

REP-PCR PROFILES OF PASTEURELLA MULTOCIDA ISOLATES FROM CASES OF HAEMORRHAGIC SEPTICAEMIA

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ABSTRACT

Five isolates of P. multocida obtained from cases of Haemorrhagic septicaemia (HS) were characterized using repetitive extragenic palindromic PCR (REP-PCR). All the isolates obtained were confirmed as serotype B P. multocida using serotype-specific (HS-B) PCR, which gave amplified product of 590 bp. The five isolates gave a similar a similar REP-PCR profile, indicating a high degree of homogeneity among them.

Key words: Haemorrhagic septicaemia, Pasteurella multocida, REP-PCR

INTRODUCTION

India has the largest population of cattle and buffaloes in the world, the most important segments of Indian livestock economy, contributing to the major portion of milk, meat, skin and manure production. Haemorrhagic septicaemia (HS) affecting cattle and buffaloes is on the top among all bacterial diseases. On the basis of distribution of disease, three distinct categories of countries have been identified (OIE, 2002). India comes under category 'A' where disease is endemic and is of utmost economic importance. The most prevalent serotype of P. multocida causing disease in India is B: 2.

The use of molecular methods to genomically characterize P. multocida strains has provided information about the relatedness between HS-causing isolates and also revealed discrimination unobtainable by conventional serotyping methods (Townsend et al., 1997).

Repetitive extragenic palindromic sequences were the first family of highly conserved repetitive DNA sequences to be identified, dispersed through-out prokaryotic genome (Higgins et al., 1982, Lupski et al., 1992). Analysis of distribution of repetitive extragenic sequences in prokaryotic genomes forms the basis of a novel PCR based DNA finger printing technique known as REP-PCR, which not only differentiates related strains but also had the potential to identify virulence associated determinants (Versalovic et al., 1991, Go et al., 1995).

Hence the aim of this study was to analyze REP-PCR finger prints generated from five isolates of P. multocida obtained from cases of haemorrhagic septicaemia.

Five isolates of P. multocida obtained from cases of HS from Palakkad district, Kerala State were used in the study. These included BP1 & BP2 isolated from cattle and BuP1 & BuP2 from buffalo

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and GP1 from goat.

All the isolates were confirmed as serotype-B by HS-B PCR using serotype specific primer pairs KTS P 61 & KTT 72 following the methods of Townsend et al., (1998a). For preparation of template DNA for PCR analysis, the isolates were grown overnight in blood agar plates at 37°C, in a candle jar and a pure colony was inoculated into five milliliters of brain heart infusion broth (BHIB) and incubated at 37 °C for 18 h. From this broth culture 1.5 ml was transferred to an Eppendorf tube and centrifuged at 3000 X g for 10 min, the supernatant was discarded, washed the pellet twice with sterile PBS and final pellet was resuspended in 100 µl of triple distilled water. The mixture was boiled for 10 min and immediately chilled on ice for 30 min. The samples were thawed and centrifuged at 3000 X g for 5 min and supernatant was stored at -20°C for further use as template for PCR reactions.

The programme of amplification followed was; initial denaturation at 95°C for 4 min; annealing at 55°C for 45 sec and extension at 72 °C for 45 sec, followed by 29 cycles of denaturation at 95 °C for 1 min; annealing at 55 °C for 1 min; and extension at 72 °C for 1 min with a single final extension at 72 °C for 9min.

The REP-PCR was carried out with primer pairs REP1 and REP 2 as per methods described (Gunawardana et al., 2000). The final volume of 25 µl reaction mix was prepared by adding, 10X PCR reaction buffer 2.5 µl, 20 pM of each primer (1µl each of forward and reverse primer), 200µM of each dNTP (2µl), 2.5mM MgCl₂ (2.5 µl), one unit of Taq DNA polymerase (0.3 µl), triple distilled water to make 20 µl and 5 µl of template DNA. The amplification were performed with an initial cycle of denaturation at 94°C for 5 min; followed by 30 repeated cycles of 94°C for 1 min; 41°C for 2 min; and 72°C for 2 min. with a final extension at 72°C for 5 min.

Reference strain of *P. multocida*, P-52

(serotype-B) obtained from IVRI; Izatnagar was used as positive control for HS-B and REP-PCR. The PCR products generated were confirmed for their expected size in 1.5 per cent agarose gel. The molecular weight marker used to assess the size of amplified product of HS-B PCR was pBR 322/Alu I digest. Standard molecular weight markers, » DNA /Hind III digest and pUC 19 DNA/ MspI digest were used to ascertain the size of the DNA fragments generated by REP-PCR.

The five isolates of *P. multocida* when subjected to HS-B PCR, using the primer pairs, KTS P61 &KTT 72 gave an amplified product of size 590 bp (Fig. 1). On the basis of observations made by earlier workers (Townsend et al., 1998a; Hunt et al., 2000 and Dutta et al., 2004) and the results obtained in the present study, it could be concluded that HS-B PCR could confirm the serotype of *P. multocida*.

Analysis of banding patterns of fragments generated by REP-PCR indicated a total of 7 bands ranging in size from 4 kbp to 242 bp. The profiles of REP-PCR of all isolates appeared to be identical (Fig. 2).

Amplification of multiple *P. multocida* genomic DNA fragments by outwardly directed primers based on the Repetitive Extragenic Palindromic sequences (REP) generated complex profiles in a PCR based finger printing method known as REP-PCR. This finger printing method is shown to be highly discriminatory that differentiates strains of related bacteria (Versalovic et al., 1991; Go et al., 1995).

REP-PCR finger prints of thirty-eight HS causing strains of *P. multocida* showed a high degree of homogeneity while that of isolates causing fowl cholera exhibited a marked heterogeneity thereby providing support for the existence of a disease associated REP profile, distinct from isolates implicated in other pasteurellosis (Townsend et al., 1997). In the present study also all the five isolates

of *P. multocida* from cases of HS showed similar REP-PCR profile.

These findings were similar to those observed by others (Gunawardana et al., 2000 and Townsend et al., 1998). They reported that isolates of *P. multocida* from HS (B: 2) from Vietnam showed only minimal variation, with a single REP-profile for each of them. The isolates used in the present study also belong to capsular serogroup B as evidenced by HS-B PCR assay. Thus the present study suggests the usefulness of REP-PCR in the epidemiology and diagnosis of HS among livestock.

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