

BIOCHEMICAL AND IMMUNOLOGICAL IDENTIFICATION OF MYCOPLASMA ISOLATES FROM BOVINE MASTITIS

M.SANJEEV KUMAR¹, SHIVARAJ MURAG¹ AND Y.HARI BABU^{*}

SRDDL, Institute of Animal Health & Veterinary Biologicals
HEBBAL, BANGALORE KARNATAKA-560024

Key words: Mycoplasma, Isolation, Immunological identification, Biochemical studies.

Mastitis of Mycoplasma etiology is highly complex condition which involves the interaction of agent, management and environment. The main source of mastitis spread is infected udder, milker's hands, milking machines which act as an important vehicle for transmitting the infection. As mastitis can't be eradicated, prevention and control by early detection holds much promise in combating the losses. Therefore isolation and identification of the causative organism is essential for achieving the ultimate aim of treating and preventing mycoplasma mastitis. This study was undertaken to identify and characterize the mycoplasma species of organisms isolated from bovine mastitis milk samples.

A total of eighty seven mastitic animals were screened for the purpose of isolation of mycoplasma species of organisms. Isolation of organisms was carried out using the methodology of Ashwini kumar and Garg (1990). Reconstituted mycoplasma agar base was supplemented with 20 ml of unactivated horse serum 10 ml of yeast extract and 0.2 ml Glucose (50%), and the milk sample was inoculated in to the medium and incubated.

Sodium polyanethol sulphonate (NPS) test was conducted as to differentiate the isolates from acheloplasma species as per Bisphing and Amsberg, (1988). Growth inhibition test was carried

out with known antiserum for identification of the isolates following the method of Clyde, (1964).

Antiserum was raised against isolate No.2 in New Zealand white rabbit (Haribabu 1995) agar gel precipitation (AGPT), Counter immunoelectrophoresis (CIE), Immunoelectrophoresis (IE) and Single radial immunodiffusion (SRID) tests were carried out based on the methodology of Uppal et al.,(1983) for immunological characterization of the isolates.

Biochemical characterization of the isolates was done using glucose fermentation test, urea hydrolysis and arginine hydrolysis tests as per the standard procedures.

In the present study out of eighty seven mastitic animals screened for the isolation of mycoplasma species. Total of four animals were found positive for mycoplasma mastitis. The pure cultures of the isolates were maintained in the laboratory for biochemical and immunological identification.

Sodium polyanethol sulphonate (NPS) test was conducted where in the results of NPS test showed growth inhibition surrounding the discs soaked in 5% NPS solution, which indicated that the isolates were of mycoplasma and not of acheloplasma species. Growth inhibition test

^{*}Dean, Veterinary College KVAFSU Bidar., I-Scientist

of the four isolates against the known antiserum of *Mycoplasma agalactiae* shown positive reaction with an inhibition zone of 2-4 mm in all the four isolates. The findings were in accordance with observation of Cottow (1974) who expressed that 1 mm inhibition zone could be taken as specific positive reaction. The results of NPS test and Growth inhibition test are given in table.

The antiserum raised in rabbit against isolate No.2 when tested on agar gel precipitation test with the mycoplasma whole cell antigen, all the four isolates developed precipitation bands after an incubation period of 24 hours in humid chambers (Fig-1). By counter immunoelectrophoresis (CIE) all the four isolates developed clear precipitation lines in 40-50 minutes in 1% agarose gel (Fig-2). These observations were in agreement with and Uppal et al, (1983), who observed that CIE is 16 times more sensitive than AGPT in detection of mycoplasma infections

Immunoelectrophoresis (IE) was carried out with whole cell antigen of isolate No.1 and 2 against the test antiserum. The test showed two clear distinct arcs of precipitation bands at the base of the trough containing antiserum (Fig-3). The findings were similar to the observations of Thirkill and Kenny, (1975). Single radial immunodiffusion (SRID) was done by using test serum, all the four isolates developed precipitation rings around the wells. The observations were in similar to that of Awati. (2003) and Hugar (2004).

Biochemical studies of all the four mycoplasma isolates of bovine origin revealed that they were similar in their biochemical activity and none of the isolates hydrolysed arginine and urea and negative for glucose fermentation test. These findings indicated that the cultures were pure and belonged to a single species of mycoplasma, the observations are in accordance with Ajuwape et al., (2003). Results of biochemical studies are shown in table

In the present study all the four isolates obtained were confirmed as *Mycoplasma agalactiae* and found to infect bovine udders frequently than other mollicute organisms. These results correlates with the findings of previous researchers who observed the association of *Mycoplasma bovis*, *Mycoplasma agalactiae*, *Mycoplasma putrefaciens*, *Mycoplasma mycoides* sub species. capri more frequently and demonstrated from diseased udders of bovines.

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Table 1

Details of Colony morphology, Biochemical and Serological characteristics of *Mycoplasma* isolates

No.	Colony morphology	API Test	fermentation test	hydrolysis test	hydrolysis test	Growth inhibition	Final identification
1.	Umbonate shaped with lace like granularity at peripheral zone	-	-	-	-	+	<i>M. agalactiae</i>
2.	Umbonate shaped with lace like granularity at peripheral zone	-	-	-	-	+	<i>M. agalactiae</i>
3.	Umbonate shaped with lace like granularity at peripheral zone	-	-	-	-	+	<i>M. agalactiae</i>
4.	Umbonate shaped with lace like granularity at peripheral zone	-	-	-	-	+	<i>M. agalactiae</i>