Short Communication

**SpeI Restriction Enzyme Displays Greater Discriminatory Power than XbaI Enzyme Does in a Pulsed-Field Gel Electrophoresis Study on 146 Clinical *Burkholderia pseudomallei* Isolates**

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**SUMMARY**: Restriction enzymes SpeI and XbaI were used in a pulsed-field gel electrophoresis (PFGE) study for molecular characterization of 146 clinical *Burkholderia pseudomallei* isolates. The PFGE parameters were optimized to enable comparable, reproducible, and robust results. The optimized parameters for both SpeI and XbaI restriction enzymes used in this study were 200 V and a pulse time of 5 to 65 s for a 28-h runtime. Using SpeI, 9 different clusters were identified, whereas 6 clusters were identified by XbaI digestion, which exhibited 85% similarity to SpeI. SpeI (discrimination index \(D = 0.854\)) showed higher discriminatory power than XbaI did (\(D = 0.464\)).

Meliodosis is a human and animal disease caused by *Burkholderia pseudomallei*, a Gram-negative soil saprophyte endemic to Southeast Asia and Northern Australia (1), and had been reported in Mauritius, South America, India, China, the Middle East, and Africa (2). Although biochemical methods remain the gold standard for diagnosing the causative agent of melioidosis, the need for diagnosing the causative agent of melioidosis, current research trends involve the use of molecular tools, such as pulsed-field gel electrophoresis (PFGE) to discriminate bacterial isolates from different sources. PFGE is a highly reproducible and discriminating tool for molecular typing of bacteria; it has been successfully applied to a broad range of Gram-negative bacteria, Gram-positive bacteria, and mycobacteria species in epidemiological studies on endemic populations as well as in outbreak situations (3).

The two most commonly used restriction enzymes for PFGE of *B. pseudomallei* are XbaI (4,5) and SpeI (6,7). However, the discriminatory abilities of XbaI and SpeI have not been previously compared and reported. Hence, in this study, we compared the discriminatory abilities of SpeI and XbaI enzymes in the subtyping of *B. pseudomallei* isolates in Malaysia.

A total of 146 *B. pseudomallei* isolates that had previously been cultured from blood, pus, tissues, and body fluids (including urine, sputum, and peritoneal dialysates) from sporadic cases of melioidosis were analyzed. PFGE was conducted according to previously published protocols (8,9).

**Materials and Methods**

**Isolates**

The study included 146 clinical *B. pseudomallei* isolates from sporadic cases of melioidosis were analyzed. The isolates had been previously cultured from blood, pus, tissues, and body fluids (including urine, sputum, and peritoneal dialysates) from sporadic cases of melioidosis. The isolates were obtained from various clinical samples, including urine, blood, sputum, and peritoneal dialysates.

**Restriction Enzymes**

The restriction enzymes SpeI and XbaI were used for the PFGE analysis. SpeI and XbaI digestion were performed using previously described protocols (8,9).

**PFGE Protocol**

The PFGE protocol used was as follows: 0.7 \(\% (w/v)\) agarose gel (Sigma, Poole, UK) in 0.5 \(\times\) \(TBE\) buffer (10 mM Tris [pH 8.0], 50 mM EDTA [pH 8.0]). Two DNA plugs (2 mm each) were digested separately overnight with SpeI and XbaI (New England BioLabs, Beverly, Mass., USA). The DNA fragments were separated by electrophoresis (CHEF DR II; Bio-Rad Laboratories, Hercules, Calif., USA) in a 1% (w/v) agarose gel (Sigma, Poole, UK) in 0.5 \(\times\) \(TBE\) buffer for 28 h at 200 V with pulse times ramped linearly from 5 s to 65 s at 14°C. The gels were stained with 0.5 \(\mu\)g/ml ethidium bromide, destained with distilled water, and photographed under UV illumination.

