Use of the Restriction Enzyme EcoRI for Pulsed-Field Gel Electrophoretic Analysis of Helicobacter pylori

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Pulsed-field gel electrophoretic (PFGE) analysis of Helicobacter pylori isolates is not commonly employed because of the inability to compare the typing with other typing systems. We adapted the PFGE analysis for H. pylori by using EcoRI and slightly modified our laboratory methods to improve the typing of isolates (typeability was 97%).

Helicobacter pylori is associated with upper gastrointestinal diseases (1, 3, 4, 5). Pulsed-field gel electrophoresis (PFGE) is usually used for typing bacteria for epidemiologic purposes. However, PFGE analysis of H. pylori isolates (2, 6, 7, 8) is not commonly used, because of the inability to compare the typing with other typing systems (2). Our laboratory previously investigated using PFGE analysis for clinically isolated bacteria (9). However, this method was not effective for the analysis of H. pylori strains. To improve typing, we selected the restriction enzyme EcoRI to cut the chromosomal DNA and also modified our laboratory methods.

A total of 71 isolates of H. pylori were collected, which were obtained from 31 patients. If H. pylori was isolated, one to four individual colonies were cultured for 4 to 7 days on agar plates for isolation (Eiken Chemical, Tokyo, Japan) in a gas mixture of 85% N2–5% O2–10% CO2 at 35°C. Colonies were purified by passage on brucella agar plates (containing 10% horse serum), harvested from the plate by scraping, and suspended in 10% glycerol for storage at −80°C.

We performed PFGE analysis as follows. H. pylori strains harvested from plates by scraping were suspended in 100 ml of brucella broth (Difco, Detroit, Mich.) supplemented with 10% horse serum (preheated to 56°C for 30 min) and cultured with shaking for 4 to 7 days at 35°C in a gas mixture (85% N2–5% O2–10% CO2). The organisms were harvested by centrifugation, washed in a saline-EDTA solution (0.15 M NaCl, 10 mM EDTA, pH 8.0), and resuspended in Pett IV solution (1 M NaCl, 10 mM EDTA, pH 8.0). An equal volume of melted 2.0% low-melting-point agarose (Low Melt Preparative Grade; Bio-Rad Laboratories, Hercules, Calif.) was added to this suspension. The mixture was poured into an insert and chilled at 4°C for 30 h. The pulse time, which changed linearly, was 0.68 to 0.86 s. A Lambda Ladder (Bio-Rad) in field inversion gel electrophoresis mode. Agarose gels (1%) were prepared with 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA, pH 8.0). Separation of 40- to 20-kb fragments was done at 9 V/cm at 14°C for 30 h 11 min. The pulse time, which changed linearly, was 0.28 to 0.53 s. A Lambda Ladder (Bio-Rad Laboratories) was used as the size standard. Next, 20- to 10-kb fragments were separated at 9 V/cm at 14°C for 26 h 3 min. The pulse time, which changed linearly, was 0.68 to 0.86 s. A Lambda Ladder was once again used as the size standard. The gel was stained for 30 min in 1 μg of ethidium bromide/ml and decolorized in distilled water for 15 min. The gel was photographed by UV transillumination.

We randomly selected three strains from our laboratory collection and tested five restriction enzymes to cut the chromosomal DNA (EcoRI, HindIII, NotI, Sau3A, and SmaI). The results of PFGE with Sau3A and HindIII showed that no PFGE profiles could be visualized. By the use of SmaI and NotI, the PFGE profiles were smeared (Fig. 1A). Only EcoRI produced satisfactory restriction patterns (Fig. 1B), and when the plugs were digested twice, we obtained more satisfactory restriction patterns (Fig. 1C).

This electrophoresis protocol appears to clearly separate restriction fragments 30 to 50 kb in size, but there is poor resolution of fragments of <2.5 kb, of which there are many. The genomic sequences for H. pylori are available, and there-
fore one can predict which restriction enzymes will give a meaningful number of fragments. Based on the sequence of the H. pylori 26695 genome, it is clear that EcoRI should yield 180 fragments. For the 20 strains obtained from patients A, B, C, D, and E and four individual colonies cultured from one patient, electrophoresis was performed under two different conditions to eliminate the need to show gel lanes with no visible fragments: separation of 40- to 20-kb fragments and separation of 20- to 10-kb fragments. Comparison of the separation of fragments under both conditions showed that PFGE profiles for strains obtained from patient A were identical to those for strains obtained from patients B, C, and D. The PFGE profiles for strains obtained from patient E were similar under both conditions. Therefore, it seems that strain typing was acceptable with separation of 40- to 20-kb fragments.

To evaluate this method, we analyzed each of 71 strains obtained from 31 patients. We obtained 33 PFGE patterns. Only two strains obtained from one patient could not be digested (typeability was 97%). Each of the independent H. pylori isolates from the 31 patients had different PFGE patterns. Based on these findings, we were able to improve the ability to type H. pylori strains with EcoRI with increased restriction enzyme activity and performance of the restriction enzyme reaction twice.

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