cyphomyrmex minutus*, mycorrhizal fungi, and *Fusarium* species by AFLP analysis has been described previously (58, 69, 91). Van der Lee et al. (108) constructed a comprehensive genetic linkage map of the plant pathogen *Phytophtora infestans*. AFLP analysis also allowed accurate strain typing of medically relevant fungi-like *Aspergillus fumigatus* and Candida spp. (107).

Bacteria. Janssen et al. (45) pioneered the AFLP analysis of bacterial genomes, optimized the experimental conditions for 147 strains of nine different bacterial species, and presented a computerized data analysis. The effects of different restriction enzymes and selective primer extensions on the discriminatory power of AFLP analysis for discriminating different species were evaluated. A clear clustering of strains of the same bacterial species was found. The results showed evidence of the potential of AFLP analysis in epidemiological and evolutionary studies. Since then, several investigators have reported on the application of AFLP analysis in bacterial genetics.

(i) Taxonomy. One of the first genera extensively studied by AFLP analysis was *Aeromonas* (39–44). Huys et al. (39) clustered the AFLP patterns of 125 *Aeromonas* strains into one DNA hybridization group, while different strains within this group could be differentiated. AFLP analysis of another comprehensive panel of 98 *Aeromonas* strains representing the 14 hybridization groups as well as four species not yet allocated to a hybridization group revealed a strong correlation with DNA hybridization, which is still considered the "gold standard" (28, 42, 120). Similarly, agreement in grouping by AFLP analysis and by DNA-DNA hybridization was found in a study of 151 strains of all described genomic species and a set of unclassified strains of the genus *Acinetobacter* (47). The results showed that all strains were allocated to the correct species, with intraspecific similarity levels ranging from 29 to 74%, with most actual values being about 45%. Closely related DNA groups were clearly distinguished, whereas a cluster of unclassified strains was found to possibly represent a yet undescribed species. Koeleman et al. (54) confirmed the grouping of several clinical isolates of *Acinetobacter baumannii* within the *A. baumannii* species. In a taxonomic study of the honeybee pathogen *Paenibacillus* AFLP analysis with the combination of biochemical and DNA typing methods and DNA hybridization resulted in a reclassification of this genus. It was shown that despite more than 90% DNA relatedness, AFLP analysis could distinguish the strains at the subspecies level (37). AFLP analysis of the recently identified species *Ornithobacterium rhinotracheale* indicated the existence of subspecies (112). Lin et al. (59) demonstrated for *Escherichia coli* and *Agrobacterium tumefaciens* strains that polymorphic AFLP bands resolved differences in F′ episomal DNA.

These studies show that AFLP analysis is a powerful method for delineation of genomic groups within genera, although definitive genomic species descriptions still rely on DNA-DNA hybridization (120).

(ii) Epidemiology. DNA fingerprinting plays an obvious role in the analysis of the spread and persistence of pathogenic bacteria in the hospital environment (101, 106). Accurate typing at the strain level requires a highly discriminatory and reproducible method. Since small genetic alterations like the spread of mobile DNA fragments may be relevant, the high discriminatory power and reproducibility of AFLP analysis become useful.

Acinetobacter baumannii is notorious as a cause of nosocomial outbreaks. As stated earlier, Dijkshoorn et al. (24), Janssen and Dijkshoorn (46), and Koeleman et al. (53, 54) demonstrated an accurate identification of all species that was concordant with the DNA hybridization grouping. In addition outbreak-related and non-outbreak-related strains could clearly be identified.

The relatedness of sequential blood culture isolates of *Staphylococcus epidermidis* obtained from single patients was confirmed by AFLP analysis (97). Unrelated strains were clearly identified as such (78 to 93% homology), as were epidemiologically related strains. Clonal transmission of an *Staphylococcus aureus* strain between different family members and their cat and dog as a cause of recurrent infection was demonstrated by AFLP analysis (96). On the basis of the AFLP analysis results, the animals were identified as the reservoir.

Legionella pneumophila strains isolated from patients and from different water sources were identified by AFLP analysis, RFLP analysis, and ribotyping (105). The origin of infection was identified by AFLP analysis. The patterns of these clinical and environmental strains clearly differed from those of the unrelated environmental strains.

Bacillus anthracis is one of the genetically most monomorphic bacterial species, and such species showed extremely low levels of molecular variation in their AFLP patterns (97% homology), whereas a great deal of diversity was found between different *Bacillus* taxa by AFLP analysis. Nevertheless, two well-defined clusters were identified. On the basis of AFLP marker similarity, the ongoing anthrax epidemic in Canada and the northern United States was shown to be due to the introduction of a single strain. This strain has remained stable for at least 30 years (52).

Salmonellae are one of the main causes of human enteric disease (15) and are among the most important causes of food poisoning worldwide. In many countries, the number of gastrointestinal infections caused by *Salmonella enteritidis* has increased in recent decades (89). Over 2,000 *Salmonella* serotypes are recognized (21, 29, 121). Although the majority of outbreaks in livestock are caused by a select number of serotypes, serotyping is not an adequate method for determination of the source of contamination during an outbreak (71).

