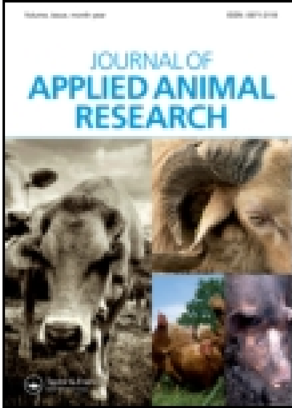
This article was downloaded by: [Central Institute of Fishery Technology]
On: 26 May 2013, At: 09:06
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954
Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Applied Animal Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/taar20

Multiplex PCR for Differentiation of Mycoplasma mycoides Cluster from other Mycoplasma

Sanjoy Das [a] & V. P. Singh [a]

[a] National Referral Laboratory on Mycoplasma Division of Bacteriology & Mycology, Indian Veterinary Research Institute, Izatnagar, 243 122, India

Published online: 11 Nov 2011.

To cite this article: Sanjoy Das & V. P. Singh (2003): Multiplex PCR for Differentiation of Mycoplasma mycoides Cluster from other Mycoplasma, Journal of Applied Animal Research, 24:1, 95-100

To link to this article: http://dx.doi.org/10.1080/09712119.2003.9706440

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable

for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013

J. Appl. Anim. Res. 24 (2003) : 95-100

Multiplex PCR for Differentiation of *Mycoplasma mycoides* Cluster from other Mycoplasma

Sanjoy Das, V. P. Singh*

National Referral Laboratory on Mycoplasma
Division of Bacteriology & Mycology
Indian Veterinary Research Institute
Izatnagar, 243 122, India

(Revised received June 6, 2003; accepted June 16, 2003)

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013

Abstract

Das, S. and Singh, V.P. 2003. Multiplex PCR for differentiation of *Mycoplasma mycoides* cluster from other mycoplasma. J. Appl. Anim. Res., 24: 95-100.

A multiplex PCR was standardized for detection of various members of ***Mycoplasma mycoides*** *cluster and their differentiation from other mycoplasma as well as some other bacteria. Using mycoplasma group specific primers GPO-1/MGSO and* ***Mycoplasma mycoides*** *cluster specific primers MC 323 / MC 358 simultaneously, two amplified products of 1.5 kbp and 715 bp were found only in members of* ***M. mycoides*** *cluster whereas, a single band of 715 bp was detected in all other non cluster mycoplasma and acholeplasma while no amplified product was detected in any other bacterial strain tested.*

Introduction

A number of important diseases viz. contagious bovine pleuropneumonia (CBPP), contagious caprine pleuropneumonia (CCPP), arthritis, mastitis, genital disorders of livestock are

*Author for correspondence.

J. Appl. Anim. Res. 0971-2119/2003/$5.00 © GSP, India.

produced by members of *Mycoplasma mycoides* cluster (Cottew *et. al.*, 1987; Thiaucourt and Bolske, 1996). Amongst them, the most important disease is CBPP, a disease of OIE list A and is also included in six priority diseases under FAO emergency prevention scheme (Rweyemamu and Benkirane, 1996). The accurate detection of the causative agent of these infections has not been possible with the help of routine conventional techniques like isolation and serodiagnosis. Isolation is laborious and often hampered by contamination with other bacteria while extreme serological cross-reactivity among different species of mycoplasma may obfuscate the serodiagnosis (Cottew *et al.*, 1987). Moreover, serological tests do not detect the positive cases in early and later stages of infection. Lately, the use of polymerase chain reaction has been found helpful in detection of *Mycoplasma mycoides* cluster as it has both specificity and sensitivity (Bashiruddin *et al.* 1994; Kumar *et. al.*, 2001). Recently, the introduction of multiplex PCR has proved to be very useful in detection and differentiation of different organisms in a single PCR reaction, which is saving both time and money (Caron *et al.*, 2000).

The present study was intended to standardize a multiplex PCR assay for detection of mycoplasma as well as members of *Mycoplasma mycoides* cluster in a single PCR reaction.

Materials and Methods

Ten strains of mycoplasma (*M.bovis, M.bovirhinis, M. bovigenitalium, M.mycoides* subsp. *mycoides* SC, *M.* sp. bovine group 7, *M. alkalescens, M. agalactiae*) and one strain each of *Acholeplasma laidlawii, Pasteurella* sp. and *Salmonella* sp. obtained from National Referral Laboratory on Mycoplasma, Division of Bacteriology and Mycology, IVRI, Izatnagar were used in this study. The strains of *M.mycoides* subsp. *mycoides* SC, *M.* sp. bovine group 7 and *M.agalactiae* were grown in MBHS-L liquid medium (Carmichael *et al.*, 1972) whereas, specially enriched B medium (Ernø and Stipkovits, 1973) was used for the growth of *M.bovis, M.bovirhinis, M.bovigenitalium* and *M. alkalesens* strains.

