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Rapid Preparation of Bacterial DNA for Pulsed-Field Gel Electrophoresis

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A disadvantage of genotyping bacterial strains by pulsed-field gel electrophoresis is that the procedure requires up to 6 days to complete. We modified a standard pulsed-field gel electrophoresis method (B. E. Murray, K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock, J. Clin. Microbiol. 28:2059–2063, 1990) so that it could be completed in less than 3 days. We successfully applied this method to the analysis of a variety of gram-positive and gram-negative bacteria.

Pulsed-field gel electrophoresis (PFGE) is a highly reproducible and discriminating tool for the molecular typing of bacteria. It has been successfully applied to a broad range of different gram-negative and gram-positive organisms and mycobacterial species in epidemiological studies of populations where diseases are endemic, as well as in outbreaks (2, 4, 5). Its major disadvantage is that it requires up to 6 days from isolation of a pure colony of a bacterial strain until results are available. Here we present a rapid PFGE method that yields results in less than 3 days from the time of isolation of the organism.

Selection of bacterial strains. The bacterial strains tested were obtained from the clinical microbiology laboratory at Rush Presbyterian St. Luke's Medical Center and were selected to represent a variety of gram-negative and gram-positive organisms. Eight to ten unique strains of each of the following bacteria were tested: *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium*. Strains were considered unique if PFGE patterns differed by more than three bands (8). DNA from each strain was analyzed in parallel by both the standard method and the rapid method.

Standard DNA extraction procedure. A modification of the method of Murray et al. was used as the standard procedure for preparation of genomic DNA (6). All gram-negative bacteria and *S. aureus* strains were incubated overnight in 5 ml of brain heart infusion broth. *Enterococcus* spp. were grown overnight in brain heart infusion broth containing 1.8% glycine. *S. pneumoniae* strains were inoculated onto tryptic soy agar containing 5% sheep blood, incubated overnight in 10% CO_2, and then suspended in 5 ml of brain heart infusion broth. All incubations were at 37°C. Cells were harvested by centrifugation and then resuspended in 2.5 ml of PIV buffer (1 M NaCl, 10 mM Tris hydrochloride [pH 7.4]). A 0.5-ml aliquot of this suspension was combined with 0.5 ml of molten 1.6% low-melting-temperature agarose (SeaPlaque GTG; FMC Bioproducts, Rockland, Maine) made up in sterile, distilled, deionized water and tempered to approximately 50°C. This mixture was then pipetted into a plug mold (Bio-Rad Laboratories, Hercules, Calif.) and allowed to solidify. Each plug was placed into 10 ml of 1× lysis solution (6 mM Tris HCl [pH 7.4], 1 M NaCl, 10 mM EDTA [pH 7.5], 0.5% Brij, 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine) to which lysozyme (Sigma, St. Louis, Mo.) at 0.5 mg/ml and RNase A (DNase free; Sigma) at 10 mg/ml were added fresh on the day of the experiment. For *S. aureus* isolates, lysostaphin (Sigma) at 100 μg/ml was also added to the lysis solution. Plugs were incubated overnight at 37°C with gentle shaking. After incubation, the lysis solution was replaced with ESP (10 mM Tris HCl [pH 7.4], 1 mM EDTA), to which proteinase K (Sigma) at a final concentration of 100 μg/ml and 1% sodium dodecyl sulfate were added fresh on the day of the experiment, and the plugs were incubated overnight at 50°C. Plugs were washed with 15 ml of dilute TE (10 mM Tris HCl [pH 7.4], 0.1 mM EDTA) twice for 30 min each time and then placed into fresh TE for storage at 4°C until restriction digestion.

Rapid DNA extraction procedure. For the rapid DNA extraction procedure, 2.5 ml of each overnight bacterial culture was harvested by centrifugation and then resuspended in 0.5 ml of 2× lysis solution containing lysozyme at 1.0 mg/ml and RNase A (DNase free) at 20 mg/ml. This mixture was immediately added to 0.5 ml of tempered 1.6% low-melting-temperature agarose, vortexed briefly, pipetted into a plug mold, and allowed to solidify. Plugs were then incubated sequentially in the following solutions for the indicated times: 3 ml of 1× lysis solution containing lysozyme at 0.5 mg/ml and DNase-free RNase at 10 mg/ml at 37°C for 2 h with gentle shaking; 3 ml of ESP containing proteinase K at 100 μg/ml and 1% sodium dodecyl sulfate at 50°C for 1 h; and 7 ml of sterile, dilute TE at 50°C for 1 h. Lysostaphin at 100 μg/ml was added to the 1× lysis solution when *S. aureus* strains were analyzed. Care was taken to remove all residual ESP solution prior to the dilute-TE wash. The TE was replaced with 5 ml of fresh dilute TE in a clean tube for storage of plugs at 4°C until restriction digestion.