Multilocus enzyme electrophoretic typing suggests that salmonellae have a clonal population structure (9). Phylogenetic analysis by DNA-based methods grouped *Salmonella* serotypes in closely related clusters (72). Both the DNA techniques and traditional typing methods differentiate strains but do not discriminate all strains within a serotype. Recent AFLP analysis of *Salmonella enterica* serovar Dublin strains with known PFGE types identified unique AFLP patterns, suggesting that AFLP analysis and PFGE have about the same discriminatory powers for salmonellae (25). Aarts et al. (1) analyzed 78 *Salmonella* strains comprising 62 different serotypes by AFLP analysis and showed that the patterns were specific for serotypes and in some cases even for strains. Duim et al. (25) obtained AFLP patterns that discriminated different *Salmonella* serotypes; however, strains within the serotype *S. enteritidis* showed similarities of 90% or more. This indicated that the AFLP analysis conditions used were not optimal for differentiation of strains within this serotype or that AFLP analysis established the clonality of *S. enteritidis* (25).

AFLP analysis of *Campylobacter*, another microorganism important in food-borne gastrointestinal infections, showed that strains from poultry were separated into two groups: *Campylobacter jejuni* and *Campylobacter coli* (26). Within the cluster of *C. jejuni* individual AFLP patterns were observed, as were groups of poultry and human strains with shared AFLP banding patterns.

For *Helicobacter pylori*, the causative agent of peptic ulcers and gastric cancer, reproducible and discriminatory results were obtained by one enzyme-adapter method and analysis on agarose gels (32). AFLP and RAPD analyses provided evidence of the existence of *H. pylori* quasispecies (57).

Streptococcus pyogenes is an important human pathogen that has reemerged over the last decade. Many typing methods have been used to study the epidemiology of streptococcal disease, and these studies suggested a clonal expansion of certain serotypes with markedly increased virulence. Serotyping requires at least 80 different antiserum specimens, and up to 50% of the strains may be nontypeable. In addition, most serotyping is based on the M-antigen or protein, the gene for which is subjected to recombination and horizontal transfer, resulting in mosaic structures. AFLP analysis of 60 strains of distinct serotypes yielded clustering of patterns that were in complete accordance with their clustering by serotype, suggesting that differentiation by AFLP typing is as good as that by serotyping. These results indicate that AFLP typing can be used to detect clonal expansion of bacterial species (93). Desai et al. (23) identified 18 AFLP and 12 PFGE profiles in 27 isolates from an outbreak caused by group A streptococci. AFLP analysis distinguished two clonal strains of serotype M77.

The presence of eukaryotic DNA and/or the small amount of microorganisms isolated may hamper the use of AFLP analysis for genotyping of intracellular microbes. Nevertheless, reproducible AFLP patterns for intracellular *Chlamydia* spp. have been obtained. These showed genomic variation (12, 63, 68). For *Chlamydia psittaci* characterization of 12 strains was possible at the infrasubspecific level. Analysis of a cluster of French isolates permitted differentiation by host origin and clinical syndrome (12). In another study the genomic relatedness of 19 *Chlamydia pneumoniae*, 21 *Chlamydia trachomatis*, and 6 *C. psittaci* isolates and 1 *Chlamydia pecorum* isolate was determined by AFLP analysis (63). Cluster analysis of all species revealed the presence of groups other than those based on sequence data from single genes, and the analysis was in accordance with available DNA hybridization data. Morré et al. (68) showed genetic heterogeneity of *C. trachomatis* strains between and within biovars and within the urogenital trachoma serovars D, E, and F.

(iii) Ecology. A library of AFLP patterns of *Aeromonas* permitted determination of the distribution of 168 *Aeromonas* isolates from drinking-water production plants (41). Of all strains tested, 86% could be allocated to the known 14 DNA hybridization groups so far recognized within this genus. The remaining strains grouped in a homogeneous AFLP cluster, which was named *Aeromonas popoffii* sp. nov. after a more elaborate study of these strains (44). The diversity and persistence of coliforms and aeromonads in a Swedish drinking well were studied (56). Most strains were transient inhabitants, but all 11 *Aeromonas hydrophilia* isolates clustered within the same hybridization group. This study suggested the persistence of a genetically stable *Aeromonas* clone that resided in the well water over the whole 4-year study period and at the same time showed the presence of transient bacterial strains in the well.

VARIATIONS ON A THEME

Several variations of the original AFLP protocol have been reported. (i) Digestion of DNA with *Bam*HI and *Bgl*II and ligation to a single adapter generated useful fingerprints for mycobacteria (76). (ii) Sequence-specific amplification polymorphisms analysis, which is a PCR between an adapter sequence and a labeled primer specific for a plant retrotransposon (27, 60, 119) or the IS*6110* element of *Mycobacteria tuberculosis* (34), is used to amplify fragments that carry the respective interspersed repeat. (iii) Differences in methylation patterns were analyzed with the restriction enzymes *Msp*I and *Hpa*II, in addition to *Eco*RI. The isoschizomers *Msp*I and *Hpa*II cleave a sequence affected by the methylation state. This approach demonstrated universal DNA methylation in three major fungal taxa (*Mucor*, *Yarrowia*, and *Ustilago*) during fungal morphogenesis (88). (iv) Amplification products obtained by AFLP analysis can be recovered and cloned for sequence analysis to identify genetic markers (18, 19). (v) AFLP analysis of cDNA is a powerful alternative to differential display for systematic analysis of differential gene expression (5, 35, 49, 66).

