NOTES

Rapid Isoelectric Focusing of Plasmid-Mediated β-Lactamases with Pharmacia PhastSystem

SAARA HUOVINEN

Department of Medical Microbiology, University of Turku, 20520 Turku, Finland

Received 11 May 1988/Accepted 9 August 1988

A modified isoelectric focusing method for rapid semiquantitative identification of plasmid-mediated β-lactamases by use of the Pharmacia PhastSystem (Uppsala, Sweden) is described. Sonication of bacterial colonies collected directly from growth plates decreased the time required for the procedure. With sonic extracts of known β-lactamase-producing strains used as controls, the assay could be completed in less than 2 h.

Isoelectric focusing (IEF) is one of the basic methods for identification and differentiation of plasmid-mediated β-lactamases (2, 3). We recently described a method for large-scale screening for plasmid-encoded β-lactamases genes that is based on DNA hybridization (1). For studies of small numbers of β-lactamase-producing strains, however, IEF has proven to be more convenient. In 1975, Matthew et al. used this system for detection and identification of β-lactamases (2). By using a chromogenic substrate, nitrocefin (2), this system permits visual comparison of patterns of β-lactamase bands produced by enzymes from different organisms. With this method, bands and their satellites can be easily recognized and compared.

Quantitative β-lactamase assays are rapid, routine methods in clinical bacteriological laboratories (6). Although classic β-lactamase IEF assays require 20 to 40 h to complete (2), IEF assays can now be performed in 1 to 1.5 h with certain equipment. In this note, I describe a rapid, semiquantitative IEF method for detection of β-lactamase that uses PhastSystem (Pharmacia, Uppsala, Sweden) and commercial PhastGels (Pharmacia). This procedure requires minimal amounts of samples and developing reagent.

The legend to Fig. 1 lists the 19 control strains (i.e., strains producing known β-lactamases) that were used (1). In addition, 47 clinical ampicillin-resistant, β-lactamase-producing isolates (36 Escherichia coli strains, 5 Pseudomonas aeruginosa strains, 5 Klebsiella pneumoniae strains, and 1 Serratia marcescens strain) were included. The presence of β-lactamase-encoding genes was also confirmed by DNA hybridization in all of the clinical isolates studied (1).

Crude extracts of bacterial strains were prepared by sonication of cultures grown overnight in brain heart infusion broth and frozen at −20°C until used (2). To develop a more rapid method for preparing sonic extracts, strains were grown on Muller-Hinton agar containing ampicillin (25 μg/ml) for 18 h at 37°C; cells were scraped from the surface of each plate, suspended in 0.5-ml volumes of 50 mM sodium phosphate buffer (pH 7.0), sonicated, and centrifuged as described by Reid et al. (5).

PhastGel IEF media are homogeneous polyacrylamide gels (43 by 50 mm) containing Pharmalyte carrier ampholytes (Pharmacia). Pharmalyte generates stable, linear pH gradients with a smooth conductivity profile across the entire pH range, which means that high field strengths of 500 V/cm and above can be used. Three PhastGel IEF media, identified according to pH gradients, are available: PhastGel IEF 3–9, PhastGel 4–6.5, and PhastGel 5–8. The gels to be used in this study were chosen according to the isoelectric points (pIs) of the β-lactamases to be studied. As controls, conventional IEF gels (165 by 215 mm) were prepared as described earlier (1, 2).

Portions of bacterial extract (1 to 3 μl) were applied to each gel according to the activity of the β-lactamase being studied. For the rapid enzyme activity test, 150 μl of nitrocefin solution (0.05 mg/ml) was added to 50 μl of bacterial sonic extract in a microdilution plate well; if there was a change in color from yellow to orange or red in less than 20 to 30 s, 1 to 3 μl of sonic extract was applied to the gel. A more accurate test for β-lactamase activity can be carried out by using spectrophotometric analysis as described by O’Callaghan et al. (4). Only 30 min was needed to complete the IEF run. Filter paper soaked in nitrocefin (500 μg/ml; BBL Microbiology Systems, Cockeysville, Md.) was laid on each gel, and photos were taken with a Polaroid MP-4 camera (type 55 film, Tiffen 658 dark-green filter).

IEF gels of selected β-lactamase-producing control strains are shown in Fig. 1. Use of gels with different pH ranges allowed characterization of all plasmid-mediated β-lactamases in the pI range of 4.3 to 7.7 (3). PhastGels were found to be less accurate than a conventional large-gel system for studies of β-lactamase migration. In Fig. 1A, the distance of migration as measured by pl was 5 mm between isolates HSM-1 (pI 5.2) and PSE-4 (pI 5.3) but only 1.5 mm between isolates PSE-4 and TEM-1 (pI 5.4).

