Available online at www.sciencedirect.com
ScienceDirect
The Veterinary Journal 178 (2008) 149–152
The Veterinary Journal
www.elsevier.com/locate/tvjl

Short Communication

A semi-defined medium without serum for small ruminant mycoplasmas

Ana S. Ramírez [a,*], Juan Luis Fleitas [a], Rubén S. Rosales [a], Carlos Poveda [a], Christian de la Fe [a], Marisa Andrada [b], Ayoze Castro [b], José B. Poveda [a]

[a] *Unidad de Epidemiología y Medicina Preventiva, Spain*
[b] *Departamento de Histología y Patología, Facultad de Veterinaria, Universidad de Las Palmas de Gran Canaria, Trasmontaña s/n, Arucas 35416, Las Palmas de Gran Canaria, Spain*

Accepted 27 July 2007

Abstract

The composition of the medium used to cultivate *Mycoplasma* species is very important. Serum is one of the most important additives as it contains lipids (cholesterol) and serum proteins, which are essential for the growth of the organisms. This work reports the development of a semi-defined medium, called MWS (Medium Without Serum) produced without animal serum and bovine serum albumin. MWS seems to be suitable for cultivating several species of caprine mycoplasma, especially *M. mycoides* subsp. *mycoides* (LC) and *M. mycoides* subsp. *capri*.

Keywords: Mollicutes; *Mycoplasma* species; Culture media; Animal sera

Mycoplasma. mycoides subsp. *capri* (Nicholas, 2002), *M. arginini* (Prasad et al., 1984), *M. agalactiae, M. mycoides* subsp. *mycoides* Large Colony (LC) type, *M. capricolum* subsp. *capricolum* and *M. putrefaciens* (Nicholas, 1998) are potentially pathogenic for small ruminants. Mycoplasmosis, and in particular contagious agalactia syndrome, causes significant economic losses worldwide (Galliard-Perrin and Lenfant, 1986).

Mycoplasmas (class *Mollicutes*) represent the smallest self-replicating life forms and have complex nutritional requirements, including a dependence on external supplies of many biosynthetic precursors (Baseman and Tully, 1997). The usual growth medium for mycoplasmas was undefined and includes animal serum to supply cholesterol and serum proteins (Miles, 1992). However, serum presents considerable batch variation and there is a potential risk of contamination with viruses, mycoplasma, prions etc. (Castle and Robertson, 1999). In addition, serum may contain substances that inhibit the isolation of mycoplasmas (Washburn and Somersom, 1979) as well as saccharolytic enzymes which can interfere in certain metabolic studies (Wadher and Miles, 1988).

When preparing mycoplasma antigens, for example in creating monoclonal antibodies, serum present in the mycoplasma medium has been considered a major contaminant (Hwang et al., 1989). Ferreira Neto and Yamamoto (1993) showed that the choice of culture medium can change the mycoplasma protein banding pattern and Thorns and Boughton (1980) found that serum proteins affect mycoplasma antigenicity. The aim of the present work is to report the development of a semi-defined, serum-free and bovine serum albumin-free medium for the growth of caprine mycoplasmas, designated MWS (Medium Without Serum) (Table 1). In addition, we have compared the growth of three mycoplasma strains in this medium with growth in a medium containing serum.

Sixteen caprine mycoplasma strains were selected. These were *M. mycoides* subsp. *mycoides* (LC) (266/94, 6P, 207/93, 2/93, 152/93, 153/93, 80X3, 83/93), *M. m.* subsp. *capri* (207/90, 260/93), *M. capricolum* subsp. *capricolum* (14B,

* Corresponding author. Tel.: +34 928 454 355; fax: +34 928 451 142.
E-mail address: aramirez@dpat.ulpgc.es (A.S. Ramírez).

1090-0233/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2007.07.023

Table 1
Composition of MWS medium

Solution A	
Bacto PPLO broth (Difco)	16.8 g
Distilled water	500.0 mL
Inorganic salts solution [a]	300.0 mL
Cholesterol (Sigma)	20 or 40 mg
Palmitic acid (Sigma)	10 mg
Oleic acid (Sigma)	10 mg
Tween 80 (Sigma)	0.2 mL
Adjust pH to 7.4. Sterilize at 121 °C for 20 min For solid medium add 9.0 g Agar No. 1	
Aseptically add the following filter-sterilized ingredients:	
Solution B	
Fresh yeast extract (50% W/V)[b]	9.0 mL
Penicillin G solution (200.000 U/mL)	9.2 mL
DNA (0.4% W/V) (Sigma)	4.4 mL
CMRL 1066 (Sigma)	1.96 g
Phenol red (1% w/v)	10.0 mL
Distilled water	167.4 mL
Adjust pH to 7.4	

[a] The inorganic salts solution contains per litre: KCl, 0.11 g; $MgSO_4 \cdot 7H_2O$, 0.0289 g; $MgCl_2 \cdot 6H_20$, 0.0289 g; $CaCl_2$, 0.04 g; $Na_2HPO_4 \cdot 12H_2O$, 0.043 g; KH_2PO_4, 0.017 g; distilled water, 1000 mL
[b] Prepared by acid extraction (Friis, 1979).