An exciting prospect is AFLP analysis of genomes that have been sequenced completely. This would yield direct localizations of genomic or expressed AFLP fragments (4) and the conversion of polymorphisms to a single nucleotide polymorphism, amenable to highly informative genotyping in a microarray format.

CONCLUSIONS

AFLP analysis has established itself as a broadly applicable genotyping method with high degrees of reproducibility and discriminatory power. Several applications in taxonomy, diagnostics, and epidemiology have already been realized. Its reproducibility may allow compilation of a database of genotypes and the exchange of data between laboratories. This requires the use of standardized reagents and protocols and international strain depositories. For a uniform interpretation of AFLP patterns, we recommend the formulation of guidelines like those developed earlier for PFGE (10, 100, 102).

ACKNOWLEDGMENTS

We thank Paul Janssen and Christina Vandenbroucke-Grauls for critically reading the manuscript and Jeroen Stoof for Fig. 2.

REFERENCES

1. **Aarts, H. J. M., L. A. J. T. van Lith, and J. Keijer.** 1998. High-resolution genotyping of *Salmonella* strains by AFLP-fingerprinting. Lett. Appl. Microbiol. **26:**131–135.

2. **Ajmone-Marsan, P., A. Valentini, M. Cassandro, G. Vecchiotti-Antaldi, G. Bertoni, and M. Kuiper.** 1997. AFLP markers for DNA fingerprinting in cattle. Anim. Genet. **28:**418–426.
3. **Akerman, S., J. Tammisola, M. Regina, and V. Kauppinen.** 1996. Segregation of AFLP markers in Betula pendula (Roth). Forest Gen. **3:**117–123.
4. **Arnold, C., L. Metherell, J. P. Clewley, and J. Stanley.** 1999. Predictive modelling of fluorescent AFLP: a new approach to the molecular epidemiology of *E. coli*. Res. Microbiol. **150:**33–44.
5. **Bachem, C. W., R. S. van der Hoeven, S. M. de Bruijn, D. Vreugdenhil, M. Zabeau, and R. G. Visser.** 1996. Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. Plant J. **9:**745–753.
6. **Ballvora, A., J. Hesselbach, J. Niewohner, D. Leister, F. Salamini, and C. Gebhardt.** 1995. Marker enrichment and high-resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene Gro1. Mol. Gen. Genet. **249:**82–90.
7. **Becker, J., P. Vos, M. Kuiper, F. Salamini, and M. Heun.** 1995. Combined mapping of AFLP and RFLP markers in barley. Mol. Gen. Genet. **249:**65–73.
8. **Beismann, H., J. H. Barker, A. Karp, and T. Speck.** 1997. AFLP analysis sheds light on distribution of two Salix species and their hybrid along a natural gradient. Mol. Ecol. **6:**989–993.
9. **Beltran, P., J. M. Musser, R. Helmuth, J. J. Farmer, III, W. M. Frerichs, et al.** 1988. Toward a population genetic analysis of Salmonella. Genetic diversity and relationship among strains of serotypes *S. choleraesuis*, *S. derby*, *S. enteritidis*, *S. heidelberg*, *S. infantis*, *S. newport*, *S. typhimurium*. Proc. Natl. Acad. Sci. USA **85:**7753–7757.
10. **Blanc, D. S., P. M. Hauser, P. Francioli, and J. Bille.** 1998. Molecular typing methods and their discriminatory power. Clin. Microbiol. Infect. **4:**61–63.
11. **Blears, M. J., S. A. De Grandis, H. Lee, and J. T. Trevors.** Amplified fragment length polymorphism (AFLP): review of the procedure and its applications. J. Ind. Microbiol. Biotechnol. **21:**99–114.
12. **Boumedine, K. S., and A. Rodolakis.** 1998. AFLP allows the identification of genomic markers of ruminant *Chlamydia psittaci* strains useful for typing and epidemiological studies. Res. Microbiol. **149:**735–744.
13. **Breyne, P., W. Boerjan, T. Gerats, M. van Montagu, A. Vangysel.** 1997. Applications of AFLP™ in plant breeding, molecular biology and genetics. Belg. J. Bot. **129:**107–117.
14. **Brigneti, G., J. Garcia-Mas, and D. C. Baulcombe.** 1997. Molecular mapping of the potato virus Y resistance gene Rysto in potato. Theor. Appl. Genet. **94:**198–203.
15. **Bryan, F. L., and M. P. Doyle.** 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food Prot. **58:**326–344.
16. **Buntjer, J. B.** 1997. DNA repeats in the vertebrate genome as probes in phylogeny and species identification. Academic thesis, Utrecht University, Utrecht, The Netherlands.

16a. **Buntjer, J. B.** 18 May 1999, revision date. [Online.] http://www.spg.wan.nl/pv/pub/CrossCheck/index.html. [28 July 1999, last date accessed.]

