

Comparison of a Commercially Available Repetitive-Element PCR System (DiversiLab) with PCR Ribotyping for Typing of *Clostridium difficile* Strains[✓]

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Received 15 February 2011/Returned for modification 10 April 2011/Accepted 11 July 2011

This study compared a repetitive-element PCR (rep-PCR) method (DiversiLab system) to PCR ribotyping. The discriminatory power of rep-PCR was 0.997. Among the PCR ribotype 027 isolates tested, different rep types could be distinguished. rep-PCR showed a higher discriminatory power than PCR ribotyping. Nevertheless, this method requires technical skill, and visual interpretation of rep-PCR fingerprint patterns may be difficult.

Clostridium difficile has been identified as the leading cause of health care-associated diarrhea among adults in industrialized countries and is responsible for outbreaks worldwide. Three methods are typically used by reference laboratories for typing *C. difficile* strains: pulsed-field gel electrophoresis (PFGE), restriction enzyme analysis (REA) corresponding to the restriction fragment pattern of whole-genome DNA digested by HindIII, and amplification of variable intergenic regions between the 16S and 23S rRNA genes (PCR ribotyping). These typing methods are used to detect the emergence of new clones of *C. difficile*, as well as to investigate outbreaks. However, each of these methods has limitations. PFGE is a long and fastidious method, REA patterns are difficult to interpret, and PCR ribotyping lacks discriminatory power (8), especially when a *C. difficile* clone is predominant in a hospital or a country (such as PCR ribotype 027). The DiversiLab system (bioMérieux) is a repetitive-element PCR (rep-PCR) typing system that has recently been applied to *C. difficile* for epidemiological purposes (9, 10). After PCR amplification of genomic DNA regions between noncoding intergenic repetitive elements, amplified fragments are separated by microfluidic electrophoresis using Agilent technology. Analysis uses the DiversiLab bacterial barcode software, which improves the normalization of the data and allows easy run-to-run comparison. The objective of this study was to compare the DiversiLab system with PCR ribotyping, which is the reference method for typing *C. difficile* strains in Europe (2, 4). We evaluated the discriminatory power and reproducibility of this method and its usefulness for subtyping 027 strains.

Sixty-nine *C. difficile* strains of well-defined PCR ribotypes were studied. PCR ribotyping was performed according to the method described by Stubbs et al. (12). These strains included

(i) 37 epidemiologically unrelated strains isolated from 35 different hospitals in 14 European countries (1); (ii) 19 strains of PCR ribotype 027 from a 4-year outbreak in a geriatric ward in a Brussels, Belgium, hospital and 1 PCR ribotype 027 isolate from Surrey, United Kingdom; and (iii) 10 Italian strains previously characterized by manual rep-PCR (11) and PCR ribotyping (2). Finally, 2 strains, CL109 (PCR ribotype 027) and FI9 (PCR ribotype 079), were tested for reproducibility studies. Isolates were subcultured on selective medium (brain heart infusion agar supplemented with defibrinated horse blood [5%, vol/vol], sodium taurocholate [0.1%, wt/vol], cefoxitin [10 mg/liter], and cycloserine [250 mg/liter]) in an anaerobic atmosphere at 37°C for 24 h. Isolates were stored at –80°C in 1 ml of brain heart infusion broth containing glycerol (10%, vol/vol). DNA was extracted from each strain using the UltraClean microbial DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's recommendations. The concentration of the extracted DNA was determined using a Nanodrop ND1000 spectrophotometer (Labtech) and adjusted to 25 to 50 ng/μl. DNA amplification was performed using the *Clostridium* Fingerprinting kit (Bacterial Barcodes Inc., Athens, GA) according to the manufacturer's procedure. After amplification, PCR products were separated by electrophoresis using microfluidic lab-on-a-chip technology (Agilent bioanalyzer 2100) and analyzed using the web-based DiversiLab software (v3.4), allowing for normalization, analysis, storage of fingerprinting patterns, and data comparison. Electropherograms were compared by calculating the similarity index of Kullback-Leibler, which places more weight on band presence than on intensity variations. To make interpretation easier, strains with a cutoff value of <95% were considered different. For strains displaying a cutoff value of ≥95%, the electropherograms were visually inspected and 2 rep-PCR types were considered different if they had at least one band difference. The discriminatory power was calculated from 37 unrelated *C. difficile* strains using the Simpson index of diversity (6). Intrarun and interrune reproducibility was assessed with strains CL109 and FI9. Strains were extracted in

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[✓] Published ahead of print on 20 July 2011.

