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Isolation and molecular characterization of *Mycoplasma synoviae* from chicken

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ABSTRACT

Samples (425) were collected for the isolation of *Mycoplasma synoviae* (MS) from chicken from commercial and government poultry farms from different parts of Karnataka, Andhra Pradesh and Tamil Nadu from both live and dead birds in Frey's mycoplasma broth. The species specific PCR was standardized for the detection of MS from chicken. Among 425 samples collected, 153 broth tubes showed characteristic signs of growth. Of the 153 broth tubes, 46 tubes were plated onto Frey's agar medium. Of these, only 27 broth tubes showed typical colonies characteristic of *Mycoplasma* species. The DNA was extracted from 153 tubes using commercially available kits and subjected for polymerase chain reaction to amplify 16S rRNA gene of MS with a set of published primer sequences. Of the 153 samples, only 3 samples yielded amplicon size of 207 bp specific for MS, which were identified by the lab numbers as KVAFSU MS R1, KVAFSU MS R4 and KVAFSU MS S9.

Key words: *Mycoplasma synoviae*, Polymerase chain reaction

Avian mycoplasmosis caused by *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) cause considerable economic losses in poultry industry (Kleven 2003, Ley 2003). MS causes respiratory disorder and synovitis in chicken, turkeys and other avian species, in conjunction with Newcastle disease, infectious bronchitis and others leading to significant drop in egg production (Stipkovits and Kempf 1996, Kleven 1997). The present study reports about isolation and molecular characterization of *Mycoplasma synoviae* from chicken with respiratory disorder and synovitis from southern India.

MATERIALS AND METHODS

Isolation of Mycoplasma: An isolate of MS, designated as MS 427 was obtained from the Department of Animal Biotechnology (ABT), Madras Veterinary College, TANUVAS. Frey's Mycoplasma broth (Frey *et al.* 1968) was used to grow and maintain the mycoplasma cultures. Clinical and postmortem samples were collected from commercial and government poultry farms from different parts of Karnataka, Andhra Pradesh and Tamil Nadu (Table 1). From live birds, swabs from choanal cleft and oropharynx were obtained, while from dead birds, samples of trachea, lungs and air sacs were obtained and immediately placed into tubes containing 5 ml of Frey's mycoplasma broth medium. The swab sticks were either left for a day or vigorously agitated once and then discarded. The medium was incubated at 37°C until the phenol red indicator changed from red to yellow color (or 5 –7 days) or characteristic whirlpool growth was seen which was suggestive of *Mycoplasma* species. When signs of growth appeared, the broth was plated into Frey's agar medium and incubated at 37°C, providing a humidified atmosphere and carbon dioxide tension by placing a vial filled with water and by flushing the container with 5–10% carbon dioxide respectively (Amin and Jordan 1978, OIE 2008).

The plates were examined daily for the appearance of typical fried egg colonies with stereoscopic/dissecting microscope at 50× magnification. The DNA was extracted from the culture using commercially available DNA extraction kit. All the samples were tested for the presence of MS by PCR using 16S rRNA species specific primers as reported by Marois *et al.* (2000).

PCR and electrophoresis: The template DNA (2 µl) was added to a mixture containing 5.00 µl of 10× PCR reaction buffer, 1.00 µl of 10 mM dNTP mix, 20 pmole of each primer

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Table 1. Particulars of samples collected and tested by cultural isolation and PCR confirmation for *Mycoplasma synoviae*

State	Place	Total no. of samples collected	No. discarded	No. chosen for DNA isolation	Positive for MS by	
					Isolation	PCR
Karnataka	Bangalore	9	8	1		
	Bellary	94	79	15	1	1
	Davanagere	22	10	12		
	Devanahally	24	16	8		
	Mysore	5	0	5		
Andhra	Chittoor	27	19	8	1	1
Pradesh	Hyderabad	11	5	6		
	Palamner	176	109	67	1	1
	Hindupur	20	16	4		
Tamil	Namakkal	28	7	21		
Nadu	Coimbatoor	9	3	6		
Total	425	272	153	3	3	

(MSF-5'-GAGAAGCAAAATAGTGATATCA-3' and MSR 5'-CAGTCGTCTCCGAAGTTAACAA-3') of 16s rRNA gene, 3 units of *Taq* DNA polymerase. The final volume was made up to 50 µl by addition of nuclease free water.