17. **Cervera, M. T., J. Gusmao, M. Steenackers, J. Peleman, V. Storme, A. Vanden Broeck, M. van Montagu, and W. Boerjan.** 1996. Identification of AFLP molecular markers for resistance against Melampsora larici-populina in Populus. Theor. Appl. Genet. **93:**733–737.
18. **Chalhoub, B. A., S. Thibault, V. Laucou, C. Rameau, H. Hofte, and R. Cousin.** 1997. Silver staining and recovery of AFLP amplification products on large denaturing polyacrylamide gels. BioTechniques **22:**216–218.
19. **Cho, Y. G., M. W. Blair, O. Panaud, and S. R. McCouch.** 1996. Cloning and mapping of variety-specific rice genomic DNA sequences: amplified fragment length polymorphisms (AFLP) from silver-stained polyacrylamide gels. Genome **39:**373–378.
20. **Cnops, G., B. den Boer, A. Gerarts, M. van Montagu, and M. van Lijsebettens.** 1996. Chromosome landing at the Arabidobsis TORNADO1 locus using an AFLP based strategy. Mol. Gen. Genet. **253:**32–41.
21. **Crosa, J. H., D. J. Brenner, W. H. Ewing, and S. Falkow.** 1973. Molecular relationships among the Salmonelleae. J. Bacteriol. **115:**307–315.
22. **Daly, A.** 1998. Evaluation of the AFLP and RAPD molecular marker technologies with regard to the genetic diversity of commercial wheat cultivars. *In* A. Karp, P. G. Isaac, D. S. Ingram (ed.), Molecular tools for screening biodiversity. Chapman & Hall, London, United Kingdom.
23. **Desai, M., A. Tanna, R. Wall, A. Efstratiou, R. George, and J. Stanley.** 1998. Fluorescent amplified-fragment length polymorphism analysis of an outbreak of group A streptococcal invasive disease. J. Clin. Microbiol. **36:**3133–3137.
24. **Dijkshoorn, L., H. Aucken, P. Gerner-Smidt, P. Janssen, M. E. Kaufmann, J. Garaizar, J. Ursing, and T. L. Pitt.** 1996. Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. J. Clin. Microbiol. **34:**1519–1525.
25. **Duim, B., E. Kruijt, W. van Leeuwen, F. van Zijderveld, and J. Wagenaar.** 1997. Application of the DNA fingerprinting method AFLP to differentiate strains of *Salmonella enteritidis* and *Salmonella dublin*, p. 81–86. *In* Proceedings of the Molecular epidemiology of *Campylobacter* and *Salmonella*.
26. **Duim, B., T. M. Wassenaar, A. Rigter, and J. A. Wagenaar.** 1999. High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with AFLP fingerprinting. Appl. Environ. Microbiol. **65:**2369–2375.
27. **Ellis, T. H. N., S. J. Poyser, M. R. Knox, A. V. Vershinin, and M. J. Ambrose.** 1998. Polymorphism of insertion sites of *Ty1-copia* retrotransposons and its use for linkage and diversity analysis in pea. Mol. Gen. Genet. **260:**9–19.
28. **Esteve, C.** 1997. Is AFLP fingerprinting a true alternative to the DNA-DNA pairing method to assess genospecies in the genus *Aeromonas*? Int. J. Syst. Bacteriol. **47:**245–246.
29. **Farmer, J. J., B. R. Davis, F. W. Hickman-Brenner, A. McWorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21:**46–76.
30. **Felsenstein, J.** 1995. PHYLIP (Phylogeny Inference Package), version 3.57c. University of Washington, Seattle.
31. **Folkertsma, R. T., J. N. A. M. Rouppe van der Voort, K. E. de Groot, P. M. van Zantvoort, A. Schots, F. J. Gommers, J. Helder, and J. Bakker.** 1996. Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. Mol. Plant-Microbe Interact. **9:**47–54.
32. **Gibson, J. R., E. Slater, J. Xerry, D. S. Tompkins, and R. J. Owen.** 1998. Use of an amplified-fragment length polymorphism technique to fingerprint and differentiate isolates of *Helicobacter pylori*. J. Clin. Microbiol. **36:**2580–2585.
33. **Greef, J. M., M. Deuter, C. Jung, and J. Schondelmaier.** 1997. Genetic diversity of European Miscanthus species revealed by AFLP fingerprinting. Gen. Res. Crop Evol. **44:**185–195.
