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METHOD

A Rapid, Simplified Method for Preparation of Chromosomal DNA from Pathogenic Bacteria for Use In Pulsed-Field Gel Electrophoresis

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Rapid, easy-to-perform subtyping methods are frequently needed for effective epidemiological surveillance and prevention and control of important bacterial infections. The development of pulsed field gel electrophoresis (PFGE), where large DNA fragments are separated after digestion of chromosomal DNA with infrequently-cutting restriction endonucleases, has provided a new approach to the molecular typing and differentiation of individual strains of a variety of bacterial pathogens (1-3). Preparation of high-quality, intact, chromosomal DNA is a prerequisite for PFGE analysis. This is achieved by first embedding the cell culture in agarose, lysing the agarose *in-situ* and digesting the chromosomal DNA with infrequently cutting restriction endonucleases (1-3,7). However, the current protocols are relatively complex, tedious and time-consuming. This paper aims to describe a simplified version of the original protocol used in our laboratory [a modified method of Smith *et al* (7)], thus reducing the preparation time from seven days to four days. This method has been tested with more than 100 *Salmonella typhi* isolates, 18 different *Salmonella* serovars, 10 *Vibrio cholerae,* and *5 Staphylococcus aureus* isolates. The chromosomal DNAs generated were intact and could be digested completely with the appropriate restriction endonucleases. Moreover, the resultant restricted DNA fragments could be clearly resolved by PFGE.

The following describes the protocol for the preparation of intact, chromosomal DNA suitable for PFGE analysis. The whole procedure, especially during the initial cell harvest, was performed on ice, unless otherwise stated. This was to prevent spontaneous autolysis and DNA degradation prior to incubation in the lysis buffer. One ml of an overnight culture (pre-chilled in ice for about 10 minutes) was harvested by centrifugation for 5 minutes at 8000 rpm at 4°C. The cell pellet was washed by resuspending it with one ml of cold SB (10 mM Tris, pH 7.5, 1 M NaCl). The cell culture was re-centrifuged and the supernatant was discarded. The cell pellet was then resuspended in an equal volume of warm SB and molten 1.5% low melt agarose (InCert agarose, FMC. Bioproducts, Rockland, Maine), before being transferred to a mold (Bio-Rad). The mold was allowed to set at 4°C for 15 minutes or on ice for 5-10 minutes. The plugs (solidified agarose-cell mixture) were then transferred to 10 ml Universal screw-cap tubes containing 2 ml of lysis solution (10 mM Tris, pH 7.6, 50 mM NaCl, 100 mM EDTA pH 7.6, 0.2% sodium deoxycholate, 0.5% Sarcosyl, 0.5% Brij-58, 1 mg/ml lysozyme and 2 µg/ml RNase) and incubated at 37°C with gentle agitation for at least 6 hours. The lysis buffer was discarded and 2 ml of ES (0.5M EDTA, 1% Sarkosyl) containing 1 mg/ml proteinase K was added to the plugs and then incubated overnight at 50°C with gentle agitation. The plugs were then dialysed 2 hourly with TE (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA, pH 8.0) for at least 5 times at room temperature. At this point, the plugs are ready for restriction endonuclease digestion. Alternately, the plugs can be stored in TE buffer (for temporary storage) or in ES (for long term storage) at 4°C.

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