

Abstract

In the present research, we standardized the pulsed-field gel electrophoresis (PFGE) technique, developed the capability to use the computer-aided software (BioNumerics) for analysis of PFGE patterns (or called DNA fingerprints), and used computer database technology, as the technical support for building a Taiwan molecular subtyping network for real time surveillance of bacterial disease, called PulseNet Taiwan. Following the standard PFGE protocols from the PulseNet group, the taskforce of the US national molecular subtyping network for surveillance of foodborne diseases, in US CDC, we standardized the PFGE protocols for *Shigella sonnei*, *Salmonella* spp., *Klebsiella pneumoniae*, and *Streptococcus pyogenes* and developed the capability of using BioNumerics to analyze PFGE patterns. We also assembled an agarose plug washing machine, which helped to shorten the washing time for PFGE plug from 4 h to 1h, to increase the handling capability to 30-60 samples a worker a day, and to obtain better DNA purity in the plugs. Therefore, the amount of restriction enzymes (NotI, XbaI, SmaI) needed for a slice digestion was lessened to less than 1 unit; the amount of the enzymes used for a digestion recommended in the PulseNet PFGE standard protocol was 40 units. The improvement helps to cut down the cost of PFGE. Because of the success made in the present research, to date, PFGE is a rapid, easy, and cost-effective method. It can be used as a routine molecular subtyping technique for bacterial pathogens. In the second year, we will standardize the PFGE protocols for *Bordetella pertussis*, *Haemophilus influenzae* serotype b, *Legionella* spp., *Neisseria meningitidis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *S. flexneria*, *Vibrio cholerae*, and *V. parahaemolyticus*. To use the standardized PFGE technique and with the aid of BioNumerics which can analyze, compare, and identify thousands of PFGE patterns in seconds, we are ready to set up a national molecular subtyping network for surveillance of bacterial infectious diseases PulseNet Taiwan.

Keywords : moped-pulsed-field gel electrophoresis (PFGE) ; molecular typing ; PulseNet Taiwan