34. **Haas, W. H., W. R. Butler, C. L. Woodley, and J. T. Crawford.** 1993. Mixed-linker polymerase chain reaction: a new method for rapid fingerprinting of isolates of the *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **31:**1293–1298.
35. **Habu, Y., S. Fukada-Tanaka, Y. Hisatomi, and S. Lida.** 1997. Amplified restriction fragment length polymorphism-based mRNA fingerprinting using a single restriction enzyme that recognizes a 4-bp sequence. Biochem. Biophys. Res. Commun. **234:**516–521.
36. **Heun, M., R. Schaefer Pregl, D. Klawan, R. Castagna, M. Accerbi, B. Borghi, and F. Salamini.** 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science **278:**1312–1314.
37. **Heyndrickx, M., K. Vandemeulebroecke, B. Hoste, P. Janssen, K. Kersters, P. De Vos, N. A. Logan, N. Ali, and R. C. Berkeley.** 1996. Reclassification of *Paenibacillus* (formerly *Bacillus*) *pulvifaciens* (Nakamura 1984) Ash et al. 1994, a later subjective synonym of *Paenibacillus* (formerly *Bacillus*) *larvae* (White 1906) Ash et al. 1994, as a subspecies of *P. larvae*, with emended descriptions of *P. larvae* as *P. larvae* subsp. *larvae* and *P. larvae* subsp. *pulvifaciens*. Int. J. Syst. Bacteriol. **46:**270–279.
38. **Hill, M., H. Witsenboer, M. Zabeau, P. Vos, R. Kesseli, and R. Michelmore.** 1996. PCR-based fingerprinting using AFLP as a tool for studying genetic relationschips in *Lettuca* spp. Theor. Appl. Genet. **93:**1202–1210.
39. **Huys, G., R. Coopman, M. Vancanneyt, I. Kersters, W. Verstraete, K. Kersters, and P. Janssen.** 1993. High resolution differentiation of aeromonads. Med. Microbiol. Lett. **2:**248–255.
40. **Huys, G., M. Altwegg, M. L. Hanninen, M. Vancanneyt, L. Vauterin, R. Coopman, U. Torck, J. Luthy Hottenstein, P. Janssen, and K. Kersters.** 1996. Genotypic and chemotaxonomic description of two subgroups in the species *Aeromonas eucrenophila* and their affiliation to *A. encheleia* and *Aeromonas* DNA hybridization group 11. Syst. Appl. Microbiol. **19:**616–623.
41. **Huys, G., I. Kersters, R. Coopman, P. Janssen, and K. Kersters.** 1996. Genotypic diversity among *Aeromonas* isolates recovered from drinking water production plants as revealed by AFLP analysis. Syst. Appl. Microbiol. **19:**428–435.
42. **Huys, G., R. Coopman, P. Janssen, and K. Kersters.** 1996. High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. Int. J. Syst. Bacteriol. **46:**572–580.
43. **Huys, G., P. Kampfer, M. Altwegg, R. Coopman, P. Janssen, M. Gillis, and K. Kersters.** 1997. Inclusion of Aeromonas DNA hybridization group 11 in *Aeromonas encheleia* and extended descriptions of the species *Aeromonas eucrenophila* and *A. encheleia*. Int. J. Syst. Bacteriol. **47:**1157–1164.
44. **Huys, G., P. Kampfer, M. Altwegg, I. Kersters, A. Lamb, R. Coopman, J. Luthy-Hottenstein, M. Vancanneyt, P. Janssen, and K. Kersters.** 1997. *Aeromonas popoffii* sp. nov., a mesophilic bacterium isolated from drinking water production plants and reservoirs. Int. J. Syst. Bacteriol. **47:**1165–1171.
45. **Janssen, P., R. Coopman, G. Huys, J. Swings, M. Bleeker, P. Vos, M. Zabeau, and K. Kersters.** 1996. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxomony. Microbiology **142:**1881–1893.
46. **Janssen, P., and L. Dijkshoorn.** 1996. High resolution DNA fingerprinting of *Acinetobacter* outbreak strains. FEMS Microbiol. Lett. **142:**191–194.
47. **Janssen, P., K. Maquelin, R. Coopman, I. Tjernberg, P. Bouvet, K. Kersters, and L. Dijkshoorn.** 1997. Discrimination of *Acinetobacter* genomic

