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RESEARCH NOTE

Optimised conditions for Pulsed Field Gel Electrophoresis (PFGE) separations of *Pasteurella multocida* B:2 DNA

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Abstract. Pulsed field conditions specific for *Pasteurella multocida* B:2 have been optimised to obtain a good separation resolution. Restriction enzyme, *Not*I, was the most suitable as it generates discernible number of bands. Other optimised electrophoretic conditions were: 1.5% agarose gel concentration, 200 V, ramped pulsed time of 1-25 s and a run time of 26 h. This research is important for fast and accurate characterisation of this bacterial strain following an outbreak in Malaysia.

Key Words: ***Pasteurella multocida*, PFGE, restriction enzymes**

Pulsed field gel electrophoresis (PFGE) has successfully shown greater discrimination in separating DNA restriction fragments ranging from less than 10 kilobase pairs (kb) to large chromosomal DNA with more than 4 million base pairs (Mb) (Smith *et al.*, 1987; Leblond *et al.*, 1990; Townsend *et al.*, 1992; Gunawardena *et al.*, 2000). PFGE is able to separate large DNA molecules by forcing them to change their direction of migration periodically (Schwartz and Cantor, 1984). Several modifications of this original technique can be observed in the literature to improve the resolution of results obtained (Hunt *et al.*, 2000). Preliminary experiments were carried out to determine the optimal pulsed times, gel concentration, voltage gradient and run time for maximum separation of DNA fragments.

When combined PFGE with restriction enzyme digestion of genomic DNA, a study of genetic variability amongst individuals is possible (Hunt *et al.*, 2000). A 'rare-cutting' enzyme is desirable as it would generate large fragments that can be separated on a PFGE ideal for analysis.

Haemorrhagic septicaemia (HS), a fatal disease of cattle and buffaloes, is caused by *Pasteurella multocida* (serotype B:2) in the tropics. There is the need to study the molecular characterisation of this organism for the development of better vaccines. Therefore the aim of this analysis was to optimise gel concentration, voltage gradient, run time and pulsed time together with restriction enzyme analysis digestions to enable a detailed molecular investigation of this bacteria.

P. multocida serotype B:2 used in this study was provided by the Veterinary Diagnostic Laboratory (VDL), Petaling Jaya and Veterinary Research Institute (VRI), Ipoh. Suspect colonies were identified by standard bacteriological techniques (Rimler and Rhoades, 1989) and confirmed as *P. multocida* serotype B:2. For DNA preparation, the cell pellet was harvested by centrifugation at 4°C for 10 min at 6000 rpm. The cell pellet was washed twice in 1 ml cold SB (10 mM Tris, pH 7.5, 1 mM NaCl) buffer and standardised to 1 X 10^7 cell/ml. Equal volume of the cell suspension and low melt agarose (1.5%) were mixed and allowed to set in a mould . The agarose plugs were then incubated in 10 ml of LB (10 mM Tris pH 7.5, 100 mM NaCl, 0.5 mM EDTA pH 8.0, 0.2% sodium deoxycholate, 0.5% Sarcosyl, 0.5% Brij-58) buffer in a 50 ml conical tube containing 0.01 g lysozyme and 10 ml RNAse (10 mg/ml) incubated overnight at 37°C with gentle agitation, followed by deproteination with 1mg/ml proteinase K at 50°C for 24 h. The plugs were then washed

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