21B), *M. agalactiae* (25/95, 97/95, L9) and *M. arginini* (33/95). The following culture media were used: PH medium (Kirchhoff and Rosengarten, 1984) containing 18% horse serum; MWS medium; four culture media obtained by mixing PH and MWS media to obtain final serum concentrations of 9%, 4.5%, 2.2% and 1.1%, respectively, and another three culture media prepared by adding serum to MWS medium to reach final serum concentrations of 1%, 0.5% and 0.25%, respectively.

Initially, all organisms were grown at 37 °C in PH medium. To adapt them to grow in MWS medium, the mycoplasmas were progressively grown with less serum by subculturing them in the range of mixed PH-MWS media with a decreasing percentage of animal serum until they reached logarithmic phase after 48 h. After the mycoplasmas had been adapted to grow in PH-MWS medium with 1.1% horse serum, they were transferred into MWS medium plus 1% horse serum. To facilitate this adaptation, the different mycoplasma species were subcultured 10 times in 1% serum MWS medium, then, five times in medium with 0.5% and 0.25% horse serum, respectively, before they were finally inoculated in MWS without serum.

In all cases, the presence of the typical mycoplasma colony morphology was checked by inoculating PH media plates with each corresponding tested culture medium. In the final stage, the MWS media were examined to assess whether they could support the growth of varied bacterial strains. This was done by transferring samples from liquid cultures onto MWS medium plates to observe typical 'fried egg' colonies. Colony counts were performed using a surface drop-plate method. The results are given in colony forming unit per millilitre (cfu/mL) (Albers and Fletcher, 1982).

Defined and semi-defined media lacking serum have been established for some *Mycoplasma* species. Cholesterol and fatty acids (palmitic and oleic), were found to be essential by Archer (1975) and Wadher and Miles (1988), although Gardella and Del Giudice (1995) did not regard oleic acid as essential. In the present study, osmotic stability was achieved by the addition of oleic and palmitic acids. These observations are compatible with the findings of other authors (Hackett et al., 1987; Razin, 1969; Razin et al., 1967) using bovine serum albumin (BSA). In addition, Rodwell (1983), Hackett et al. (1987), Razin and Tully (1970) and Muñoz and Sotomayor (1990) suggested that cholesterol and fatty acids can act as a serum substitute.

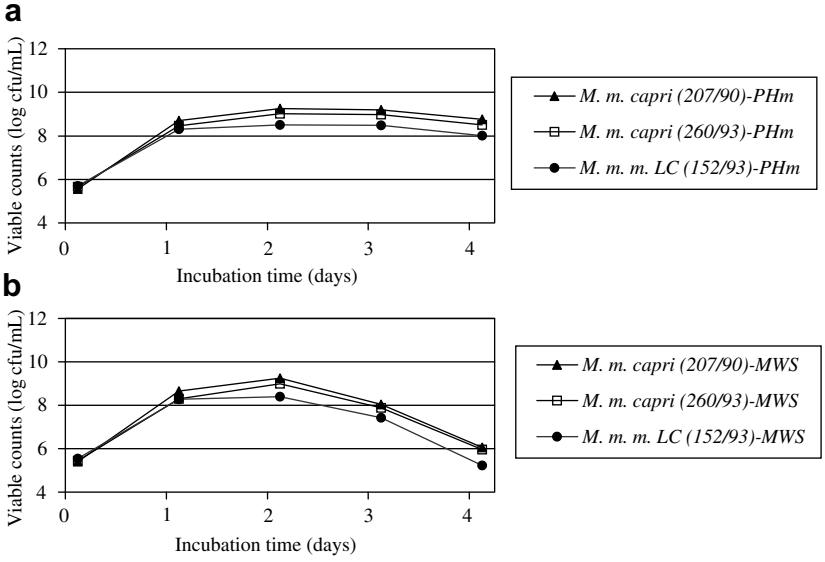

Fig. 1. Comparative bacterial titre of *M. mycoides* subsp. *capri* (207/90, 260/93) and *M. mycoides* subsp. *mycoides* (LC) (152/93) in PH medium (PHm) and MWS medium.