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Molecular detection and characterization of Indian isolates of *Pasteurella multocida* serogroup 'D'

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ABSTRACT

Five *Pasteurella multocida* serogroup D isolated from pig (n=4, serotype D: 1) and sheep (n=1, serotype D: 3) were tested for molecular identification and characterization. All the isolates were detected by species specific PCR (PM-PCR) and ~460 bp products were obtained. RE analysis of the isolates using enzymes H*ha* I and H*pa* II generated very clear and readable discriminatory banding profiles and 3 different RE profiles obtained out of 4 *P. multocida* isolates of serotype D: 1. Among the 2 enzymes used in this study, H*pa* I had better discriminatory power than H*ha* I. Single primer based RAPD-PCR assay generated readable and distinct banding patterns between serotypes, but failed to differentiate 4 isolates of *P. multocida* serotype D: 1. Molecular detection using species specific PCR and characterization by molecular techniques like REA and RAPD-PCR could enable typing of *P. multocida* strains as a complement of classical capsular and somatic typing in preliminary characterization.

Key words: *Pasteurella*, PCR, REA, Serogroup D

Pasteurella multocida is a common commensal and pathogen of the respiratory tract of animals. Five capsular serogroups (A, B, D, E, F) based on indirect haemagglutination test (Carter 1955) and 16 somatic serogroups (1–16) based on agar gel precipitation test (Heddelston *et al.* 1972) are currently used for serotyping. Considerable variation has been observed among serotypes with respect to host predilection, pathogenicity and biochemical, cultural and antigenic specificity (Carter and Chengappa 1981).

Specific PCR assays, restriction endonuclease analysis (REA), randomly amplified polymorphic DNA (RAPD)-PCR and ribotyping have been successfully used in a variety of bacterial infections, including some of those caused by *P. multocida* (Bhat *et al*. 2004).

The objective of this study was to apply the species specific PCR for detection of *P. multocida* and to utilize REA and RAPD-PCR techniques to differentiate isolates of *P. multocida* serogroup D isolated from pigs and sheep.

MATERIALS AND METHODS

Identification of P. multocida: *P. multocida* isolates (4 from pigs and 1 from sheep) were studied and all the strains were subjected for identification based on cultural, morphological and biochemical characteristics as described in the standard bacteriological methods (Cruickshank *et al*. 1975). The smears were prepared from single non-haemolytic colony and Gram stained. Biochemically, indole production, presence of ornithine decarboxylase and urease activity were determined. Carbohydrate fermentation test of the isolated organisms were performed using D-glucose, D-arabinose, L-arabinose, lactose, maltose, dulcitol, mannitol, D-sorbitol, D-trehalose, D-xylose and L-xylose.

Serotyping of P. multocida: Serological determination of all the *P. multocida* isolates was performed at the Veterinary Research institute, Peradeniya, Sri Lanka using the indirect haemagglutination (IHA) test (Carter 1955) and agar gel precipitation test (AGPT) (Heddelston *et al.* 1972).

Detection of P. multocida *isolates by species specific PCR: P. multocida* isolates were further detected and confirmed by species specific PCR (PM-PCR) as described by Townsend *et al*. (1998). The PCR assay was carried out using primer set KMT1T7 (5'- ATC CGC TAT TTA CCC AGT GG – 3') and KMT1SP6 (5'- GCT GTA AAC GAA CTC GCC AC–3'). A colony touch PCR was carried out in a PCR mixture of 25 µl contained each primer at a concentration of 3.2 µM, each dNTPs at a concentration of 200µM, 1× PCR buffer, 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase. The following standard cycling procedure was used for amplification of the amplicon: an initial denaturation at 95°C

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