species by AFLP fingerprinting. Int. J. Syst. Bacteriol. **47:**1179–1187.
48. **Jones, C. J., K. J. Edwards, S. Castaglione, M. O. Winfield, F. Sala, C. van de Wiel, G. Bredemeijer, B. Vosman, M. Matthes, A. Daly, R. Brettschneider, P. Bettini, M. Buiatti, E. Maestri, A. Malceschii, N. Marmiroli, R. Aert, G. Volckaert, T. Rueda, R. Linacero, A. Vazques, and A. Karp.** 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European Laboratories. Mol. Breed. **3:**381–390.
49. **Jones, J. T., and B. E. Harrower.** 1998. A comparison of differential display and cDNA-AFLPs as tools for the isolation of differentially expressed parasite genes. Fundam. Appl. Nematol. **21:**81–88.
50. **Kaloshian, I., J. Yaghoobi, T. Liharska, J. Hontelez, D. Hanson, P. Hogan, T. Jesse, J. Wijbrandi, G. Simons, P. Vos, P. Zabel, and V. M. Williamson.** 1998. Genetic and physical localization of the root-knot nematode resistance locus mi in tomato. Mol. Gen. Genet. **257:**376–385.
51. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Achterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
52. **Keim, P., A. Kalif, J. Schupp, K. Hill, S. E. Travis, K. Richmond, D. M. Adair, M. Hugh-Jones, C. R. Kuske, and P. Jackson.** 1997. Molecular evolution and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. J. Bacteriol. **187:**818–824.
53. **Koeleman, J. G. M., G. A. Parlevliet, L. Dijkshoorn, P. H. M. Savelkoul, and C. M. J. E. Vandenbroucke-Grauls.** 1997. Nosocomial outbreak of multi-resistant *Acinetobacter baumannii* on a surgical ward: epidemiology and risk factors for acquisition. J. Hosp. Infect. **37:**113–123.
54. **Koeleman, J. G. M., J. Stoof, D. J. Biesmans, P. H. M. Savelkoul, and C. M. J. E. Vandenbroucke-Grauls.** 1998. Comparison of ARDRA, RAPD and AFLP fingerprinting for identification of *Acinetobacter* genomic species and typing of *Acinetobacter baumannii*. J. Clin. Microbiol. **36:**2522–2529.
55. **Kühn, I., M. J. Albert, M. Ansaruzzaman, N. A. Bhuiyan, S. A. Alabi, M. S. Islam, P. K. Neogi, G. Huys, P. Janssen, K. Kersters, and R. Mollby.** 1997. Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls, and from surface water in Bangladesh. J. Clin. Microbiol. **35:**369–373.
56. **Kühn, I., G. Huys, R. Coopman, K. Kersters, and P. Janssen.** 1997. A 4-year study of the diversity and persistence of coliforms and Aeromonas in the water of a Swedish drinking water well. Can. J. Microbiol. **43:**9–16.
57. **Kuipers, E. J., N. Dawn, A. Israel, J. G. Kusters, M. M. Gerrits, J. Weel, A. van der Ende, R. W. M. van der Hulst, S. A. Thompson, H. P. Wirth, J. Höök-Nikanne, and M. J. Blaser.** Evidence for quasispecies development of *H. pylori* by study of paired isolates obtained years apart in the same host. Submitted for publication.
58. **Leissner, C. E. W., M. L. Niessen, and R. F. Vogel.** 1997. Use of the AFLP technique for the identification and discrimination of *Fusarium graminearum*. Cereal Res. Commun. **25:**555–556.
59. **Lin, J. J., J. Kuo, and J. Ma.** 1996. A PCR-based DNA fingerprinting technique: AFLP for molecular typing of bacteria. Nucleic Acids Res. **24:**3649–3650.
60. **Lu, J., M. R. Knox, M. J. Ambrose, J. K. M. Brown, and T. H. N. Ellis.** 1996. Comparative analysis of genetic diversity in pea assessed by RFLP- and PCR-based methods. Theor. Appl. Genet. **93:**1103–1111.
61. **Mackill, D. J., Z. Zhang, E. D. Redona, and P. M. Colowit.** 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome **39:**969–977.
62. **Maslow, J. N., M. E. Mulligan, and R. D. Arbeit.** 1993. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. Clin. Infect. Dis. **17:**153–164.
63. **Meijer, A., S. A. Morré, A. J. C. van den Brule, P. H. M. Savelkoul, and J. M. Ossewaarde.** 1999. Genomic relatedness of *Chlamydia* isolates determined by amplified fragment length polymorphism analysis. Submitted for publication. J. Bacteriol. **181:**4469–4475.
64. **Michelmore, R. W., I. Paran, and R. V. Kesseli.** 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis; a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA **88:**9828–9832.
65. **Milbourne, D., R. Meyer, J. E. Bradshaw, N. Bonar, J. Provan, W. Powell, and R. Waught.** 1997. Comparison of PCR based marker systems for the analysis of genetic relationships in cultivated potato. Mol. Breed. **3:**127–136.
66. **Money, T., S. Reader, L. J. Qu, R. P. Dunford, and G. Moore.** 1996. AFLP-based mRNA fingerprinting. Nucleic Acids Res. **24:**2616–2617.
67. **Morel, V.** 1997. Bacteria diversify through warfare. Science **278:**575.
68. **Morré, S. A., T. M. Ossewaarde, P. H. M. Savelkoul, J. Stoof, C. J. L. M. Meijer, and A. J. C. van den Brule.** Analysis of genetic heterogeneity in *Chlamydia trachomatis* biovars and clinical isolates of the serovars D, E and F by amplified fragment length polymorphism. Submitted for publication.
69. **Mueller, U. G., S. E. Lipari, and M. G. Milgroom.** 1996. Amplified fragment length polymorphism (AFLP) fingerprinting of symbiotic fungi cultured by the fungus-growing ant *Cyphomyrmex minutus*. Mol. Ecol. **5:**119–122.