Restriction digestion and PFGE of DNA. A plug slice 2 to 4 mm wide was suspended in a total volume of 227 μl of the manufacturer's recommended restriction buffer and 20 U of *Xba*I (Gibco Bethesda Research Laboratories, Gaithersburg, Md.) (for *E. coli*, *K. pneumoniae*, and *S. marcescens* DNAs) or 20 U of *Sma*I (Gibco Bethesda Research Laboratories) (for *S. aureus*, *S. pneumoniae*, *E. faecalis*, and *E. faecium* DNAs). Digestion mixtures were incubated at 25°C for 2 h for the rapid procedure and overnight for the standard procedure. After digestion, plug slices prepared by the standard procedure were washed in dilute TE for 1 h at 37°C before loading onto the gel. The digested DNA was electrophoresed in a 1.2% agarose

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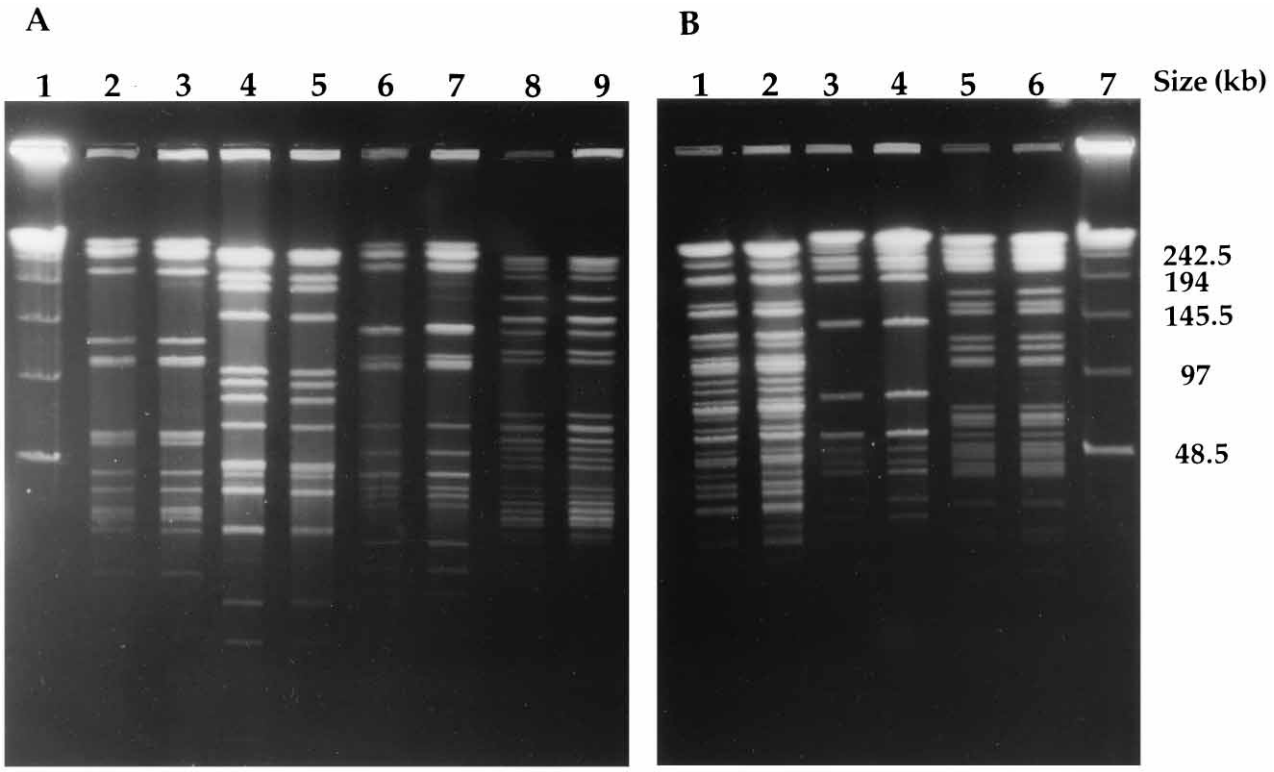

FIG. 1. PFGE of restriction enzyme-digested total genomic DNA extracted by standard and rapid methods from representative gram-positive (A) and gram-negative (B) bacterial strains. Gram-positive bacterial DNA was digested with *Sma*I, and gram-negative bacterial DNA was digested with *Xba*I. Shown are paired DNA preparations from each bacterial strain. The first lane of the pair contains DNA prepared by the standard procedure, and the second lane contains DNA prepared by the rapid procedure. (A) Lanes: 1, lambda concatemers used as molecular size markers; 2 and 3, *Staphylococcus aureus* DNA; 4 and 5, *Streptococcus pneumoniae* DNA; 6 and 7, *Enterococcus faecalis* DNA; 8 and 9, *Enterococcus faecium* DNA. (B) Lanes: 1 and 2, *Serratia marcescens* DNA; 3 and 4, *Klebsiella pneumoniae* DNA; 5 and 6, *Escherichia coli* DNA; 7, lambda concatemers used as molecular size markers.