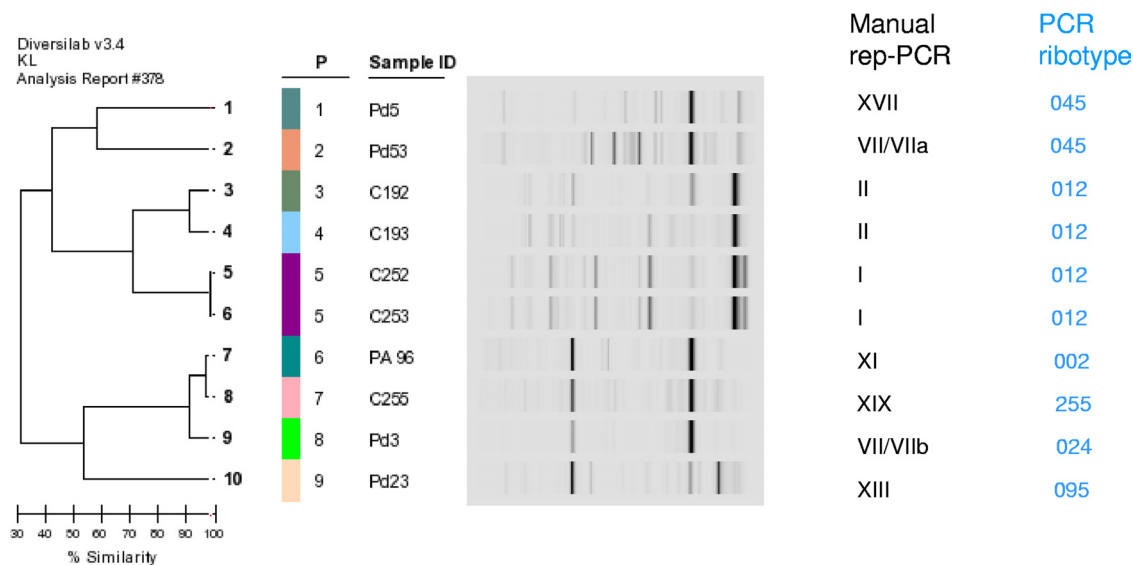


FIG. 1. rep-PCR-generated dendrogram for 10 Italian strains. Fingerprint types (P) are color coded. Results of manual rep-PCR and PCR ribotyping are indicated on the right.

duplicate, two separate PCR runs were performed on each extract, and each amplification product was loaded in duplicate on two chips.

Mean reproducibility gave a similarity index of 96.3% for strain FI9 and 98.6% for strain CL109 (results not shown). Stability of rep-PCR patterns was previously demonstrated (7). Among the 37 unrelated strains, 35 rep types and 23 PCR ribotypes could be differentiated and the discriminatory power

using the Simpson index of diversity was 0.997 and 0.959 for rep-PCR and PCR ribotyping, respectively. Our results are consistent with those of Healy et al., who found a higher level of discrimination for the rep-PCR method (0.97) than for PCR ribotyping (0.86) (5). Among the 10 Italian strains, 9 rep-PCR fingerprint types were defined (Fig. 1) and the results of the rep-PCR method (DiversiLab) were consistent with those of PCR ribotyping and manual rep-PCR. However, the rep-PCR