For MS the DNA amplification was performed in a thermal cycler with an initial denaturation of 94°C for 5 min followed by 35 cycles of, denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min and 1 cycle of final extension at 72°C for 7 min (Marois *et al*. 2000).

The amplicons obtained were checked in 2% agarose gel electrophoresis with 100 bp ladder as a marker, they were detected by UV transillumination with ethidium bromide staining and gel documentation was done. The positive results were recorded based on the appearance of a fragment of 207 bp for MS (Marois *et al.* 2000).

RESULTS AND DISCUSSION

Among 425 samples collected into Frey's broth medium, 153 samples were chosen for cultural isolation and PCR of which only 3 samples were found positive (Table 1).

The cultural isolation of mycoplasma was slow, difficult, expensive and requires sterile conditions, and isolations need to be carried out in conjunction with serology and growth inhibition tests to confirm the isolates. Further, the isolation may not be successful due to overgrowth of faster growing nonpathogenic mycoplasmas (Hyman *et al*. 1989, Zhao and Yamamoto 1990, Yoder 1991). In the present study, although a complex medium was used by incorporating NAD and swine serum, due to fastidious growth requirements of avian mycoplasma, their isolation from the field samples was difficult and tedious.

Further, *in vitro* DNA amplification by using published primer sequences designed on the basis of the 16S rRNA gene of MS was found to be very specific (Lauerman *et al*. 1995, Kempf 1998, Ramadass *et al*. 2006). Although 153 samples suggestive of mycoplasma growth in Frey's broth media with a characteristic whirlpool appearance were subjected for PCR analysis, only 3 samples yielded amplicon size of 207 bp specific for MS (Fig. 1).

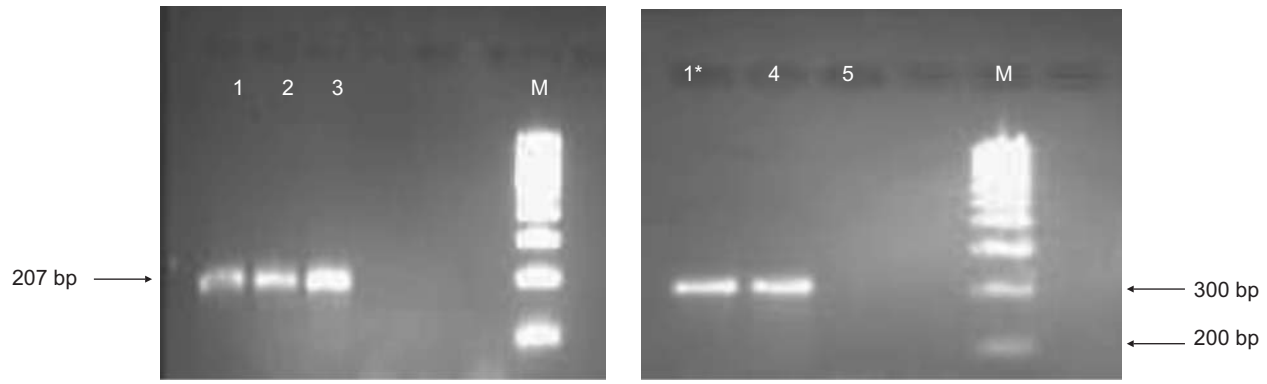


Fig 1. Agarose gel electrophoresis of PCR amplified products of three *Mycoplasma synoviae* field isolates.

Lane 1and1*: KVAFSU MS R4, *Mycoplasma synoviae* field isolate; Lane 2: KVAFSU MS R1, *Mycoplasma synoviae* field isolate; Lane 3: KVAFSU MS S9, *Mycoplasma synoviae* field isolate; Lane 4: Positive control MS 427; Lane 5: Negative control; Lane M: 100 bp DNA ladder.

Less number of organisms may be responsible for very few samples getting detected by PCR leading to false negative results in the present study. During our investigation, the farm reports were made available to us by farm supervisors (unpublished data) and it was noticed that most chicken were seropositive for MS or MG. So, it is no surprise that chicken could be serologically positive, but culture or DNA probe test negative, because tracheal levels of mycoplasma increase, peak and decrease with time after infection as reported by Yagihashi and Tajima (1986) and Levisohn and Dykstra (1987).The 16s RNA gene amplification was found to be a method of choice for confirmation of MS.

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