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High-Resolution Genotyping of *Campylobacter* Strains Isolated from Poultry and Humans with Amplified Fragment Length Polymorphism Fingerprinting

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For epidemiological studies of *Campylobacter* infections, molecular typing methods that can differentiate campylobacters at the strain level are needed. In this study we used a recently developed genotyping method, amplified fragment length polymorphism (AFLP), which is based on selective amplification of restriction fragments of chromosomal DNA, for genetic typing of *Campylobacter jejuni* and *Campylobacter coli* strains derived from humans and poultry. We developed an automated AFLP fingerprinting method in which restriction endonucleases *Hin*dIII and *Hha*I were used in combination with one set of selective PCR primers. This method resulted in evenly distributed band patterns for amplified fragments ranging from 50 to 500 bp long. The discriminatory power of AFLP was assessed with a *C. jejuni* strain, an isogenic flagellin mutant, and distinct *C. jejuni* strains having known pulsed-field gel electrophoresis and *fla* PCR-restriction fragment length polymorphism genotypes. Unrelated *C. jejuni* strains produced heterogeneous patterns, whereas genetically related strains produced similar AFLP patterns. Twenty-five *Campylobacter* strains obtained from poultry farms in The Netherlands grouped in three *C. jejuni* clusters that were separate from a *C. coli* cluster. The band patterns of 10 *C. jejuni* strains isolated from humans were heterogeneous, and most of these strains grouped with poultry strains. Our results show that AFLP analysis can distinguish genetically unrelated strains from genetically related strains of *Campylobacter* species. However, desirable genetically related strains can be differentiated by using other genotyping methods. We concluded that automated AFLP analysis is an attractive tool which can be used as a primary method for subtyping large numbers of *Campylobacter* strains and is extremely useful for epidemiological investigations.

Thermophilic *Campylobacter jejuni* and *Campylobacter coli* are important human pathogens and are common causes of gastrointestinal diseases in both developed and developing countries (21). Complications of *C. jejuni* infections, such as reactive arthritis and pancreatitis, have been described, and clinical evidence strongly suggests that infection with *C. jejuni* may be a precipitating factor for the development of polyneuropathies, such as Guillain-Barré syndrome (13). *Campylobacter* is widespread in nature and can be isolated from the gastrointestinal tracts of many animal species, as well as from freshwater. The major infection route for humans supposedly is consumption of contaminated poultry products, although epidemiological data suggest that there are other sources of infection (23). In order to better understand the epidemiology of *Campylobacter* infections in both poultry and humans, reproducible typing methods which can distinguish individual strains are necessary. Preferably, methods that also determine genetic distances between different but related strains should be developed in order to obtain valuable information concerning the spread and stability of bacterial populations.

Several phenotypic methods for typing *C. jejuni* and *C. coli* have been described; these methods include serotyping, phage typing, and biotyping. However, they are not generally available due to a lack of specific reagents. Some other disadvantages of phenotypic methods are that they have restricted differentiation powers and a high proportion of strains are nontypeable. Recently, researchers have developed molecular techniques for genetic subtyping; these methods include pulsed-field gel electrophoresis (PFGE), *fla* PCR-restriction fragment length polymorphism (RFLP) analysis, ribotyping, and randomly amplified polymorphic DNA analysis (2, 6, 11, 15, 19). The advantage of these genotypic methods is that they are more generally available and applicable; however, the methods that have been described often lack adequate discriminatory power, and the reproducibility of some is poor (19). Consequently, there is an increasing need for highly sensitive and reliable genomic typing methods for *Campylobacter* strains.

Amplified fragment length polymorphism (AFLP) is a recently developed method for genotyping (28). This method is based on selective amplification of restriction fragments generated from total genomic DNA. AFLP fingerprinting has been shown to have potential for strain identification, as well as high-resolution differentiation of genetically related bacterial strains (5, 8–10, 22). This technique can be easily automated, which allows standardization and high throughput of strains in epidemiological investigations. Moreover, digitization of the data results in easy storage, cross-referencing, and exchange of data between laboratories. Therefore, we investigated whether AFLP could be adapted for fingerprinting and epidemiological analysis of *Campylobacter* isolates.

The parameters that determine the discriminatory power of AFLP are the restriction enzymes and selective amplification primers used. The enzymes and primers were selected and optimized in this study by using a set of genetically defined *C. jejuni* strains. The method was then used with randomly obtained *Campylobacter* strains isolated from poultry and from human patients with gastroenteritis. Reproducible AFLP fin-

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