Similarly, the distance between OXA-7 (pI 7.65) and OXA-6 (pI 7.68) was 2.5 to 3 mm (Fig. 1C), whereas the distance between OXA-5 (pI 7.62) and OXA-7 was only 1 mm. This inaccuracy was found in all types and lots of PhastGels tested. To avoid the inaccuracy it was always necessary to study known β-lactamase-producing controls as well as the isolates being tested for production of β-lactamase. The inaccuracies in β-lactamase migration found with PhastSystem were not found when a conventional large-gel system was used. Thus, the PhastSystem procedure does not generate an accurate pI and can be used to tentatively identify a β-lactamase only if one of the controls produces that β-lactamase.
Figure 1. IEF of plasmid-mediated β-lactamases produced by selected control strains. The bacteria used as sources of extracts were obtained from plates. pI values according to linear measurement of the gel are indicated on the left. For strain and plasmid designations, see reference 1. (A) PhastGel IEF, pH range of 4 to 6.5. Lanes: a, HMS-1 (pI 5.2); b, PSE-4 (pI 5.3); c, TEM-1 (pI 5.4); d, TLE-1 (pI 5.5); e, TEM-2 (pI 5.6); f, PSE-1 (pI 5.7). (B) PhastGel IEF, pH range of 3 to 8. Lanes: a, LCR-1 (pI 5.85); b, AER-1 (pI 5.9); c, PSE-2 (pI 6.1); d, PSE-3 (pI 6.9); e, OXA-3 (pI 7.1); f, OXA-1 (pI 7.4). (C) PhastGel IEF, pH range of 3 to 9. Lanes: a, OXA-4 (pI 7.5); b, SHV-1 (pI 7.6); c, OXA-5 (pI 7.62); d, OXA-7 (pI 7.65); e, OXA-6 (pI 7.68); f, OXA-2 (pI 7.7); g, ROB-1 (pI 8.1). Chromosomal β-lactamases are visible above pI 8.0 in lanes a, b, c, and e.

Figure 2. IEF of β-lactamases extracted from clinical strains producing the plasmids noted in parentheses. (A) PhastGel IEF, pH range of 3 to 9. Lanes: a, control (PSE-4; pI 5.3); b, P. aeruginosa (PSE-4); c, control (TEM-1; pI 5.4); d, E. coli (TEM-1); e, control (PSE-1; pI 5.7); f, P. aeruginosa (PSE-1). (B) PhastGel IEF, pH range of 3 to 9. Lanes: a, control (OXA-1; pI 7.4); b, E. coli (OXA-1); c, control (OXA-2; pI 7.7); d, E. coli (OXA-2 and TEM-1; pI 5.4); e, control (SHV-1; pI 7.6); f, K. pneumoniae (SHV-1).

Extracts of six β-lactamase-producing clinical strains were run in conjunction with control strains (Fig. 2). The “bird figure” of enzyme bands seen in Fig. 2B was also often seen in large IEF gels.

All 47 clinical strains producing plasmid-mediated β-lactamases showed evidence of producing the same enzyme in PhastGels regardless of whether colonies were collected for sonication directly from plates or after overnight growth in broth.

In addition to inaccuracy, some other disadvantages of PhastSystem are worth mentioning. When enzyme activity was low, the small amount of extract (1 to 3 μl) that could be applied limited the usefulness of PhastSystem. Larger gels allow inoculation of up to 100 μl of extract per lane. For testing extracts of weak β-lactamase-producing strains, highly concentrated samples are needed if PhastSystem is used. In addition, PhastSystem allowed only eight samples per gel. Bands at the edges of the gels were often bent, making interpretation more difficult. In some cases, pI values close to the top border (pH 6.5, 8, or 9, depending on the gel type) were also difficult to estimate.

Pharmacia PhastSystem IEF has clear advantages over the conventional IEF method: only 10% as much expensive nitrocefin is consumed, results can be obtained more quickly, and several gels can be run on one day. If the disadvantages are kept in mind, research laboratories and centers studying resistant clinical bacterial isolates can consider Pharmacia PhastSystem as a supplement to DNA
hybridization tests for rapid identification of plasmid-mediated β-lactamases.

I thank G. A. Jacoby and P. Huovinen for critical reading of the paper, A. Kauppi-Grönroos and T. Laine for technical assistance, and Wallac-Pharmacia (Espoo, Finland) for providing the gels used in this study.

This work was supported by grants from the University Foundation of Turku, the Research and Science Foundation of Farmos, the Cultural Foundation of South-Western Finland, and the Sigrid Jusélius Foundation.

LITERATURE CITED
1. Huovinen, S., P. Huovinen, and G. A. Jacoby. 1988. Detection of plasmid-mediated β-lactamases with DNA probes. Antimicrob. Agents Chemother. 32:175-179.
2. Matthew, M., A. M. Harris, M. J. Marshall, and G. W. Ross. 1975. The use of analytical isoelectric focusing for detection and identification of β-lactamases. J. Gen. Microbiol. 88:169-178.
3. Medeiros, A. A., and G. A. Jacoby. 1986. Beta-lactamase-mediated resistance, p. 49-84. In S. F. Queener, J. A. Webber, and S. W. Queener (ed.), Beta-lactam antibiotics for clinical use. Marcel Dekker, Inc., New York.
4. O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler. 1972. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. 1:283-288.
5. Reid, A. J., I. N. Simpson, P. B. Harper, and S. G. B. Amyes. 1987. Ampicillin resistance in Haemophilus influenzae: identification of resistance mechanisms. J. Antimicrob. Chemother. 20:645-656.
6. Schoenknecht, F. D., L. D. Sabath, and C. Thornsberry. 1985. Susceptibility tests: special tests, p. 1000-1008. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.