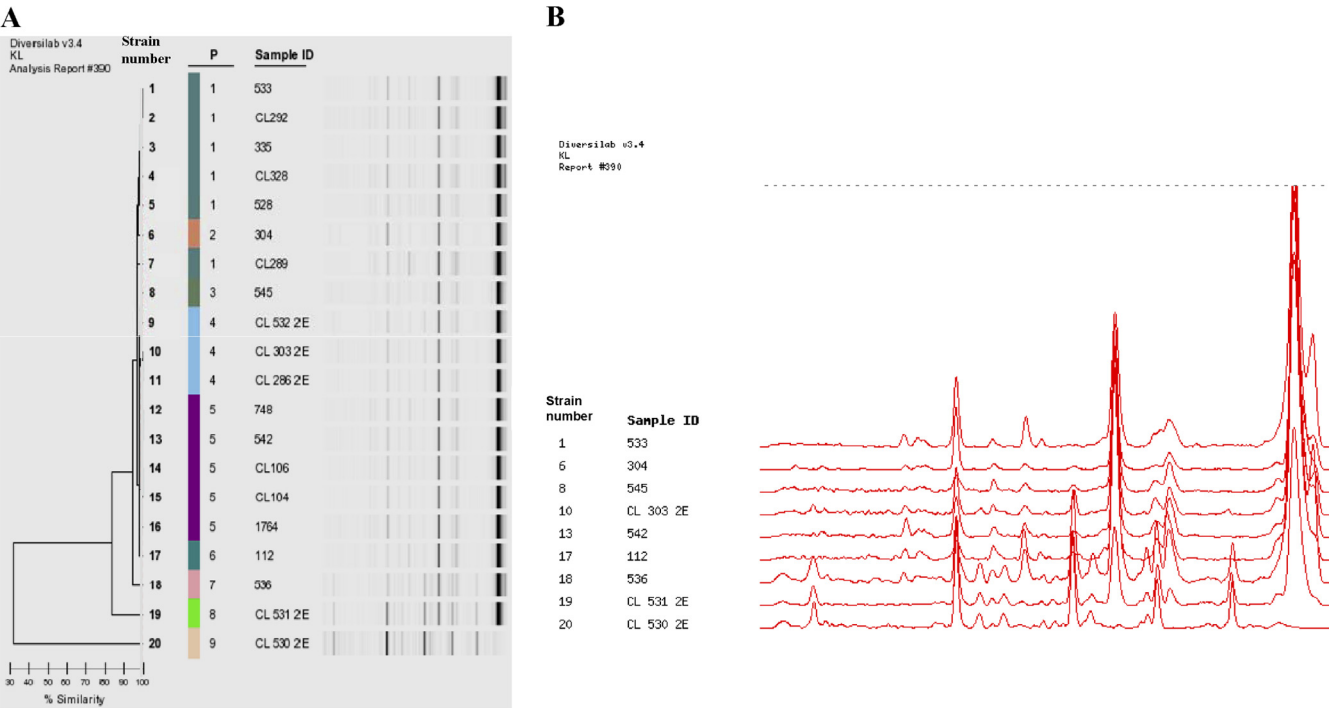


FIG. 2. (A) rep-PCR profile of 20 strains of PCR ribotype 027. Strains are numbered 1 to 20, and fingerprint types (P) are color coded. (B) rep stacker showing the profile of one representative strain of each fingerprint type.

method (DiversiLab) was more discriminatory than both other methods, which distinguished 6 and 8 different types, respectively. One reason might be that the discriminatory criterion previously adopted for manual rep-PCR was less restrictive (11); indeed, 2 isolates were assigned to the same rep-PCR group if their patterns differed by fewer than three bands, so the types identified were probably underestimated.

Among the PCR ribotype 027 strains, 9 rep-PCR fingerprint types were distinguished by the rep-PCR method (DiversiLab) (Fig. 2A). However, the differences between patterns 1 (strains 1, 2, 3, 4, 5, and 7), 4 (strains 9, 10, and 11), and 5 (strains 12, 13, 14, 15, and 16) were extremely slight (Fig. 2B). This method was helpful in distinguishing several rep types of hypervirulent PCR ribotype 027, which is in accordance with results reported by Frye et al. showing that rep-PCR provided multiple fingerprints for this PCR ribotype (3). Similar results were described for a previous study showing that different subgroups could be distinguished for PCR ribotype 001 strains (10).

In conclusion, compared to both manual rep-PCR and PCR ribotyping, the rep-PCR method (DiversiLab) showed a higher discriminatory power in typing *C. difficile*. This high discriminatory power may be helpful for investigating outbreaks and strain transmission from patient to patient, more particularly when a clone is predominant within a hospital, such as 027. This rep-PCR method (DiversiLab) requires good standardization. Additionally, technical skill is necessary for all of the steps, particularly for the visual analysis of rep-PCR fingerprint patterns, which may be difficult for little variations that can differentiate the electropherograms characterizing some *C. difficile* strains. Interlaboratory reproducibility should be assessed before using this technique for national or international surveillance of *C. difficile* genotypes.

We are indebted to bioMérieux for providing the DiversiLab equipment.

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