70. **Nandi, S., P. K. Subudhi, D. Senadhira, N. L. Manigbas, S. Sen-Mandi, and N. Huang.** 1997. Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol. Gen. Genet. **255:**1–8.
71. **Olsen, J. E., D. J. Brown, M. N. Skov, and J. P. Christensen.** 1993. Bacterial typing methods suitable for epidemiological studies. Applications in investigations of salmonellosis among livestock. Vet. Q. **15:**125–135.
72. **Olsen, J. E., M. N. Skov, E. J. Threlfall, and D. J. Brown.** 1994. Clonal lines of *Salmonella enterica* serotype Enteritidis documented by IS-200, ribo-, pulsed-field gelelectrophoresis and RFLP typing. J. Med. Microbiol. **40:**15–22.
73. **Otsen, M., M. den Bieman, M. T. Kuiper, M. Pravenec, V. Kren, T. W. Kurtz, H. J. Jacob, A. Lankhorst, and B. F. van Zutphen.** 1996. Use of AFLP markers for gene mapping and QTL detection in the rat. Genomics **37:**289–294.
74. **Otsen, M., et al.** Unpublished data.
75. **Pakniyat, H., W. Powell, E. Baird, L. L. Handley, D. Robinson, C. M. Scrimgeour, E. Nevo, C. Hacket, and P. D. Caligari.** 1997. AFLP variation in wild barley (Hordeum spontaeum C. Koch) with reference to salt tolerance and associated ecogeography. Genome **40:**332–341.
76. **Palittapongarnpim, P., S. Chomyc, A. Fanning, and D. Kunimoto.** 1993. DNA fingerprinting of *Mycobacterium tuberculosis* isolates by ligation-mediated polymerase chain reaction. Nucleic Acids Res. **21:**761–762.
77. **Paul, S., F. N. Wachira, W. Powell, and R. Waugh.** 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (Camellia sinensis (L.) O. Kuntze) revealed by AFLP markers. Theort. Appl. Genetics **94:**255–263.
78. **Pearson, K.** 1926. On the coefficient of racial likeness. Biometrika **18:**105–117.
79. **Picardeau, M., G. Prod'Hom, L. Raskine, M. P. LePennec, and V. Vincent.** 1997. Genotypic characterization of five subspecies of *Mycobacterium kansasii*. J. Clin. Microbiol. **35:**25–32.
80. **Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey, and A. Rafalski.** 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breeding **2:**225–238.
81. **Qi, X., and P. Lindhout.** 1997. Development of AFLP markers in barley. Mol. Gen. Genet. **254:**330–336.
82. **Rademaker, J. L. W., F. J. Louws, U. Rossbach, P. Vinuesa, and F. J. de Bruijn.** 1999. Computer-assisted pattern analysis of electrophoretic fingerprints and database construction, p. 1–33. *In* A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn (ed.), Molecular microbial ecology manual, Suppl. 4. Kluwer Academic Publishers, Dordrecht, The Netherlands.
83. **Rademaker, J. L. W., and F. J. de Bruijn.** 1997. Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer-assisted pattern analysis, p. 151–171. *In* G. Caetoan-Anollés and P. M. Gresshoff (ed.), DNA markers: protocols, applications and overviews. John Wiley & Sons, Inc., New York, N.Y.
84. **Rademaker, J. L. W., B. Hoste, K. Kersters, F. J. Louws, J. Swings, L. Vauterin, P. Vauterin, and F. J. de Bruijn.** Comparison of AFLP and rep-PCR genomic fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. Int. J. Syst. Bacteriol., in press.
85. **Ranade, S. A., and P. V. Sane.** 1996. Analysis of genetic diversity in plants with molecular techniques. Trop. Ecol. **37:**31–37.
86. **Reineke, A., P. Karlovsky, and C. P. Zebitz.** 1998. Preparation and purification of DNA from insects for AFLP analysis. Insect Mol. Biol. **7:**95–99.
87. **Replogle, J., W. D. Lord, B. Budowle, T. L. Meinking, and D. Taplin.** 1994. Identification of host DNA by amplified fragment length polymorphism analysis: preliminary analysis of human crab louse (Anoplura: Pediculidae) excreta. J. Med. Entomol. **31:**686–690.
88. **Reyna-Lopez, G. E., J. Simpson, and J. Ruiz-Herrera.** 1997. Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. Mol. Gen. Genet. **253:**703–710.
89. **Rodrique, D. C., R. V. Tauxe, and B. Rowe.** 1990. International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol. Infect. **105:**21–27.
90. **Rohlf, F. J.** 1993. NTSYS-pc (ver. 1.80). Applied Biostatistics Inc., New York, N.Y.
91. **Rosendahl, S., and J. W. Taylor.** 1997. Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP. Mol. Ecol. **6:**821–829.
92. **Russell, J. R., Fuller, J. D. Fuller, M. Macaulay, B. G. Hatz, A. Jahoor, W. Powell, and R. Waugh.** 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet. **95:**714–722.
93. **Schouls, L.** Unpublished data.
94. **Sensi, E., R. Vignani, W. Rohde, and S. Biricolti.** 1996. Characterization of genetic biodiversity with *Vitis vinifera* L, Sangiovese and Colorino genotypes by AFLP and ISTR DNA marker technology. Vitis **35:**183–188.
95. **Sharma, S. K., M. R. Knox, and T. H. N. Ellis.** 1996. AFLP analysis if the diversity and phylogeny of *Lens* and its comparison with RAPD analysis. Theor. Appl. Genet. **93:**751–758.
96. **Simoons-Smit, A. M., P. H. M. Savelkoul, T. M. Starink, and C. M. J. E. Vandenbroucke-Grauls.** Typing of clinical and domestic animal strains of *Staphylococcus aureus* by AFLP. Submitted for publication.

