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DIRECT DETECTION OF MYCOPLASMA GALLISEPICUM (S6 STRAIN) BY POLYMERASE CHAIN REACTION (PCR)

(With 5 Fig.)

By

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استخدام سلسلة تفاعل أنزيم البوليميريز للكشف السريع للميكوبلازما جاليسبتكم عتره S6

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تم استخدام إختبار سلسلة تفاعل أنزيم البوليميريز للتفرقه والكشف السريع بين عترات الميكوبلازما المختلفه . ثم استخدام نوعين من البادئ كان النوع الاول يحتوى على 22 زوج من النيوكلوتيدات والنوع الاخر يحتوى على 23 زوج من النيوكلوتيدات .

وقد أظهرت النتائج الاتى :-

وجود حزمه من النيوكلوتيدات وكان وزنها الجزئى 960 زوج من النيوكلوتيدات عند الكشف على الميكوبلازما جاليسبتكم عتره S6 .

عند استخدام باقى عترات الميكوبلازما : ميكوبلازما أورال - ميكوبلازما أرجينينى - ميكوبلازما سلفاريم - ميكوبلازما نيمونى لم تظهر أى منهما وجود حزمه من النيوكلوتيدات عند 960 وزن جزئى .

ويعتبر هذا الاختبار بسيط وعالى الحساسيه فى التفرقه بين العترات المختلفه للميكوبلازما .

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SUMMARY

The polymerase chain reaction was used for rapid detection and differentiation between different strains of Mycoplasma gallisepticum. Two oligonucleotide primers of 22 and 23 base pair in length were constructed. The PCR amplification products were visualized by ethidium bromide and ultraviolet exposure. The results were as follows: 1. A positive single PCR product 960 bp in size was detected for Mycoplasma gallisepticum (S6 strains). 2. Other strains of mycoplasma (oral, arginini, salivarium and pneumonia) showed no band at 960 bp molecular weight. This technique provides a simple and extremely sensitive method of identifing isolates of M. gallisepticum and other Mycoplasmas.

INTRODUCTION

Mycoplasma gallisepticum (MG S6) is responsible for chronic respiratory disease, an economically significant disease of chickens and turkeys. Losses are due to poor feed conversion, secreased egg production, increased embryo emryo death and culling of harched progeny and carcass condemnation at processing. The diagnosis of M.gallicepticum infection traditionally has been done by serology and / or culturing of the organism from clinical specimens *(YODER, 1984)*. However, problems with false positive and false negative reactions have been encountered with serologic tests in recent years *(KLEVEN and SOLIMAN, 1988 and YODER, 1989)*. Furthermore, M.gallceptilum is one of the more fastidious avian mycoplasma and is difficult to grow in artificial media. A recent technique was developed in the field of molecular biology depenoing on gene amplification. This NDA amplification technique has been termed the polymerase chain reaction or PCR *(MULLIS and FALOONA, 1987)*. Since the PCR technique allows the specific amplification of discrete fragments of DNA, target material that is initially present in only picogram quantities can be detected *(OSTE, 1988)*. DNA amplification techniques have been used only in the basic research areas of cloning *(SCHARF et al., 1986)* and sequencing *(WONG et al., 1987)* but also in the development of diagnostic tests *(KWOK et al., 1987; OLIVE, 1989; MOSER et al., 1989 and VAN KYS et al., 1989)*. A recent approach to diagnosis of mycoplasma infection involves the use of polymerase chain reaction *(NASCIMENTO et al., 1991)*. The polymerase chain reaction (PCR) has been proven to be a very

specidic and sensitive method for amplifying low amount of nucleic acid to a level that can be easily detected *(INNIS and GELFAND, 1990).*

The present study describes the application of the PCR for the identification of M.gallisepticum (S26 strain).

MATERIAL AND METHODS

Mycoplasma cultures and PCR preparation:

Five Mycoplasma strains: M. orale, M. arginini, M. salivarium, M. pneumoniae and M. gallisepticum (S6 strain) used in the present study were obtained from Dr. *S.J. GEORY,* Department of pathobiology, university of connecticut, USA All species were grown in PPLO broth base with 12% horse serum and 12% fresh yeast extract. The chromosomal DNA of all strains were isolated by *MARMUR (169).*

Primer determination and synthesis:

Based on analysis of DNA sequencing data of M.gallisepticum MG (S6), the base sequences of the left (L) and right (R) oligonucleotide primers were determined according to the criteria outlined by *INNIS and GELFAND (1990).* Thus primer sequences were selected that had the highest possible guanine plus cytosine (G + C) content, that had a minimum of complementary bases between them and that did not have poly Cs and poly Gs at their ends. The primers were synthesized by Molecular Biology at the university of Connecticut. The 22 bp and 23 bp primers were constructed to a target aa 910 bp region of the M.gallisepticum (S6).

