Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 770 - 774

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

DEVELOPMENT OF A PCR BASED ASSAY FOR THE DETECTION OF *MYCOPLASMA PNEUMONIAE*

ABOO THAR ALI HAMMADI AND SONIA SHARMA*

Department of Microbiology, Indian Academy Degree College Bangalore, India

ABSTRACT

In the present study we collected seven blood samples from patients infected with *M. pneumonia.*Genomic DNA was isolated from these blood samples and specific primers were designed for *p*1 gene coding for cytadhesin protein P1 of the *M. pneumonia.* The amplified product was cloned into pTZ57R/T cloning vector and transformed into *E. coli* strain DH5α. Plasmid was isolated from the transformed cells, digested and checked for gene product release. Also, colony PCR was used to confirm the cloning and transformation. The released gene product was eluted and sequenced. The obtained sequence was 99% matching with a part of *p*1 gene available in the public nucleotide date base. In conclusion, this report describes a genomics-based PCR that provided rapid detection of *M. pneumoniae*. This PCR assay was developed for testing blood specimens suspected to harbor *M. pneumoniae* and is highly sensitive.

KEYWORDS: ***M. pneumonia,*** **p1 gene, PCR, Cloning, Sequencing**

SONIA SHARMA
Department of Microbiology, Indian Academy Degree College Bangalore, India

INTRODUCTION

Mycoplasmas,members of the class *Mollicutes* are the smallest free living organism and unique species of bacteria[1].They are characterized by their small genomes of single circular chromosome, low GC content and the permanent lack of a cell wall[2].Lack of cell wall, inability to grow in simple media and resistance to penicillium is the major difference between mycoplasmas and other bacteria[3].*Mycoplasma* can detect in various micro environments such as blood, tissue fluid, urine, saliva and bone marrow. Therefore mycoplasma can cause pneumonia responsible of trachiobronchitis, bronchitis,and less severe upper respiratory tract infections in children as well as young adults[4, 5]. *M. pneumoniae* is one of the most common causes of pneumonia worldwide[6]. Most widely available methods for *M. pneumoniae* diagnosis are culture methods and serology; however, there is no single standard for its laboratory diagnosis. Culture method can take several weeks and serological method takes two to four weeks which depends on antibody or acute and convalescent serumsamples[7]. PCR techniques are very sensitive and more rapid than culture or serology for detection of acute *M. pneumoniae* infections but are less widely available[8].Much of the information about metabolic properties of *M. pneumoniae* has been through annotation of its genome and direct identification of the genes coding for metabolic pathways enzyme[9].The p1 adhesin gene is codes for cytadherence, necessary for colonization and infection of host cells by *M. pneumonia*[10]. In this study we applied the Development of molecular-based testing such as the PCR assay for the detection of *M. pneumoniae*. The sensitivity was confirmed by cloning and sequencing of the PCR product.

MATERIALS AND METHODS

DNA Isolation and PCR

DNA was isolated from blood sample using Qiagen DNA Mini Kit as per the manufacturer's instructions. The PCR was performed using the following conditions: complete denaturation (95°C for 3 min), followed by 30 cycles of amplification (94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min) and the final elongation step (72°C for 2 min). PCR products were separated on 1 % Agarose gel.

Cloning

The PCR product was eluted from the gel and cloned in ready to ligate cloning vector (pTZ57R/T) as per manufacturer's instructions. The positive clones were picked from antibiotic plate and colony PCR was performed for confirmation of cloning.

Plasmid Isolation and Digestion

From the transformed cells Plasmid was isolated by alkaline lysis method[11]and electrophorized on 1% Agarose gel. The purified plasmid was subjected to restriction digestion using *Bam*H1 and *Eco*R1. The release of the gene product was checked on 1% agarose gel.

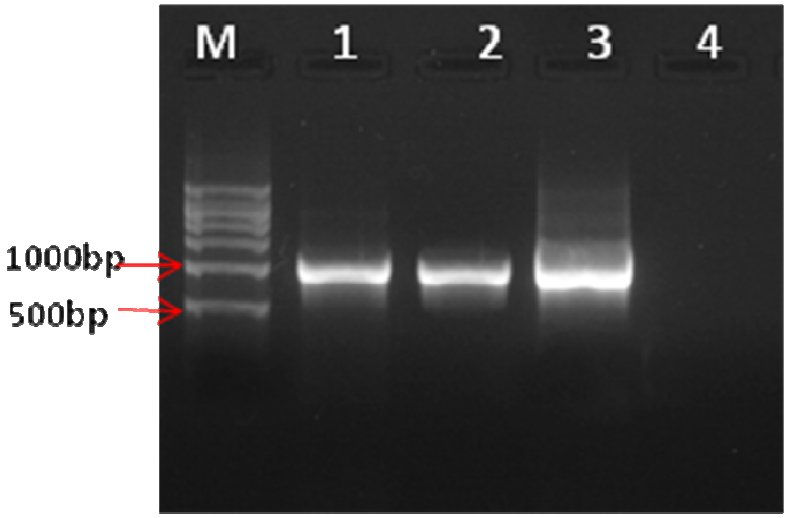
Sequencing and Data Analysis

The released gene product was eluted from the gel and sent for sequencing at Eurofins, Bangalore. The obtained DNA sequence was compared to GenBank sequences for identification.