97. **Sloos, J. H., P. Janssen, C. P. A. van Boven, and L. Dijkshoorn.** 1998. AFLP™ typing of *Staphylococcus epidermidis* in multiple sequential blood cultures. Res. Microbiol. **149:**221–228.
98. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco, Calif.
99. **Speijer, H., P. H. M. Savelkoul, M. J. Bonten, E. E. Stobberingh, and H. T. Tjhie.** Application of different genotyping methods for *Pseudomonas aeroginosa* in an endemic setting on an intensive care. J. Clin Microbiol., in press.
100. **Struelens, M. J., et al.** 1996. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin. Microbiol. Infect. Dis. **2:**2–11.
101. **Tenover, F. C., R. Arbeit, G. Archer, J. Biddle, S. Byrne, R. Goering, G. Hancock, G. A. Hébert, B. Hill, R. Hollis, W. R. Jarvis, B. Kreiswirth, W. Eisner, J. Maslow, L. K. McDougal, J. M. Miller, M. Mulligan, and M. A. Pfaller.** 1994. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. J. Clin. Microbiol. **32:**407–415.
102. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
103. **Thomas, C. M., P. Vos, M. Zabeau, D. A. Jones, K. A. Norcott, B. P. Chadwick, and J. D. G. Jones.** 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to *Cladosporium fulvum*. Plant J. **8:**785–794.
104. **Travis, S. E., J. Maschinski, and P. Keim.** 1996. An analysis of genetic variation in Astrgalus cremnophylax var. Cremnophylax, a critically endangered plant, using AFLP markers. Mol. Ecol. **6:**735–745.
105. **Valsangiacomo, C., F. Baggi, V. Gaia, T. Balmelli, R. Peduzzi, and J.-C. Piffaretti.** 1995. Use of amplified fragment length polymorphism in molecular typing of *Legionella pneumophila* and application to epidemiological studies. J. Clin. Microbiol. **33:**1716–1719.
106. **Van Belkum, A.** 1994. DNA fingerprinting of medically important microorganisms by use of PCR. Clin. Microbiol. Rev. **7:**174–184.
107. **Vandenbroucke-Grauls, C. M. J. E., A. M. Simoons-Smit, and P. H. M. Savelkoul.** 1997. DNA fingerprinting of *Candida* spp. by AFLP, abstr. C-238, p. 162. In Abstracts of the 97th General Meeting of the American Society for Microbiology. 1997. American Society for Microbiology, Washington, D.C.
108. **Van der Lee, T., I. De Witte, A. Drenth, C. Alfonso, and F. Govers.** 1997. AFLP linkage map of the oomycete *Phytophthora infestans*. Fungal Genet. Biol. **21:**278–291.
109. **van Eck, H. J., J. R. van der Voort, J. Draaistra, P. van Zandvoort, E. van Enkevort, B. Regers, J. Peleman, E. Jacobsen, and J. Helder.** 1995. The inheritance and chromosomal localisation of AFLP markers in a non-inbred potato offspring. Mol. Breed. **4:**397–410.
110. **Vaneechoutte, M.** 1996. DNA fingerprinting techniques for microorganisms. A proposal for classification and nomenclature. Mol. Biotechnol. **6:**115–142.
111. **Van Embden, J. D. A., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, T. M. Shinnick, and P. M. Small.** 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31:**406–409.
112. **van Empel, P.** 1998. *Ornithobacterium rhinotracheale*. Academic thesis. Utrecht University, Utrecht, The Netherlands.
113. **van Lith, L. A. J. T.** Unpublished data.
114. **Vantoai, T. T., St. Peng JiQing, S. Martin, and J. Q. Peng.** 1996. Using AFLP markers to determine the contribution of parental genomes during selection. Soybean Gen. Newsl. **23:**214–216.
115. **Versalovic, J., T. Koeuth, and J. R. Lupski.** 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. **19:**6823–6831.
116. **Versalovic, J., C. R. Woods, Jr., P. R. Georghiou, R. J. Hamill, and J. R. Lupski.** 1993. DNA-based identification and epidemiologic typing of bacterial pathogens. Arch. Pathol. Lab. Med. **117:**1088–1098.
117. **Voorrips, R. E., M. C. Jongerius, and H. J. Kanne.** 1997. Mapping of two genes for resistances to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor. Appl. Genet. **94:**75–82.
118. **Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau.** 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:**4407–4414.
119. **Waugh, R., K. McLean, A. J. Flavell, S. R. Pearce, A. Kumar, B. B. Thomas, and W. Powell.** 1997. Genetic distribution of Bare-1-liek retrotransposable elements in the barely genome revealed by sequence specific amplification polymorphisms (S-SAP). Mol. Gen. Genet. **253:**687–694.
120. **Wayne, L. G., R. C. Good, E. C. Bottger, R. Butler, M. Dorsch, T. Ezaki, W. Gross, V. Jonas, J. Kilburn, P. Kirschner, M. I. Krichevsky, M. Ridell, T. M. Shinnick, B. Springer, E. Stackebrandt, I. Tarnok, Z. Tarnok, H. Tasaka, V. Vincent, N. G. Warren, C. A. Knott, and R. Johnson.** 1996. Semantide- and chemotaxonomy-based analyses of some problematic phenotypic clusters of slowly growing mycobacteria, a cooperative study of the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **46:**280–297.
121. **World Health Organization, Centre for Reference and Research on** *Salmonella.* 1980. Antigenic formulae of the *Salmonella*. World Health Organization International Salmonella Center, Institut Pasteur, Paris, France.
122. **Zabeau, M., and P. Vos.** 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. Publication 0 534 858 A1, bulletin 93/13. European Patent Office, Munich, Germany.