1999
Malays. Appl. Biol. (2000) 28(1&2): 9-12

DNA DIVERSITY AMONG POULTRY ISOLATES OF *SALMONELLA CHINCOL* DETECTED BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FINGERPRINTING

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Salmonella species are very important agents of food-borne infections all over the world (Archer and Young, 1988; Chalker and Blaser, 1988; Ingham *et al.*, 1990). Consequently, the typing of *Salmonella* spp. is of considerable importance in the surveillance of possible community food-borne outbreaks of salmonellosis and as a research tool. Several typing systems have been developed, but none has given more than a partial understanding of the ecology and epidemiology of *Salmonella* spp. (Ridley *et al.*, 1996). Recently, genotypic methods such as randomly amplified polymorphic DNA (RAPD) technique has been successfully used for a wide variety of organisms concerning DNA diversity, mapping and molecular typing (Tibayrence *et al.*, 1993). The discriminatory power of the RAPD technique depends on the selected primer sequences. The method exploits the fact that for any given short oligonucleotide sequence, the genomes of bacteria and higher organisms are likely to contain many sequences with partial, rather than complete, homology to the primer. If two such complementary sequences are located close together on the genome on opposite strands, and both have the same polarity, then PCR amplification of the intervening sequence can occur under conditions that permit the primer to anneal to both sequences. The distribution of these partially complementary sequences is random, and hence the result of PCR is a set of randomly amplified polymorphic DNA sequences (William *et al.*, 1990; Welsh and McClelland, 1990). Single base changes may destroy the ability of a sequence to anneal to the primer, or may create a new primer binding site. Hence, the pattern of amplified sequences obtained is primer and strain specific, and constitutes an identity profile of the organism. Clonal identity is reflected in isolates having the same band patterns with any particular primer. Genetic drift is seen as discrete changes to the band patterns (Welsh and McClelland, 1990).

In this paper we report on the application of RAPD analysis to the typing of *Salmonella chincol*. The 15 isolates of *S. chincol* examined in this study were isolated from poultry at the Bangi wet market, Selangor, Malaysia. Details of the laboratory methods used for isolation have been described previously (Rusul *et al.*, 1996). All isolates were grown in Luria-Bertani (LB) broth (Sambrook *et al.*, 1989) at 37°C overnight with shaking at 200 rpm. Bacterial cells harvested by centrifugation from 1.5 ml of liquid culture were washed in TE (10 mM Tris-HCl, pH8.0, and 1 mM EDTA) and resuspended in 700 ml of the same buffer containing 1 mg/ml lysozyme. Subsequently, 5 ml of 25% sodium dodecyl sulphate and 10 ml of 20 mg/ml proteinase K were added to the mixed cell solution, and the cells lysed for 20 min at 50°C. After incubation, the chromosomal DNA was extracted with an equal volume of TE saturated phenol/chloroform/isoamyl (25:24:1) alcohol. The supernatant fluid was collected in an Eppendorf tube and then equal volumes of sodium acetate and isopropanol were added. The precipitated DNA was recovered by centrifugation, and washed with 70% ethanol and the pellet was resuspended in 50 ml of distilled water. The purified DNA concentrations were estimated by comparison with known DNA markers on agarose gels.

A randomly designed 10-mer oligonucleotide set, designated as Gen1-50-01 to Gen1-50-10 was obtained from Genosys Biotechnologies Inc, USA. The Gen1-50-06 (5'-GTGCAATGAG-3') and Gen1-50-09 (5'-CAATGCGTCT-3'), were chosen

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for RAPD analysis because they yielded clear patterns. These primers all had a G+C content of 50% and Marmur *et al.* (1963) found that *Salmonella* spp. have an average genome GC content of 50-52%. PCR was carried out in a final volume of 25 ml containing 30 ng of template DNA, 1x PCR buffer containing 20 mM Tris-HCl (pH8.4), 50 mM KCl, 2 mM $MgCl_2$, 100 mM (each) deoxynucleotide triphosphate, 0.2 mM primer, and 1 U of *Taq* polymerase (Research Instruments, USA). RAPD was carried out using a thermal cycler (Perkin Elmer 2400). The cycling parameters were 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C for a total of 40 cycles, with a final cycle extending amplification conditions to 72°C for 5 min. The PCR amplification products were visualized by running 10 ml of the reaction on a 1.2% agarose gel, which was stained with ethidium bromide and examined over u.v. light. A lambda ladder (Promega, USA) were used as DNA size markers.

Initially, ten primers were screened with a subsample of four isolates to detect polymorphism within *S. chincol*. However, only two oligonucleotide showed DNA polymorphism between the isolates. These two discriminating primers, Gen1-50-06 and Gen1-50-09, were selected to analyse the whole set of the 15 *S. chincol* isolates. The remaining primers (Gen1-50-01, -02, -03, -04, -05, -07, -08 and -10) gave bands with only some of the isolates or had poor reproducibility and were not tested further. Such diversity between primers has been previously recorded (Oakey *et al.*, 1995). Figures 1 and 2 show the RAPD profiles of *S. chincol* obtained with oligonucleotide primer Gen1-50-06 and Gen1-50-09 respectively. Five different RAPD profiles were apparent from primer Gen1-50-06, and at least eight RAPD profiles from primer Gen1-50-09, with molecular sizes ranging from 280 to 3800 base pairs (all isolates were analysed at least three times with both primers). However, no band was produced with isolates SC7, SC9, SC12 and SC13 (Figure 1) with primer Gen1-50-06, which could be interpreted as the loss of specific sites for primer binding in the chromosomal DNAs of these isolates. The combination of the two amplification patterns allows identification of thirteen RAPD types. Consequently for these two primers, variability in the RAPD profiles consisted of gain or loss of a fragment (Figures 1 and 2).