Primer	base sequence	No.of basis	G+C%
L22	5 TAA GAA TCC AGG GTG AGC AAT 3	22	40.9
R23	5 TCC TCC ACT AAA TAA ATT GAC CCG 3	23	39.1

Amplification:

The PCR was performed with thermostable taq DNA polymerase (Amplitaq, Perkin Elmer Cetus Norwalk, Conn.) in an automated DNA thermal cycler (Perkin Elmer).

DNA amplification was done in 100 ul total reaction volume. Each reaction mixture consisted of 10 ul of 10 X reaction buffer (progema, Madison, Wis), 16 ul of 10 m.M. of each nucleotide (dATP, dCTP, dGPT and dTTP; pharmacia), 2 ul (containing 400 ng / of each primer (L and R), 0.5 ul (2 units)

of Taq DNA polymerase (Amplitaq; Perkin Elmer), 8 ul 25 mM $MgCl_2$, 2 ul DNA templet containing 40 ng DNA) and complete the mixture with 59.5 ul distilled water.

Each reaction mixture was overlaid with 50 ul of sterile mineral oil. The amplification was performed by heating the sample for 5 minutes at 97 °C then using thirty cycles of denaturation for one minute at 94 °C, annealing for one minute at 55 °C and extension for one minute at 72 °C with the exception that the final extension step was held for 10 minutes.

Detection of PCR products:

After amplification a 5 ul portion from the PCR prooducts was withdrawn and mixed with equal volumes of loading buffer and subjected to electrophoresis at electrophoresis at 80 V for one hour on a 0.8% agarose gel. Gels were stained with ethidium bromide and photographed on a short wave length UV transilluminator.

RESULTS

Fig. (1): showed that of S6 strains tested (7) produced 960 by size DNA PCR product which was detected by ethidium bromide stained agarose electrophoresis gel.

Fig. (2): Cleared that the PCR product of M. orale strains was 615 bp.

Fig. (3): Showed that the PCR products of M. arginini strains was 307 bp.

Fig. (4): Showed that the PCR product of M. salivarium strains was 369 bp.

Fig. (5): revealed that the PCR product of M. pneumonia 260 bp.

DISCUSSION

In early Mycoplasma gallisepticum infection or during a carrier stage in which traditional serologic and cultural procedures may fail, a sensitive method for detection of the organism would facilitate diagnosis. The PCR was able to detect DNA in fraction of the total chromosomal contents of one mycoplasma cell *(NASCIMENTO et al., 1991).*

In the present study, the MG amplified product was obtained as a 960 bp fragment which allowed identification by a simple agarose gel electrophoresis / ethidium bromide / ultraviolet exposure. No amplified products were detected in other tested mycoplasma strains. The PCR method was found to be

rapid, inexpensive, sensitive and specific for the detection of Migallisepticum *(NASCIMENTO et al., 1991).*

Standardization of Mgallisepticum - PCR at the level of 30 cycles agrees with published protocols *(INNIS and GELFAND, 1990).*

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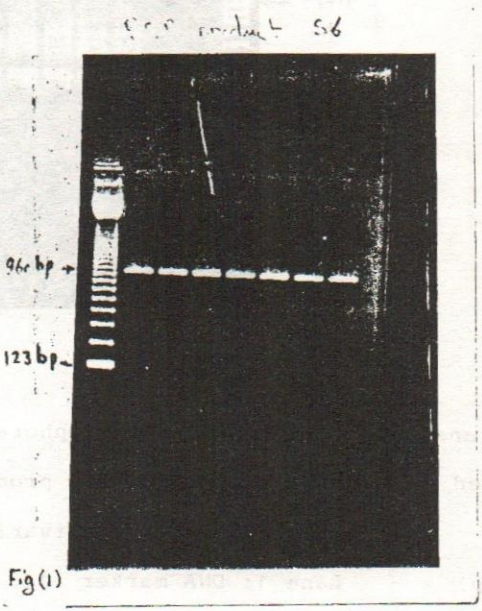


Fig(1)

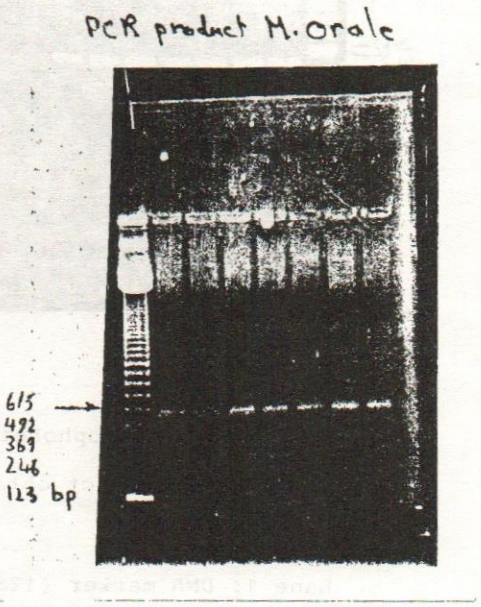
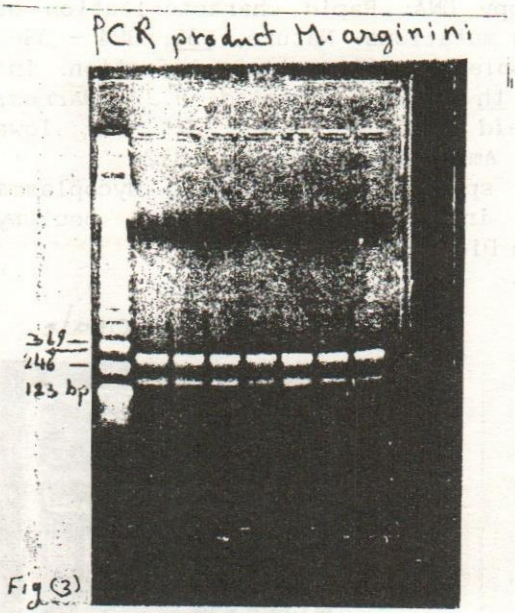