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013

Isolation of genomic DNA

The genomic DNA of all the strains were isolated as per the protocol of Wilson (1987) and the concentration of genomic DNA was determined by measuring the optical density at 260 nm in a UV spectrophotometer. Finally, these were diluted in sterile distilled water to make the concentration of 10 ng/μl of DNA.

Multiplex PCR assay

Mycoplasma group specific primers GPO-1[5'-ACT CCT ACG GGA GGC AGC AGT A-3'] and MGSO [5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC -3'] (Kuppeveld *et al.*, 1992) as well as *Mycoplasma mycoides* cluster specific primers viz. MC 323 [5'-TAG AGG TAC TTT AGA TAC TCA AGG -3'] and MC 358 [5'- GAT ATC TAA AGG TGA TGG T - 3'] (Bashiruddin *et al.*, 1994) were used simultaneously. A 20 ng of genomic DNA of each strain was used separately as a template in 50 μl reaction mixture consisting of 10mM Tris-HCl, pH 8.8; 50 mM KCl, 1.5 mM $MgCl_2$; 0.08% nonidet P40; 200 μM dNTP mixture (MBI Fermentas). The programme consisted of initial denaturation at 94C for 5 min followed by 30 cycles of denaturation at 94C for 45 sec, annealing at variable temperatures (50, 53, 55 and 57C) for 1 min and extension of 1 min 20 sec at 72C. The final extension was carried out at 72C for 5 min.

A 10 μl of each PCR product was analysed on 1.4% agarose gel electrophoresis at 7 volts/cm for 1 h 30 min in 0.5x TBE buffer (45 mM Tris, 45 mM boric acid and 2 mM EDTA, pH 8.0) alongwith the DNA maker (pBR 322 DNA digested with AluI enzyme). The gel was strained with ethidium bromide and finally, it was visualized and photographed under UV gel documentation system.

Results and Discussion

Out of the four annealing temperatures, only 53C was found optimum in the present multiplex PCR assay as evidenced by the presence of intense bands of both the amplified products. On using both sets of primers GPO-1/MGSO and MC 323/MC 358, a single band of 715 bp was found in all the non-cluster mycoplasma and acholeplasma strains whereas, two amplified products of 1.5 kbp

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013

and 715 bp were detected only in the strains of *Mycoplasma mycoides* cluster viz. *M.mycoides* subsp. *mycoides* SC (PG1 and NCVP-1/86) and *M.* sp. bovine group 7 (PG50). No amplified product was detected in other bacteria viz. *Pasteurella* sp. and *Salmonella* sp. (Fig.1). Similar types of findings were also reported by Kuppeveld *et al.* (1992) using GPO-1/MGSO primers, which amplify a 715 bp region of 16S rRNA gene of mycoplasma, acholeplasma, spiroplasma and ureaplasma. While Bashiruddin *et al.* (1994) observed 1.5 kbp product in the region of CAP-21 sequence of all the members of *Mycoplasma mycoides* cluster group (*M. mycoides* subsp. *mycoides*

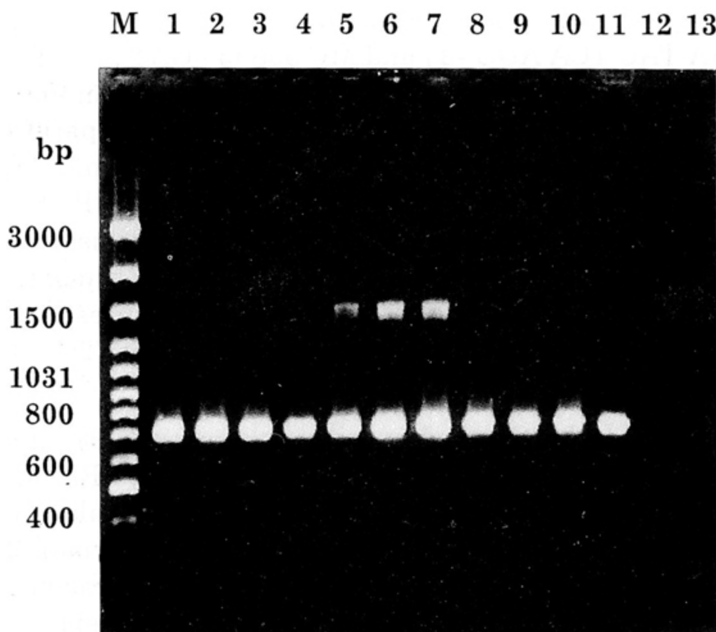


Fig. 1 : Multiplex PCR using primers GPO-1, MGSO, MC323 and MC358: Lane M: Marker (pBR322DNA digested with AluI), Lane1: *M. bovis* (NC317), Lane2: *M. bovis* (23K), Lane3: *M. bovirhinis* (NC448), Lane4: *M. bovigenitalium* (NC58), Lane 5: *M. mycoides* subsp. *mycoides* SC (PG1), Lane 6: *M. mycoides* subsp. *mycoides* SC (NCVP-1/86), Lane7: *M. sp.* bovine group7 (PG50), Lane8: *M. alkalescens* (NC242), Lane9: *M. agalactiae* (RPNS216), Lane 10: *M. agalactiae* (RPNS 200), Lane 11 : *A. laidlawii* (NC 313), Lane 12 : *Pasteurella sp.*, Lane 13 : *Salmonella sp.*

