Journal of Animal and Veterinary Advances 4 (2) : 210-213, 2005

DNA characterization of *Mannheimia haemolytica* A2, A7, A9 and *Pasteurella trehalosi*

[1]M.Y. Sabri, [4]M. Zamri-Saad, [2]R. Son and [3]Sharifah
[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia,
[2]Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia,
[3]Veterinary Research Institute, 59 Jalan Sultan Azlan Shah 31800 Ipoh, Perak, Malaysia,
[4]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract: Whole-cells DNA of *Mannheimia haemolytica* A2, A7, A9 and *Pasteurella trehalosi,* isolated from goats were subjected to plasmid screening and random amplified polymorphic DNA (RAPD) analysis. The plasmid DNA screening revealed the presence of plasmid only in *P. trehalosi* isolated from goat. The plasmid was 4825 bp. The RAPD analysis indicated similarities and differences among isolates *M. haemolytica* and *P. trehalosi*. However, OPA16 primer revealed the DNA fingerprinting pattern that was able to clearly distinguish the various isolated while OPA14 primer revealed less distinguishable pattern. OPA20 revealed a fairly distinguishable DNA pattern.

Key words: RAPD, Plasmid, Mannheimia haemolytica, Pasteurella trehalosi

Introduction

Pneumonic mannheimiosis is one of the most common respiratory diseases of goats and sheep caused by the bacteria *Mannheimia* (Gilmour, 1993). *M. haemolytica* comprises two biotypes; A and T, based on their fermentation of arabinose and trehalose. The trehalose-negative organisms represent a distinct genus of Mannheimia with five species; *M. glucosidal*, *M. granulomatis*, *M. haemolytica*, *M. ruminalis*, and *M. varigena*. They are now classified as *M. haemolytica* and contain serovars 1, 2, 5-9, 12-14, 16 and 17. The trehalose-positive organisms are known as *Pasteurella trehalose*.

The random amplify polymorphic DNA (RAPD) analysis is one of the methods used to characterize and differentiate *Mannheimia* and *Pasteurella* isolates (Chaslus-Dancla *et al*., 1996 and Kodjo *et al*., 1999). This study describes the plasmid screening and the use of RAPD in characterisation of *M. haemolytica* and *P. trehalose* isolates.

Materials and Methods

Bacterial Strains, Growth Conditions and DNA / Plasmid DNA Extraction : *Mannheimia haemolytica* A2, A7, and A9 that were isolated earlier from pneumonic lungs of goats and two *Pasteurella trehalosi* isolated from goats (5600) and chicken (4293) were used. All isolates were grown in brain-heart infusion broth for 24 h at 37°C with gentle shaking. Whole-cell DNA and plasmid were isolated and purified using the Wizard Genomic DNA Purification Kit System and Wizard Plus Minipreps DNA Purification System (Promega). Following the extraction, all samples were kept at -20°C until further use. Plasmid DNA of each isolates were electrophoresed in 1% agarose with 4 μl 6X loading dye and compared with the Lambda DNA/*Hind*III marker.

RAPD Analysis: Three 0.25 μM arbitrary 10-mer RAPD primer (sequence 5'-3', OPA-14: TCTGTGCTGG, OPA-16: AGCCAGCGAA and OPA-20: GTTGCGATCC) and 1 μl DNA of each strain were added to a PCR cocktail mixture, each deoxynucleotide triphosphate at a concentration of 200 μM, in a 25-μl final reaction volume. RAPD amplification reactions were performed in the Minicycler apparatus (MJ Research, USA) with the following conditions: 1 cycle at 94°C for 2 min, followed by 45 cycles at 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min. Final elongation at 72°C for 5 min. RAPD products were resolved by electrophoresis on a 1% agarose gel in Tris-borate-EDTA (TBE) buffer at 100 V for 1.5 h. The preliminary experiments using eleven primers were evaluated; therefore the reaction conditions and the reproducibility of the patterns can be optimized. All results were compared with a 1 kb DNA ladder marker.

Results

Comparative Characterization Between M. Haemolytica and P. Trehalose: Characterizations using plasmid DNA screening and RAPD were carried out in M. haemolytica A2, A7, A9 and *P. trehalosi* isolates from goats and chicken. Following plasmid extraction, only *P. trehalosi* from goats showed the presence of plasmid with a band of 4825 bp (Fig. 1).

Table 1 shows the RAPD analysis of *M. haemolytica* A2, A7, A9 and *P. trehalosi*. Generally, OPA16 primer gave the best differentiation between the five isolates followed by OPA20 primer. The OPA14 primer failed to differentiate clearly the various isolates. The OPA14 primer revealed slight differences between the isolates. Among the three *M. haemolytica* isolates, *M. haemolytica* A9 failed to produce the 1400bp band but had the 1752bp band. *M.*

haemolytica A2 was clearly different from A7 and A9 by having the 1036 and 192bp bands, which were absent in the other *M. haemolytica* isolates. However, only *M. haemolytica* A7 had the 484bp band (Table 1, Fig. 2). Both isolates of *P. trehalosi* revealed similar results and closely resembled *M. haemolytica* A7.