DNA products produced in RAPD analysis depend on the primer used, with different primers producing different banding patterns. Therefore, the more primers used, the greater the likelihood of demonstrating strain dissimilarities. Analysis of additional isolates from other sources may reveal additional RAPD groups, but this technique is only one measure of genetic relatedness. Thirteen variations were detected among the 15 isolates, confirming the relatively high diversity within *S. chincol* studied. As far as we could determine, each isolate was from a different poultry source in Peninsular Malaysia (Rusul *et al.*, 1996). We do not know whether the variation identified in this study was due to genomic instability or whether substantial genetic evolution has occurred in some *S. chincol* populations because of selective pressure. This could be ascertained by studying a single well-defined strain as it progresses through a poultry farm to determine how rapidly genomic variation occurs in this species.

RAPD-PCR is a rapid and simple technique which requires no previous knowledge of nucleotide sequence, does not rely on the actual transcription and translation of proteins, highly sensitive, requires a minimum amount of template DNA and because it potentially analyse the whole genome, is highly discriminatory. Due to the sensitivity of this method, reproducibility can be affected by small variations in the reaction mixture and temperature cycles (Ellesworth *et al.*, 1993; MacPherson *et al.*, 1993; Schierwater and Ender, 1993); hence, care is needed to standardize the procedure completely if it is to be used for routine analysis. The discriminatory power of RAPD-PCR will undoubtedly be an aid in epidemiology studies. More specifically, the unique fingerprints will help map sources and occurrences of outbreaks and the relatedness of isolates from different sources. However, care needs to be taken in checking the reproducibility of the fragments as variability obtained may probably result from the low stringency condition used in RAPD-PCR which will increase the occurrence of mismatching between the target sequence and the primers (Ellesworth *et al.*, 1993). The number of potential polymorphic bands suggest that RAPD fingerprinting is a sensitive method for strain differentiation. Our results must still be interpreted cautiously since two distinct PCR products may resolve together and variability may not be revealed by agarose gel electrophoresis. Meunier and Grimont (1993) have express some concern about the reproducibility of RAPD profiles but concluded that reproducibility was excellent as long as the methodology was standard.

In this paper, the potential of the RAPD analysis of *Salmonella chincol* to identify the genomic differentiation has been demonstrated. The scattered RAPD profiles of *S. chincol* demonstrated their genomic variety. Thus, genomic heterogeneity of *S. chincol* found in this study confirms the usefulness of RAPD for the rapid differentiation of this pathogen.