**Dendrogram Construction**

Recorded DNA fingerprints were exported to BioNumerics software version 5.1 (Applied Maths, Kortrijk, Belgium), which created a dendrogram using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA). Genetic diversity and **B. pseudomallei** isolates relatedness were compared at 85% similarity at 1% optimization and 1.8% tolerance. Simpson's index of diversity was applied to measure and compare the diversity of the PFGE banding patterns.

**Results**

All bacterial isolate pulsotypes were reproducible upon one repeated PFGE experiment. SpeI endonuclease produced 13 to 23 bands that ranged in size from 20 kb to 1,100 kb, while XbaI generated 13 to 18 bands that ranged from 20 kb to 800 kb in size. XbaI restriction on all 146 *B. pseudomallei* isolates generated 117 pulsotypes, whereas SpeI restriction generated 126 pulsotypes.

**Discussion**

The discriminatory power of SpeI was superior to XbaI, as evidenced by the higher number of distinct clusters generated by SpeI (9) compared to XbaI (6). The SpeI restriction enzyme displayed a discrimination index of 0.854, which is significantly higher than the discrimination index of XbaI (0.464).

**Conclusion**

The results of this study highlight the potential of SpeI as a superior restriction enzyme for the PFGE analysis of *B. pseudomallei* isolates, particularly for diagnostic and epidemiological purposes in melioidosis cases.
Fig. 1. Dendrogram of SpeI-generated PFGE and cluster analysis.

*pseudomallei* isolates did not belong to any cluster; they harbored unique profiles.

*XbaI* generated 6 clusters as shown in Table 1. The largest cluster was BX1 with 71.9%, followed by BX4 with 12.3%. Other smaller clusters (BX2, BX3, BX5, and BX6) were less than 7% each. An unnamed cluster of 2 isolates contributed 1.4%. In Table 1, column BX1 clearly shows that the largest cluster, and BX1 from *XbaI* could be further differentiated into 7 clusters using the SpeI enzyme. This also indicates that all the
bacteria grouped under cluster BX1 could be further subdivided on the basis of the different pulsotypes generated using SpeI. Therefore, the use of the DNA restriction enzyme SpeI was found to be more discriminatory in PFGE typing than XbaI was for B. pseudomallei. The overall result was also supported by Simpson’s index diversity analysis; SpeI-digested B. pseudomallei isolates resulted in a discriminatory index of 0.854, compared to 0.464 for XbaI.

In general, the choice of restriction enzymes depends on the GC content of the particular organism. The GC content of B. pseudomallei is 64%; therefore, SpeI and XbaI are suitable for generating a reasonable number of bands for easy analysis. Based on the findings of previ-
ous studies, optimal PFGE parameters for both XbaI and SpeI were defined as 5–65 s at 28 h. The pulse time is relatively long since both enzymes gave rise to fragments with large molecular weights of 700 kb and 1,135 kb, respectively. Pulse time and runtime were maintained throughout the *B. pseudomallei* study for band separation. Matsumoto et al. reported that it is very important to standardize the PFGE protocol to obtain reproducible and comparable results (3).

As shown in Table 1, 34 isolates were from fatal
cases. With use of XbaI, 76.5% ($n = 26$) of the isolates were grouped under BX1, and 1 to 3% were scattered throughout BX2, BX4–BX6, and the unnamed cluster. However, with use of SpeI, the 34 isolates spread out among the 7 different pulsotypes from BS3 to BS9 as well as in the unnamed cluster. Fatal cases that were grouped under pulsotypes BS3 and BS5 were from the hyperendemic state of Pahang, but those from Kedah were from clusters BS5, BS6, and an unnamed cluster. Therefore, the molecular epidemiological benefits and advantages of using SpeI include increased discriminatory power; correlation with patient outcome, source, and origin may be less accurate with the use of the XbaI enzyme.
Based on our results, the restriction enzyme $\text{Spe}I$ was determined to be more suitable and accurate for PFGE profiling and typing of $B.\ pseudomallei$ for future molecular and epidemiological studies.

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**Conflict of interest** None to declare.

**REFERENCES**


