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Short Communication

A SIMPLE AND COST-EFFECTIVE METHOD FOR EXTRACTION OF GENOMIC DNA FROM *MYCOPLASMA GALLISEPTICUM* CULTURE

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SUMMARY

The present report describes a simple and cost-effective method for isolating genomic DNA from *Mycoplasma gallisepticum* cultures. The method described here requires only five reagents or solutions *viz*. lysis buffer, NaCl solution, chloroform, ammonium acetate, and ethanol. Using this method, the concentration of DNA from 0.5 ml cultures containing ~10^5 CCU/ml *M. gallisepticum* was ~1.1 ng/µl. Also, no PCR inhibitors were present in the extracted DNA as it produced good amplification in PCR. This method is advantageous as it is time saving, doesn't require extended digestion with proteinase K, and only organic solvent required is chloroform.

Keywords: *Mycoplasma gallisepticum*, PCR, DNA, Poultry

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Mycoplasma gallisepticum (MG), an economically important poultry pathogen, causes chronic respiratory disease (CRD) in chickens (Ferguson-Noel *et al.*, 2020). MG infection causes economic losses to the poultry industry due to carcass condemnations, reduced feed efficiency and egg production, treatments, and control costs (Ferguson-Noel *et al.*, 2020). MG infection can be diagnosed based on clinico-pathological findings as well as with laboratory tests. Cultural isolation is the gold standard method for MG diagnosis, but this procedure is laborious, time-consuming, expensive, and requires serial passages (OIE, 2018). In rare cases, the growth of MG isolates can take up to 30 days. Furthermore, cultural isolation may be unsuccessful if MG is overgrown by other contaminating bacteria and/or inhibitory effects of antimicrobials used to control the infection (Hossam *et al.*, 2016; OIE 2018; Sawicka *et al.*, 2020). Serological tests (rapid plate agglutination test (RPA), haemagglutination-inhibition (HI), enzyme-linked immunosorbent assay (ELISA) are used for detection of antibodies against MG, however there are problems with specificity and sensitivity (OIE, 2018). Also, serology can yield false positive results if birds have been previously vaccinated with oil-emulsion vaccines against other avian pathogens (Bencina, 2009). The polymerase chain reaction (PCR) technique has been recommended as a reliable method for the detection of MG infection by World Organization for Animal Health (OIE) and National Poultry Improvement Plan (NPIP) (NPIP, 2006; OIE, 2018). In the present investigation, we describe a simple and cost-effective method to extract genomic DNA from MG cultures. This method minimizes the requirement of organic solvents (only chloroform is needed), doesn't require proteinase K enzymatic digestion, and yields DNA of good purity.

DNA was extracted as follows: 0.5ml culture containing ~10^5 CCU/ml was harvested with centrifugation for 5 min at 21,000gin CPR-24 plus (REMI, India). The cell pellet was resuspended in 200 µl lysis buffer (40 mM tris acetate, 20 mM sodium acetate, 1mM EDTA, 1% SDS) and lysed by vigorous pipetting. Proteins and cell debris were removed by addition of 66µl of 5M NaCl solution. The contents of micro centrifuge tube were mixed well, and then centrifuged at 21,000 g for 10 min at 4°C. The clear supernatant was transferred into a new vial, an equal volume of chloroform was added, and the tube was gently inverted for 50 times until a milky solution was formed. The tube was thereafter centrifuged at 16,000 g for 3 min and the supernatant (upper layer) was transferred to new vial. The DNA was precipitated with equal volume of 5M Ammonium acetate and two volumes of 100% ethanol. The tube was kept at -20 ºC for 20 min and centrifuged at 15,000 rpm for 5 min at 4 ºC. The pellet was washed twice with 70% ethanol, air dried and dissolved in 50µl of TE buffer. Quantification of extracted DNA was done using BioPhotometer plus (Eppendorf, USA) for the determination of sample concentration and purity. The U.V absorbance was checked at 260 and 280 nm for determination of the sample concentration and purity. The DNA isolated from MG cultures were screened by PCR using MG specific primer pairs (MGF primer-GAG-CTA-ATC-TGT-AAA-GTT-GGT-C and MGR primer-GCT-TCC-TTG-CGG-TTA-GCA-AC)(OIE, 2018). The MG specific amplification PCR product of 185 bp were detected by loading the PCR products in 1.5% agarose gel containing ethidium bromide.