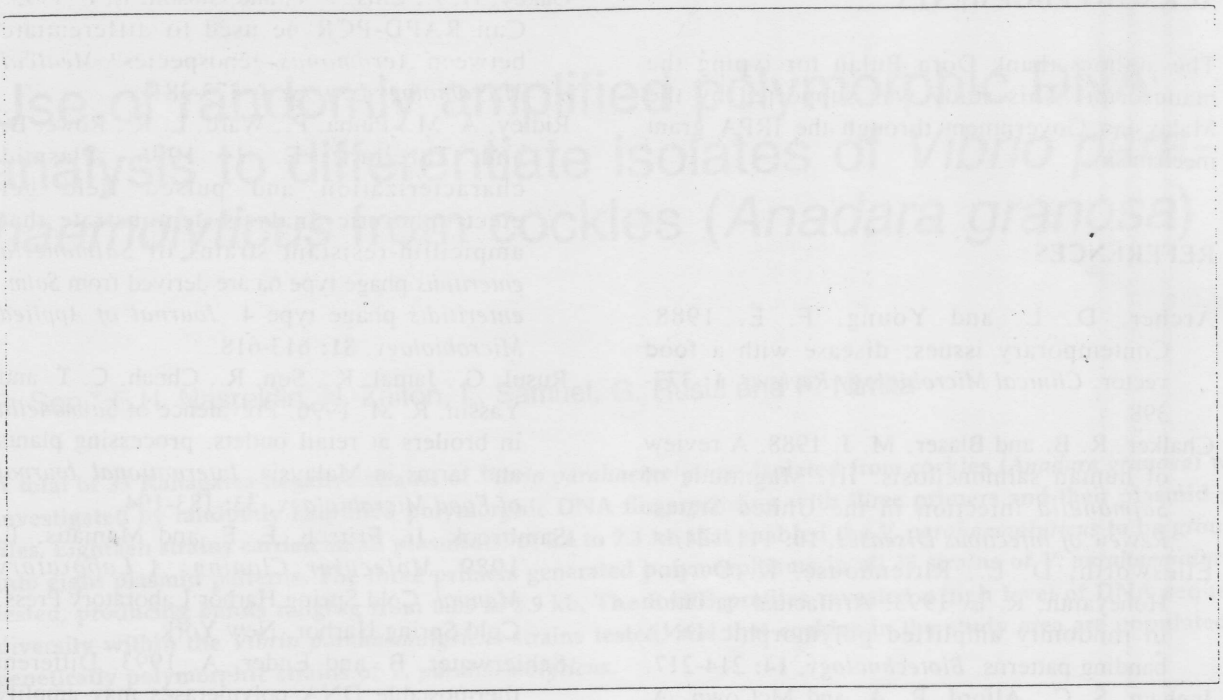

Fig. 1: RAPD profiles obtained using primer Gen1-50-06 on various *S. chincol* strains. Lanes: 1, SC1; 2, SC2; 3, SC3; 4, SC4; 5, SC5; 6, SC6; 7, SC7; 8, SC8; 9, SC9; 10, SC10; 11, SC11; 12, SC12; 13, SC13; 14, SC14; 15, SC15; and 16, Lambda DNA ladder molecular sizes marker.
RAPD-types: I, SC1; II, SC2; III, SC3, SC6, SC10, SC11, SC14 and SC15; IV, SC4 and SC5; V, SC8; Untypeable, SC7, SC9, SC12 and SC13.

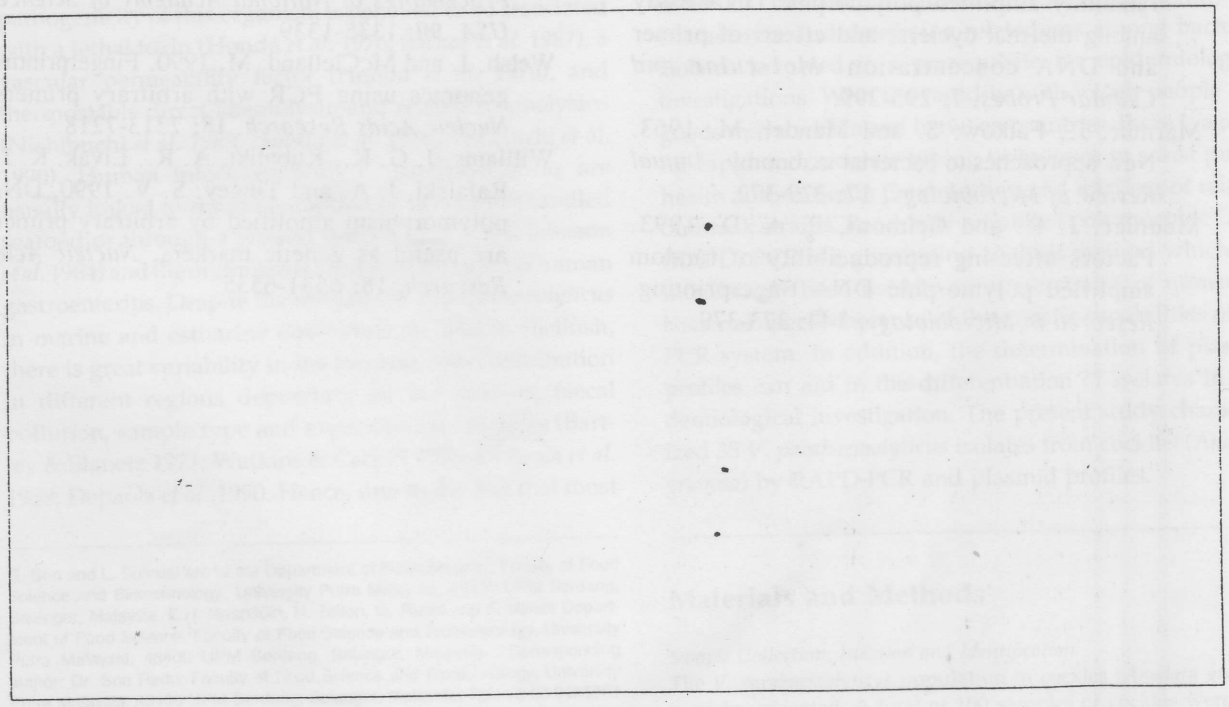

Fig. 2: RAPD profiles obtained using primer Gen1-50-09 on various *S. chincol* strains. Lanes: 1, SC1; 2, SC2; 3, SC3; 4, SC4; 5, SC5; 6, SC6; 7, SC7; 8, SC8; 9, SC9; 10, SC10; 11, SC11; 12, SC12; 13, SC13; 14, SC14; 15, SC15; and 16, Lambda DNA ladder molecular sizes marker.
RAPD-types: I, SC1, SC2, SC4, SC5, SC6 and SC9; II SC3 and SC8; III, SC7; IV, SC10; V, SC11; VI, SC12; VII, SC13; VIII, SC14 and SC15.

ACKNOWLEDGEMENTS

The authors thank Dora Bulan for typing the manuscript. This study was supported by the Malaysian Government through the IRPA grant mechanism.

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