Random amplified polymorphic DNA (RAPD) analysis of *Pasteurella multocida* and *Manheimia haemolytica* strains isolated from cattle, sheep and goats

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ABSTRACT: In this study, 30, 15 and 1 strains of *Pasteurella multocida* and 9, 8 and 6 strains of *Mannheimia haemolytica* from cattle, sheep and goats isolated in Elazig province located in the East of Turkey, respectively were typed by random amplified polymorphic DNA (RAPD) assay using a random primer (OPA-11). By RAPD assay, two and three distinct band profiles were obtained in the examination of *P. multocida* isolates from cattle and sheep, respectively. However, *M. haemolytica* isolates from cattle, sheep and goats showed only one profile and these strains were not discriminated by RAPD. This study showed that little genetic heterogeneity exists among *P. multocida* and *M. haemolytica* isolates from lungs of cattle, sheep and goats.

Keywords: *Pasteurella multocida*; *Mannheimia haemolytica*; random amplified polymorphic DNA; cattle; sheep and goats; typing

Pasteurella spp. are important primary and oppurtunistic pathogens as well as common commensals of the upper respiratory tract of various domestic and wild animals (Loubinoux *et al*., 1999). Pasteurellosis is one of the most common disease of cattle, sheep and goats throughout the world where outbreaks usually lead to high mortality and great economic loss to the ruminant industry (Gilmour *et al*., 1991; Links *et al*., 1992).

Phenotypic methods, mainly biotyping and serotyping, have been widely used in the taxonomy and epidemiological studies of this species (Rimler and Brogden, 1986; Rhoades and Rimler, 1987, 1990; Mohan *et al*., 1994, 1997; Fegan *et al*., 1995). Due to shortfalls associated with phenotypic techniques, genotyping techniques have been used extensively to differentiate epidemiologically significant strains of *P. multocida* (Lainson *et al*., 2002). Both ribotyping (Snipes *et al*., 1992; Jaworski *et al*., 1993; Murphy *et al*., 1993) and restriction enzyme analysis (REA) (Snipes *et al*., 1989; Kim and Nagaraja, 1990; Christiansen *et al*., 1992; Magarinos *et al*., 1992; Wilson *et al*., 1993) have been shown to be useful for epidemiological studies involving Pasteurellosis studies. Random amplified polymorphic DNA (RAPD) analysis has been applied for the distinction of strains belonging to the same species (Welsh and McClelland, 1990; Williams *et al*., 1990). This method has been widely used in a variety of bacteria (Lam *et al*., 1995; Lin *et al*., 1996; Chaslus-Dancla *et al*., 1996; Chatellier *et al*., 1997; Lee and Mize, 1997; Tambic *et al*., 1997; Zhang *et al*., 1997; Maurer *et al*., 1998; Charlton *et al*., 1999; Dziva *et al*., 2001). It is a fast, sensitive method for the epidemiological studies and PCR-based method of genetic typing based on genomic polymorphisms (Weigler *et al*., 1996; Huber *et al*., 2002).

The aim of this study was to investigate the genetic differences among *P. multocida* and *M. haemolytica* isolates from cattle, sheep and goats by RAPD.

MATERIAL AND METHODS

Bacterial isolates and species confirmation

A total of 46 *P. multocida* and 23 *M. haemolytica* obtained from lung of cattle, sheep and goats were used in this study. Isolates were identified as *P. mul-*

tocida and *M. haemolytica* biochemically (Quinn *et al*., 1994). The identification of *P. multocida* and *M. haemolytica* strain were confirmed by PCR using PMOut (5′- AGG TGA AAG AGG TTA TG-3′ and 5′- TAC CTA ACT CAA CCA AC-3′) and PHSSA primers (5′-TTC ACA TCT TCA TCC TC-3′ and 5′-TTT TCA TCC TCT TCG TC-3′) (Neumann *et al*., 1998; Leeb, personal communication) derived from *Omp* and *ssa* gene, respectively.

DNA extraction

A few colonies from *P. multocida* and *M. haemolytica* cultures were transfered into an Eppendorf tube containing 300 µl distilled water. The tubes were vortexed and incubated at 56°C for 30 min. The suspension was then added in 300 µl of K-buffer (20 mM Tris pH 8.0 + 150 mM NaCl + 10 mM EDTA + 0.2% SDS) and 200 µg/ml Proteinase K. Following 30 min boiling, an equal volume of phenol was added to the suspension which was shaken vigorously by hand for 5 min and then, centrifuged at 11 600 g for 10 min. The upper phase was transferred into a new Eppendorf tube. Genomic DNA was precipitated with absolute ethanol and 0.3 M sodium acetate at –20°C for one hour. The mixture was then centrifuged at 11 600 g for 10 min and the upper phase discarded. The pellet was washed twice with 300 µl of 90% and 70% ethanol, respectively, each step followed by 5 min centrifugation. The pellet was dried, and resuspended in 50 µl sterile distilled water and used as a template DNA in PCR.

Primers

A random OPA-11 primer (5′-CA AT CG CC GT-3′) was used to determine genetic differences among *P. multocida* and *M. haemolytica* isolates.