In order to determine the efficiency of current

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Table 4.1. Concentration and purity of DNA of *M. gallisepticum* extracted by two methods

Sr.No.	Method and isolate number	Field sample	
		Conc. of DNA (ng/µl)	Purity (O.D. 260/280)
1.	B-MG-1	1.1	1.76
2.	B-MG-2	1.2	1.78
3.	C-MG-1	1.1	1.75
4.	C-MG-2	1.2	1.76

B- Bayatzadeh *et al.*, 2011 and C- method of current study

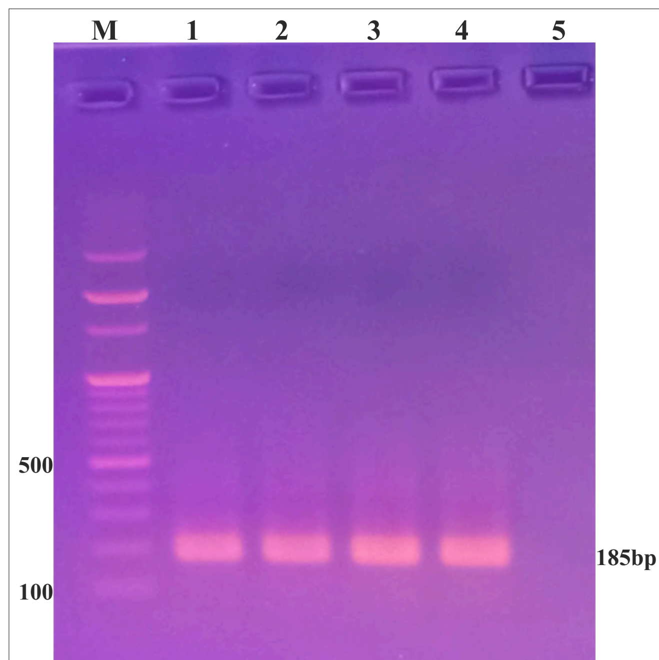

Fig. 1. MG specific 16S rRNA PCR: Lane M (DNA ladder), Lane 1 (MG1 isolate DNA by Bayatzadeh *et al.*, 2011 method), Lane 2 (MG2 isolate DNA by current study method), Lane 3 (MG2 isolate DNA by Bayatzadeh *et al.*, 2011 method, Lane 4 (MG2 isolate DNA by current study method), Lane 5 (Negative control)

method to extract DNA from field *M. gallisepticum* cultures we compared it with published procedure (Bayatzadeh *et al.*, 2011). Not only the yield of DNA from M. gallisepticum cultures was similar but the 260/280 nm ratio was ~ 1.8 by both methods (Table 1). To check if the extracted DNA contained any inhibitors which could hamper PCR amplification, PCR reactions were carried out using specific primers (OIE, 2018). Good amplification was obtained from DNA samples of both methods (Fig. 1). Specific amplicons of 185 bp were obtained from DNA samples of both methods while non-specific were absent. Further, the total time required to complete the DNA extraction by current method is less (~ 1hr) compared to previous method (~ 5h 30 min) (Bayatzadeh *et al.*, 2011).

CONCLUSION

The method described in the current report is simple, time saving and cost-effective. Using this method and 0.5ml cultures of M. gallisepticum, a genomic DNA of good purity (260/280 nm ratio was ~ 1.8) and concentration ~ 1.1 ng/µl was obtained. Because of its merits viz. time saving (~ 1hr), proteinase K digestion not required, and requirement of less reagents; this method may be helpful in future research on this mycoplasma organism.

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