The OPA 16 primer revealed clear differences among the three isolates of *M. haemolytica*. *M. haemolytica* A2 showed several bands which were absent in other isolates of *M. haemolytica*. They were 1606, 1048, 798, 699 and 521bp bands. *M. haemolytica* A9 had a unique 440bp band (Table 1, Fig. 3). *M. haemolytica* A7 produced only two bands at 3240 and 2500bp. The *P. trehalosi* isolated from chickens was different from the goat isolate by the presence of the 2789, 2379, 643 and 427bp bands, which were absent in *P. trehalosi* isolated from goat.

OPA 20 primer differentiated the three *M. haemolytica* fairly well. *M. haemolytica* A2 showed the 3636 and 1450bp bands, which were absent in other isolates of *M. haemolytica*. Both *M. haemolytica* A7 and A9 showed the 700bp band but A7 revealed the 2823bp band instead of the 2323bp band of A9 (Table 1, Fig. 4). The bands produced by both isolates of *P. trehalosi* were completely different from each other and from the three isolates of *M. haemolytica*.

Discussion

The purpose of this study was to differentiate the various isolates of *Mannheimia* and *Pasteurella* through the screening of plasmid DNA and RAPD analysis. Characterization of multiple bacterial isolates has been carried out using the plasmid profile analysis and become a useful tool even on the same species (Schaberg *et al*., 1981 and Farrar, 1983). In this study, what we found that only *P. trehalosi* isolated from goats showed plasmid band. Since the presence of plasmid has been associated with the resistance to certain antibiotic, we believe that the *P. trehalosi* containing plasmid was isolated from a farm that practicing regular antibiotic use (Zhao *et al*., 1992).

Table 1: RAPD analysis with primers OPA 14, OPA 16 and OPA 20 revealing similarities and differentiation among the isolates.

	A2	A7	A9	T	4293 T
OPA 14	2320 bp	2296bp	2345bp	2247bp	2200bp
	1438	1424	1752	1409	1351
	1036	484	484	500	192
OPA 16	3393	3240	3393	3393	3290
	1606	2500	2500	2500	2789
	1048		440	1331	2379
	798		316		1299
	699				643
	521				427
	321				
OPA 20	3636	2823	2323	2880	1340
	2823	759	777	2222	825
	2385			695	712
	1450				425

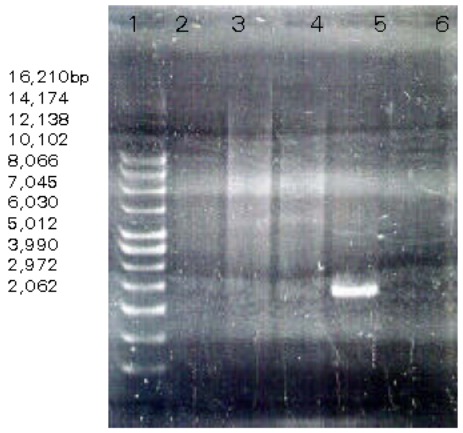

Fig. 1: Agarose gel electrophoresis of plasmid DNA from *M. haemolytica* and *P. trehalosi*. Lane 1: Supercoiled DNA ladder, lane 2: *M. haemolytica* A2, lane 3: *M. haemolytica* A7, lane 4: *M. haemolytica* A9, lane 5: *P. trehalosi* (goats isolate) and lane 6: *P. trehalosi* (chicken isolate)

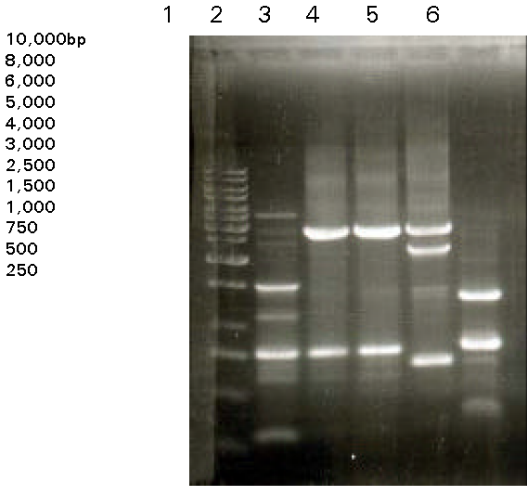

Fig. 2: RAPD analysis of *M. haemolytica* A2, A7, A9 and *P. trehalosi*.using OPA 14 primer. Lane 1: 1 kbDNA ladder, lane 2: *M. haemolytica* A2, lane 3: *M. haemolytica* A7, lane 4: *M. haemolytica* A9, lane 5: *P. trehalosi* (goats isolate) and lane 6: *P. trehalosi* (chicken isolate)