RESULTS AND DISCUSSION

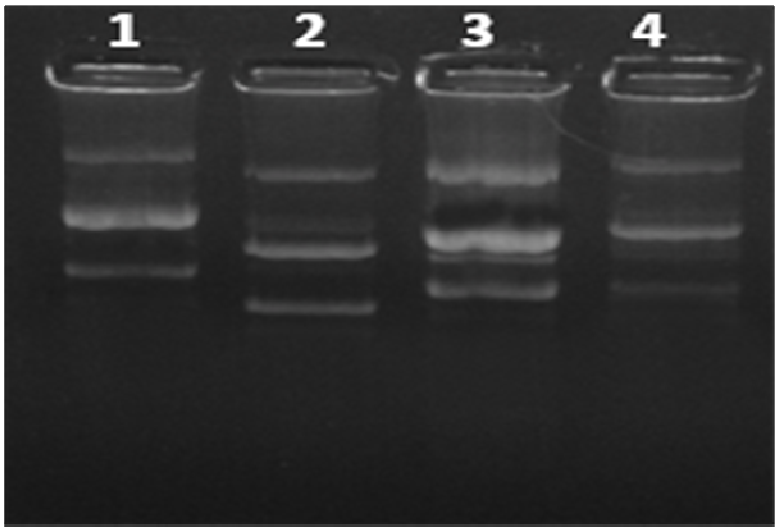
Pneumonia caused by *Mycoplasma pneumoniae*has been difficult to diagnose because of both clinical and laboratory diagnostic problems of its identification. In this study, species specific primers were designed for the p1 gene of*M. pneumoniae* coding for cytadhesin protein using Primer 3 Software. The designed primers were validated initially *in silico* and subsequently in wet lab. The primers were found to produce ~1000 bpampliconas shown in the figure 1.We tested seven blood samples in which only 3 samples showed the positive bands.

Figure 1
PCR amplification of the p1 gene by specific primers a)
(M- 500 bp ladder, Lane 1, 2, 3-Blood Samples, 4- Negative control)



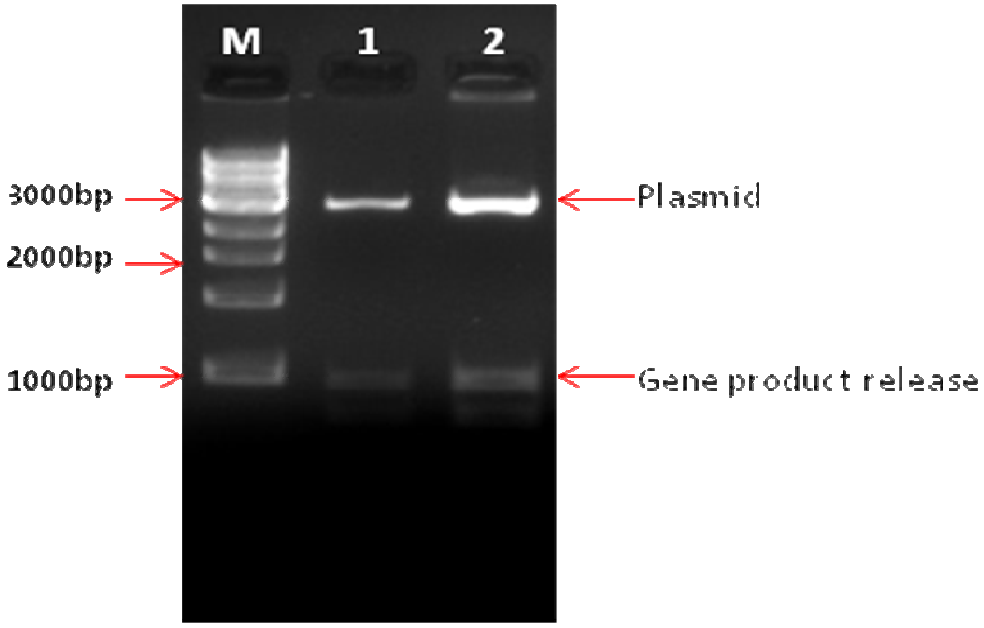
The PCR Product was eluted and ligated to cloning vector (pTZ57R/T). The ligated plasmid was transformed in to DH5α and the white colonies were cultured in Ampicillin containing LB broth. The Plasmids was isolated from the transformed cells and checked on 1% Agarose gel (Figure 2).