RAPD analysis

The RAPD reaction was performed in a total volume of 50 µl consisting of 5 µl template DNA, 10 × PCR buffer (750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 3.5 mM $MgCl_2$, 200 µM deoxynucleoside triphosphates, 1.25 U of Taq DNA polymerase (Fermentas, Lithuania), and 1 µM of OPA-11 primer. The amplification was done in a Touchdown Thermal Cycler (Hybaid, England). PCR cycling procedures were as follows; 50 cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 1 min. A final step of extension was applied at 72°C for 10 min. Fifteen microliters of PCR products were analysed by electrophoresis in a 1.5% agarose gel with TBE buffer.

RESULTS AND DISCUSSION

An example of the electrophoretic profiles generated by RAPD analysis using OPA-11 primer of *P. multocida* and *M. haemolytica* isolates from cattle, sheep and goats is shown in Figures 1 and 2, respectively. Five different profiles were found in this study. Profiles A and B were found in cattle, profiles A, C and E were found in sheep by RAPD analysis of

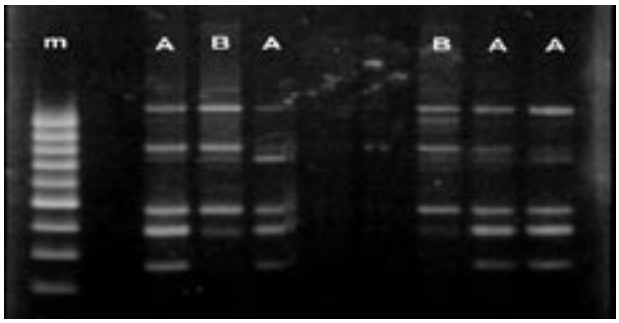

Figure 1. RAPD analysis of *P. multocida* isolates from cattle; m – 100 bp DNA ladder, A, B – profiles

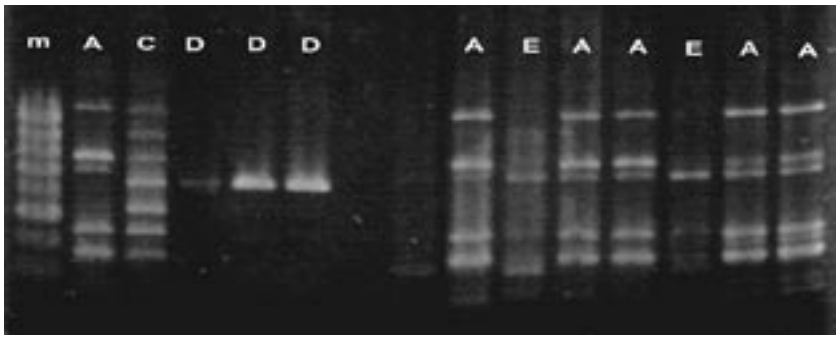

Figure 2. RAPD analysis of *P. multocida* and *M. haemolytica* isolates from sheep and goats; m – 100 bp DNA ladder, A, C, D, E – profiles

P. multocida isolates. Profile D was obtained as only profile in *M. haemolytica* isolates by RAPD analysis with OPA-11 primer.

In contrast to the uncertainties of characterization based on variable phenotypic characteristics, the application of molecular typing methods can provide a stable and highly discriminatory analysis of bacterial isolates (Hilton, 1997).

In order to explain genetic differences among the strains isolated from sheep and goat lungs, RAPD typing was used in this study. All the isolates were successfully typed using RAPD method. RAPD recognized two and three distinct profiles among *P. multocida* isolates obtained from cattle and sheep, respectively. However, the *M. haemolytica* isolates showed only one profile and these isolates were not discriminated by RAPD. Results of the RAPD assay demonstrated that little genetic heterogeneity exists among *P. multocida* and *M. haemolytica* isolates from cattle, sheep and goats. These results are in agreement with those of a previous study which reported the presence of only one profile among the bovine isolates of *M. haemolytica* by RAPD (Chaslus-Dancla *et al*., 1996). However, Chaslus-Dancla *et al*. (1996) showed that a relatively large amount of genetic heterogeneity existed among *P. multocida* strains isolated from rabbits. Kodjo *et al*. (1999) reported that slight differences were present among *M. haemolytica* isolates in RAPD analysis and suggested that PFGE is more efficient than ribotyping and RAPD for distinguishing *M. haemolytica* isolates of different origins. The use of different and more than one RAPD primers may improve differentiation power of RAPD process. Chaslus-Dancla *et al*. (1996) used two different random primers and obtained 7 different profiles with *P. multocida* strains. Only one random primer was used and 3 different profiles were observed in this study and this may be cause of less RAPD profiles than other studies.

Dziva *et al*. (2001) typed 81 *P. multocida* isolates of animal origin isolates by both capsular typing and RAPD analysis. Nine different groups of strains with identical RAPD profiles (100% similarity) were also observed. These findings implicated that there was significant relationship between phenotypes and RAPD profiles. This relationship could not be demonstrated in this study since phenotyping was not performed in this study. However, Wilson *et al*. (1993) observed that DNA fingerprint profiles of 50 isolates did not match profiles of the somatic type reference strains. These contradictory results between phenotyping and genetic analysis could be result of using different RAPD primers in different studies.

The results of this study indicated that little genetic heterogeneity exists among *P. multocida* isolates from cattle and sheep and RAPD analysis using OPA-11 is not efficient in differentiating strains of *M. haemolytica*.

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