Fig. (1): Electrophoresis analysis of the PCR product obtained with MG - S6.
Lane 1: DNA marker (123 bp)
Lanes 2-7: 7 strains of MG S6
the arrow corresponds to 960 bp.

Fig. (2): Electrophoretic analysis of PCR product obtained with M. orale.
Lane 1: DNA marker (123 bp)
Lanes 2-7: 7 strains of M. orale, the arrow corresponds to 615 bp.



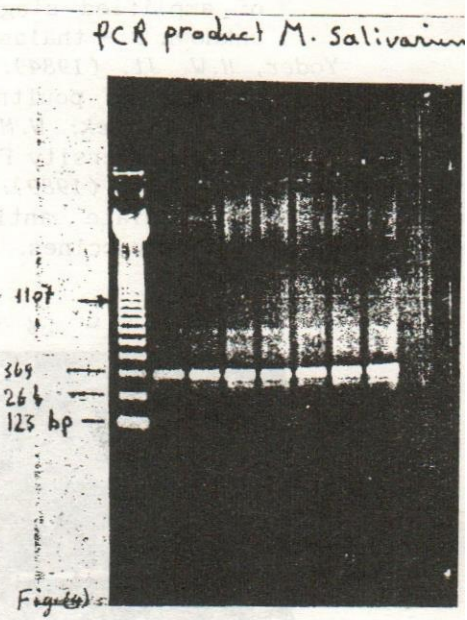


Fig. (3): Electrophoretic analysis of the PCR product obtained with M. arginini
Lane 1: DNA marker (123 bp)
Lanes 2-7: 7 strains of M. arginini, the arrow corresponds to 307 bp.

Fig. (4): Electrophoretic analysis of PCR product obtained with M. salivarium.
Lane 1: DNA marker (123 bp)
Lanes 2-7: 7 strains of M. salivarium, the arrow corresponds to 369 bp.

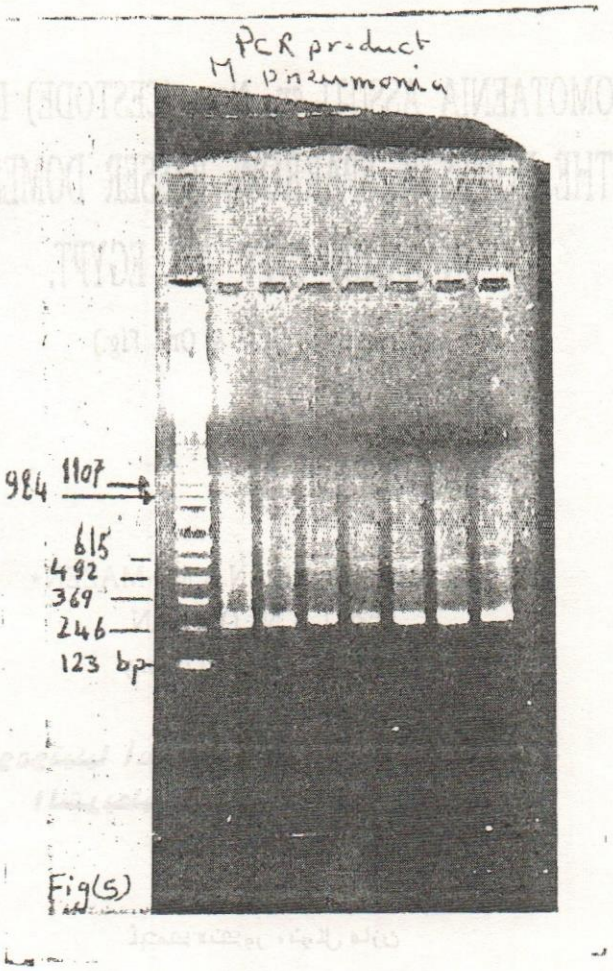


Fig. (5) : Electrophoretic analysis of the PCR product obtained with M. pneumonia.

Lane 1 : DNA marker (123 bp)

Lanes 2-7 : Strains of M. pneumonia, the arrow corresponds to 260 bp.