(SeaKem LE; FMC Bioproducts)–0.5× TBE (1× TBE is 89 mM Tris HCl [pH 7.4], 89 mM boric acid, and 25 mM EDTA [pH 8.0])–0.05% ethidium bromide gel with a CHEF-DRII apparatus (Bio-Rad) for 21 h at 200 V with switching times ramped from 1 to 20 s.

The standard and rapid methods gave essentially indistinguishable results for all of the strains tested (Fig. 1). In some examples, more DNA was extracted by one method, resulting in resolution of additional bands on the gel. DNA extraction did not appear to be consistently more efficient for one or the other procedure, however.

Although rapid PFGE methods have been developed for *S. aureus*, *Staphylococcus epidermidis*, and *Enterococcus* spp. (1, 3, 4, 7), the procedure outlined in this report is the first we know of that has been successfully applied to multiple different bacterial genera. We shortened the standard PFGE assay primarily by reducing the time allowed for all enzymatic reactions, a modification employed by others to condense similar procedures (1, 3, 4, 7). Long incubation periods were initially recommended so that buffers and enzymes used in DNA extraction and restriction digestion steps would have sufficient time to diffuse into the plug. We did not attempt to determine the minimum incubation times needed to give adequate results, but very lengthy incubations appear to be unnecessary for most of the bacterial strains evaluated. However, 2 of 20 *S. marcescens* strains and 1 of 4 *Enterococcus avium* strains subsequently tested required overnight incubation in 1× lysis solution before interpretable results were obtained (data not shown).

A second alteration made in the standard procedure was to electrophorese the DNA in agarose and buffer containing a low concentration of ethidium bromide, thus eliminating the need to stain and destain the gel. A disadvantage of this approach is that it results in an increase in ethidium bromide waste. However, by making this alteration and shortening incubation times, we were able to complete the entire protocol in fewer than 3 days. As the rapid procedure is written, the steps performed on the second day take approximately 9 h to complete. If this work day needs to be shortened, restriction digestion can proceed overnight rather than for 2 h and electrophoresis can begin on the morning of the third day. Even with this modification, the procedure can be completed in approximately 72 h.

Other rapid PFGE methods have been shortened by eliminating proteinase K digestion of crude, agarose-embedded DNA extracts (1, 3). We were unable to obtain satisfactory results for all strains when applying this modification; a smear suggestive of degraded or randomly sheared DNA was sometimes seen. This may have been due to degradation of the DNA sample by residual cellular endonucleases in our preparations that were destroyed by proteinase K in the standard method.

Occasionally, it may be necessary to extend the electrophoresis period beyond 21 h to discern minor banding differences between bacterial strains. We generally maintain the electrophoresis time between 21 and 27 h by varying the switching times if finer separation of bands is desired. For example, separation of 150- to 300-kb fragments of *Sma*I-

digested *E. faecium* DNA is enhanced if switching times are changed to 5 and 35 s and the run time is increased to 24 h.

PFGE is a powerful tool for subtyping of bacteria. We hope that this modified rapid PFGE procedure will prove useful to hospital epidemiologists and others who wish to quickly determine the genetic relatedness of bacterial isolates during an outbreak investigation. It should also allow more practical and timely evaluation of large numbers of bacterial isolates.

REFERENCES

1. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J. Clin. Microbiol. **33:**551–555.
2. **Goering, R. V.** 1993. Molecular epidemiology of nosocomial infection: analysis of chromosomal restriction fragment patterns by pulsed-field gel electrophoresis. Infect. Control Hosp. Epidemiol. **14:**595–600.
3. **Goering, R. V., and M. A. Winters.** 1992. Rapid method for epidemiological evaluation of gram-positive cocci by field inversion gel electrophoresis. J. Clin. Microbiol. **30:**577–580.
4. **Leonard, R. B., J. Mayer, M. Sasser, M. L. Woods, B. R. Mooney, B. G. Brinton, P. L. Newcomb-Gayman, and K. C. Carroll.** 1995. Comparison of MIDI Sherlock system and pulsed-field gel electrophoresis in characterizing strains of methicillin-resistant *Staphylococcus aureus* from a recent hospital outbreak. J. Clin. Microbiol. **33:**2723–2727.
5. **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.
6. **Murray, B. E., K. V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock.** 1990. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. **28:**2059–2063.
7. **Rasmussen, S. K., J. C. McLaughlin, M. Puerzer, L. W. Massie, D. L. Palmer, and G. D. Overturf.** 1995. A condensed, three-day pulsed-field gel electrophoresis procedure used to investigate a methicillin-resistant *Staphylococcus aureus* outbreak in a regional hospital, abstr. C66, p. 6. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology. American Society for Microbiology, Washington, D.C.
8. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. A. 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.