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013

type SC, *M. mycoides* subsp. *mycoides* type LC, *M. mycoides* subsp. *capri*, *M. capricolum* subsp. *capricolum*, *M. capricolum* subsp. *capripneumoniae* and *M.* sp. bovine group 7) with MC 323 / MC358 primers.

The results of present study revealed that the multiplex PCR can be used for the detection of members of *Mycoplasma mycoides* cluster and it also differentiates them from other non cluster mycoplasma and bacteria simultaneously in a single PCR assay. This ultimately saved the time and money for detection of mycoplasma upto the species level in a single PCR reaction.

Acknowledgement

The authors are thankful to the Director and Head, Division of Bacteriology & Mycology, IVRI, Izatnagar for providing necessary facilities.

References

Bashiruddin, J.B., Taylor, T. K. and Gould, A.R. 1994. A PCR based test for specific identification of *Mycoplasma mycoides* subsp. *mycoides* SC. J. Vet. Diagn. Invest., 6: 428-434.

Caron, J., Ouardani, M. and Dea, S. 2000. Diagnosis and differentiation of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infections in pigs by PCR amplification of the p36 and p46 genes. J. Clin. Microbiol., 38: 1390-1396.

Carmichael, L.E. St., George, T.D., Sulliva, M.D. and Horsfall, N. 1972. Isolation and characterization of an ovine mycoplasma responsible for proliferative interstitial pneumonia. Cornell Vet., 62: 654-679.

Cottew, G.S., Bread, A., DaMassa, A.J., Erno, H., Leach, R.H., Lefevre, P.C., Rodwell, A.W and Smith, G.R. 1987. Taxonomy of the *M.mycoides* cluster. Isr. J. Med. Sci., 23: 632-635.

Ernø , H. and Stipkovits, L. 1973. Bovine mycoplasma, cultural and biochemical studies. Acta. Vet. Scand., 14: 436-449.

Kumar, M.M., Singh, Vijendra P., Srivastava, N.C., Singh, V.P., Sharma, B., Sundar, J. and Kumar, A.A. 2001. Rapid and specific detection of *Mycoplasma mycoides* cluster and differentiation of mycoides group from capricolum group by polymerase chain reaction. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 22: 118-121.

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013

Kuppeveld, F.J.M., Logt, J.T.M.V., Angulo, A.F., Zoest, M.O.V., Quint, W.G.V., Neisters, H.G.M., Galama, J.M.D and Melchers, W.J.G., 1992. Genus and species specific identificaition of mycoplasmas by 16s rRNA amplification. Appl. Environ. Microbiol., 58: 2606-2615.

Rweyemamu, M. and Benkirane, A. 1996. Global impact of infections with organisms of *Mycoplasma mycoides* cluster in ruminants. COST 826. Agric. and Biotech. Luxenbourg, pp. 1-11.

Thiaucourt, F. and Bolske, G. 1996. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev. Sci. Tech. Off. Int. Epiz., 15: 1397-1414.

Wilson, K. 1987. *Preparation of genomic DNA from bacteria. Current protocols in molecular biology.* Unit 2: 4-1. Wiley, New York.

संजय दास, वी.पी. सिंह। अन्य माइकोप्लाज्मा से माइकोप्लाज्मा माइकायडिस समूह का बहुघटकी पीसीआर द्वारा विभेदीकरण।

अन्य माइकोप्लाज्मा और सूक्ष्माणुओं से माइकोप्लाज्मा माइकायडिस का पता लगाकर विभेदीकरण के लिए एक बहुघटकी पीसीआर का मानकीकरण किया गया। माइकोप्लाज्मा वर्ग विशिष्ट प्राइमर, जीपीओ-1/एमजीएसओ और माइकोप्लाज्मा माइकायडिस वर्ग विशिष्ट प्राइमर एमसी 323/एमसी 358 को साथ-साथ प्रयोग करके 1.5 केबीपी और 715 बीपी के दो दीर्घीकृत उत्पाद केवल माइकोप्लाज्मा माइकायडिस वर्ग से मिले, जबकि अन्य असमूही माइकोप्लाज्मा और एकोलिप्लाज्मा में केवल 715 बीपी की एक पट्टी और अन्य परीक्षित सूक्ष्माणुओं में कुछ भी नहीं मिला।

Downloaded by [Central Institute of Fishery Technology] at 09:06 26 May 2013