Abstract

The purpose of research is to build a DNA fingerprint database for clinically important bacteria for epidemiological investigation. To carry out the goal, protocols for pulsed-field gel electrophoresis (PFGE) for various bacterial species have to be standardized and analysis procedures for pattern identification and comparison, a fingerprint database and computer network system need to be set up. During the period of study, we standardized the PFGE protocols for nine bacterial species: Shigella spp., Escherichia coli O157, Salmonella spp., Vibrio parahaemolyticus, Klebsiella pneumoniae, Neisseria meningitidis, Streptococcus pyogenes, Bordetella pertussis, Legionella spp. We also assemble a gel plug washer. The washer helped to ease the washing procedures, to save washing time, and to improve the DNA purity. The restriction enzyme used for each digestion could be reduced to one fourth of the quantity recommended by US CDC due to good DNA quality. The improvement on the PFGE operation procedures has resulted in reducing PFGE analysis cost and increasing the capability to handle large number of bacterial isolates in the laboratory. Moreover, the reference size markers used in the US CDC？HHH？HHHs PulseNet were used here that made the PFGE patterns comparable with those produced in the laboratories using the same markers and electrophoretic conditions. BioNumerics software was used to analyze PFGE images and create fingerprint database, but its functions on database management was insufficient. In order to store large amount of data and to search and retrieve data via the Intranet and Internet, a PFGE image file subdatabase, BioNumerics DNA fingerprint subdatabase, and epidemiological information subdatabase were created using MySQL and SQL sever 2000 database softwares. Two servers were set up for the database and to provide an intranet website for data processing and an internet website for data requesting from general laboratories. To date, the fingerprint database has contained DNA fingerprints of 522 Shigella spp., 4 E. coli O157, 160 N. meningitidis, 597 S. pyogenes, 385 Salmonella spp., 52 B. pertussis, 25 V. parahaemolyticus, and 330 K. pneumoniae isolates. The database, improved PFGE analysis techniques, and computer network will be used to build a laboratory-based molecular subtyping network (PulseNet Taiwan) for real time surveillance of bacterial pathogens. This database has already exhibited its usefulness to the epidemiological investigation on the shigellosis outbreaks occurring on the Bali travel tours and on a family in an aboriginal village in Eastern Taiwan.

Keywords：Pulsed-Field Gel Electrophoresis；DNA fingerprint database；Shigella；PulseNet Taiwan