Figure 2
Plasmid isolated from the transformed bacterial cells



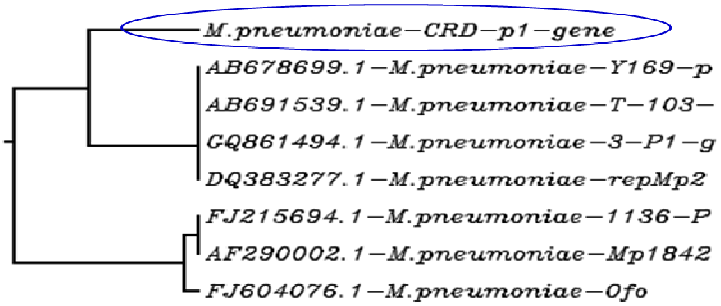
The purified plasmid was subjected to double restriction digestion using *Bam*H1 and *Eco*R1. The restricted product was checked on 1% Agarose gel and the release of the gene product was visualized (Figure 3).

Figure 3
Restriction digestion of ligated plasmid using BamH1 and EcoR1



The released gene product was eluted from the gel and sequenced for identification. The sequence was compared to Gen Bank sequences which showed 99% similarity with corresponding gene (*p*1) as determined by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The N-J tree with branch length was plotted using ClustalW sequence alignment (http://align.genome.jp/), showing the relationship of *p*1 gene among the closest *M.pneumoniae*strains in the NCBI database (Figure 4).

Figure 4
A tree plot constructed with the NJ method using sequence of the p1 gene



It has been realized for quite some time that the detection of *M. pneumoniae*is greatly enhanced by the use of the PCR methodology. In our study this method was found to be twice as sensitive as culture, and as expected for a PCR procedure, it is extremely rapid, especially when compared to the speed of culture. Rapid etiologic diagnosis of these infections should allow initiation of proper antibiotic treatment, resulting in a reduced antibiotic pressure. In future, availability of the complete genome sequence of these pathogens will provide an alternative approach for identification of novel targets for new antimicrobial therapies[12].

REFERENCES

1. Quirk J, Kupinski J and Dicioccio R., Detection of Mycoplasma ribosomal DNA sequences in ovarian. Gynecol. Onc., 83: 560- 562, (2001)
2. K.E. Johansson KE B. Pettersson B. Taxonomy of mollicutes. In: S. Razin and R. Herrman (eds.), *Molecular biology and pathogenicity of mycoplasmas*, Kluwer Academic/Plenum Publishers, New York, 2002, pp.1-29.
3. Held P., Clean culture. Drug. Disco. Develop. Maga., 11: 30-32, (2008)
4. Esposito S., BlasiF., Bosis S., Droghetti R., Faelli N., Lastrico A. and Principi N. Etiology of acutepharyngitis: the role of a typical bacteria. Gen. Micro., 53: 645-651, (2004)
5. Burder J., Godry R., Talkei K. and Cunha F. Characterization of *Mycoplasma peneterans*and*Mycoplasma fermentans*Immunodominatprotiens. Brazilian. J. Microbiol., 36: 131-136, (2005)
6. Luby JP. Pneumonia caused by mycoplasma pneumoniaeinfection. Clin Chest Med., 12(2): 237-244, (1991)
7. Loens K., Goossens H. andIeven M. Acute respiratory infection due to Mycoplasma pneumoniae: current status of diagnostic methods. European Journal of ClinicalMicrobiology and Infectious Diseases,29:1055-1069, (2010)
8. Nilsson AC, Bjorkman P andPersson K. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiology, 8: 93, (2008)
9. Bork P., Dandekar T., Diaz-Lazcoz Y., Eisenhaber F., Huynen M. and Yuan Y. Predicting function: from genes to genomes and back. Journal of molecular biology, 283(4): 707-725,(1998).
10. Waites K.B., Katz B. andSchelonka R.L. Mycoplasmas and ureaplasmas as neonatal pathogens. ClinMicrobiol Rev, 18: 757-789, (2005)
11. Joseph Sambrook and David W. Russell, Ed. Molecular cloning: a laboratory manual, 2nd edn, 3vol, Cold Spring Harbor Laboratory Press, New York, (2001).
12. Angamuthu K. and Piramanayagam S. Insilico identification of putative drug targets in mycoplasma hominis using differential metabolic pathway analysis. Int J Pharm Bio Sci, 3(4): (B) 391 - 398, (2012).