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# STRAIN DIFFERENTIATION OF MYCOPLASMA MYCOIDES SUBSP. MYCOIDES SC BY RAPAD-PCR

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*Mycoplasma mycoides* subsp. *mycoides* type small colony is the causative organism of contagious bovine pleuropneumonia (CBPP), which is included in the list A diseases of *Office Internationale-des-epizooties* (OIE) and also among the 6 priority diseases by FAO emergency prevention scheme (Rweyemamu and Benkirane, 1996). The disease until 17th century was limited to Alps region of Europe, but later on it was introduced into most of the European countries, North America, Australia and Asia by 18th and 19th centuries. In past, a number of outbreaks have been reported in many of the European countries ( Provost *et al.*, 1987). However, in India, CBPP is mainly restricted to certain parts of Assam (Chowdhary *et al.*, 1987). Recently Srivastava *et al.* (2000) has also isolated this organism from goat.

Number of molecular techniques *viz.* restriction endonuclease analysis, randomly amplified polymorphic DNA-PCR or RAPD-PCR, ribotyping, SDS-PAGE profile etc. have been reported for typing mycoplasmas by number of workers (Poumarat and Solsona, 1995; Nagai *et al.*, 1995; Rosati *et al.*, 1999; Butler *et al.*, 2001). Amongst them, RAPD-PCR or arbitrarily primed PCR (AP-PCR) has been proved to provide a reproducible typing method of different mycoplasma species as it is very simple and rapid (Charlton *et al.*, 1999; Butler *et al.*, 2001). In this study, an attempt was made to differentiate the Indian isolate from standard strain of *M. mycoides* subsp. *mycoides* SC by RAPD-PCR.

## Materials and Methods

Two strains (PG 1 and NCVP-1/86) of *M. mycoides* subsp. *mycoides* SC used in this study were taken from National Referral Laboratory on Mycoplasma, India Veterinary Research Institute, Izatnagar. Both the strains were grown in MBHS-L liquid media prepared according to the method of Carmichael *et al.* (1972). The strains were characterised by digitonin sensitivity, biochemical tests and growth inhibition test as per the method of Singh (1990). The genomic DNA of the strains were isolated according to the protocol of Wilson (1987) and the concentration of genomic DNA was determined by taking OD at 260 nm. Finally, they were diluted in sterile distilled water to make the concentration 100ng/µl.

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RAPD-PCR was performed using genomic DNA of both the strains of *M. mycoides* subsp. *mycoides* SC as template along with 4 different decamer primers as listed in Table. The PCR was carried out in 25 µl reaction mixture, which contained 100ng of genomic DNA; 50 mM KCl; 10 mM Tris-HCl, pH 8.8; 0.08% nonidet P40;2.5 mM $MgCl_2$; 250 µM of each dNTP; 1.5 unit of *Taq* DNA Polymerase (MBI Fermentas) and 50 pmol of decamer random primer using a programme with an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation (95°C for 45 seconds), annealing (36°C for 45 seconds) and extension (72°C for 45 seconds). Final extension was carried out at 72°C for 6 minutes. The PCR products were characterised by electrophoresis on 1.4% agarose gel containing ethidium bromide (0.3 µg/ml). A 10 µl of each PCR product was run along with suitable DNA marker (Generuler) at 7 volts/cm for 1 hour 30 minutes in 0.5 x TBE buffer (45 mM Tris, 45 mM boric acid and 2mM EDTAS, pH 8.0). Finally, the gel was visualised and photographed under a UV-gel documentation system.

## Results and Discussion

Out of the 4 primers, only OPG 10 yielded the polymorphic bands in both (PG 1 and NCVP-1/86) strains of *M. mycoides* subsp. *mycoides* SC strains. More or less similar RAPD pattern has been observed with an approximately 20 and 22 bands in PG 1 and NCVP -1/86 strain, respectively. A 360 bp long fragment was found in PG 1 strain, but not in NCVP-1/86 strain. Similarly, the bands of 1.48 kbp and 410bp were found in NCVP -1/86 strain but absent in PG 1 strain of *M. mycoides* subsp *mycodes* SC. The intense amplified products *viz.* 500 bp, 620 bp and 920 bp were found in PG 1 strain whereas, 320 bp, 920 bp, 1.19 kbp and 1.5. kbp long intense bands in case of NCVP -1/86 strain (Fig.).

The present findings revealed that two strains of *M. mycoides* subsp. *mycodes* SC can easily be distinguished very well by RAPD-PCR. Earlier, Poumarat and Solsona (1995) also differentiated 12 strains of *M. mycoides* subsp. *mycoides* SC by restriction endonuclease analysis (REA) using *Pst*I and *Bam*HI enzymes. The strain differentiation by RAPD-PCR is more characteristic than other typing techniques (Butler *et al.*, 2001), but it requires further study using a large number of mycoplasmal strains, which will be useful in future for molecular epidemiological investigations.

**Table - Arbitrary primers used in this study**

| Primers | Sequence |
|---|---|
| OPG 10 | 5'-AGG GCC GTCT-3' |
| OPG 13 | 5'-CTC TCC GCCC A-3' |
| OPA 04 | 5'-AAT CGG GCTG-3' |
| OPA 14 | 5'-TCT GTG CTG G-3' |

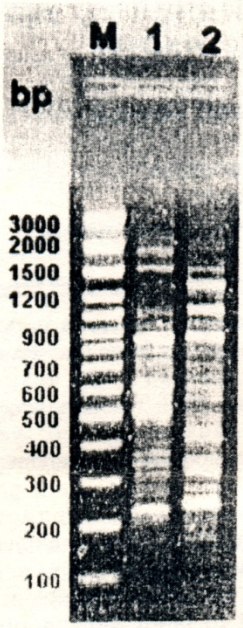


Fig. RAPD-PCR using 10 mer random primer OPG 10. Lane M : Marker (Generuler), Lane 1 : *M. mycoides* subsp. *mycoides* SC (PG 1), Lane 2 : *M. mycoides* subsp. *mycoides* SC (NCVP - 1/86)

## Summary

A randomly amplified polymorphic DNA PCR was carried out to differentiate the Indian isolate of *M. mycoides* subsp. *mycoides* SC (MmmSC) from the standard strain. Using OPG10 primer, more or less similar RAPD pattern was obtained in both the strains. The bands of 1.48 kbp and 410 bp were present in Indian isolate (NCVP-1/86) but not found in standard PG 1 strain of MmmSC. Similarly an approximately 360 bp product was found in PG1 strain, but absent in NCVP-1/86 strain. The prominent bands (500bp, 620bp and 920bp) were found in PG1 strain while 320bp, 920bp, 1.19kbp and 1.5kbp long intense bands were detected in case of NCVP-1/86 strain.

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