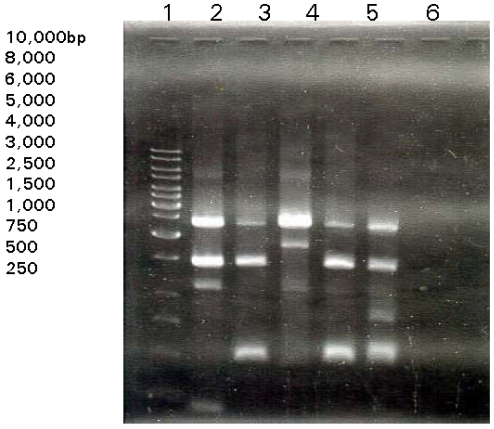

Fig. 3: RAPD analysis of *M. haemolytica* A2, A7, A9 and *P. trehalosi* using OPA 16 primer. Lane 1: 1 kbDNA ladder, lane 2: *M. haemolytica* A2, lane 3: *M. haemolytica* A7, lane 4: *M. haemolytica* A9, lane 5: *P. trehalosi* (goats isolate) and lane 6: *P. trehalosi* (chicken isolate)

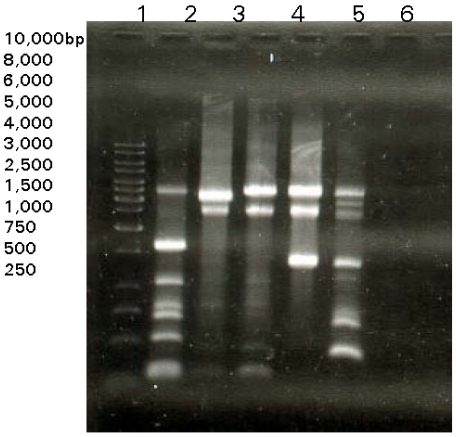

Fig. 4: RAPD analysis of *M. haemolytica* A2, A7, A9 and *P. trehalosi* using OPA 20 primer. Lane 1: 1 kbDNA ladder, lane 2: *M. haemolytica* A2, lane 3: *M. haemolytica* A7, lane 4: *M. haemolytica* A9, lane 5: *P. trehalosi* (goats isolate) and lane 6: *P. trehalosi* (chicken isolate)

The RAPD fingerprinting of the five isolates of *M. haemolytica* and *P. trehalosi* was obtained using primers OPA 14, 16 and 20. Each isolate produced unique and distinct fingerprint patterns. However OPA 16 primer produced the most distinguishable pattern while OPA 14 produced less distinguishable pattern. Thus, RAPD fingerprinting was found to be a rapid and sensitive method for serotype identification. It can be used to distinguish among the isolates and is valuable in surveillance and epidemiological studies.

Acknowledgement
The study was financially supported by IRPA grant from the Ministry of Science, Technology and Environment, Malaysia.

References

Chaslus-Dancla, E., M. C. Lesage-Decauses, S. Leroy-Setrin, S. L. Martel, P. Coudert and J. P. Lafont, J. P., 1996. Validation of random amplified polymorphic DNA assays by ribotyping as tools for epidemiological surveys of *Pasteurella* from animals. Vet Microbiol 52: 91-102.

Farrar, W. E. Jr., 1983. Molecular analysis of plasmids in epidemiologic investigations. J. Infect. Dis. 148:1-6.

Gilmour, N. J. L., 1993. Pasteurellosis: the disease. In: Pasteurellosis in production animals. Eds. BE Patten, TL Spencer, RB Johnson, D Hoffmann, L Lehane, pp79-82, ACIAR Proceedings No43.

Kodjo, A., L. Villard, C. Bizet, J. L. Martel, R. Sanchis, E. Borges, D. Gauthier, F. Maurin and Y. Richard, 1999. Pulsed-field gel electrophoresis is more efficient than ribotyping and random amplified polymorphic DNA analysis in discrimination of *Pasteurella haemolytica* strains. J. Clin. Microbiol. 37: 380-385.

Schaberg, D. R., L. S. Tompkins and S. Falkow, 1981. Use of agarose gel electrophoresis of plasmid deoxyribonucleic acid to fingerprint gram negative bacilli. J. Clin. Microbiol. 13: 1105-1108.

Zhao, G., C. Pijoan, M. P. Murtaugh and T. W. Molitor, 1992. Use restriction endonuclease analysis and ribotyping to study epidemiology of *Pasteurella multocida* in closed swine herds. Infect. Immun. 60: 1401-1405.