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# Plasmid DNA Profilling of *Pasteurella multocida* Serotype A, B, D and Untypable (U) Isolates from Animals

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**Abstract:** A total of two-hudred (200) *P. multocida* isolates obtained from Veterinary Diagnostic Laboratory, Petaling Jaya were screened for their presence of plasmid via alkaline lysis method (Sambrook, *et al.* 1989). The size, number and profile of plasmids for each isolate were estimated by agarose gel electrophoresis. 37% were noted to harbor small plasmid DNA, ranging in size from 1.5 to 3.6 Mda and there was no presence of large plasmids in all isolates. No plasmids could be detected for serotype A. The plasmid size ranged from 1.5 to 3.6 megadaltons (Mda) with seven different plasmid profiles. All isolates of *P. multocida* serotype B were found to contain a common plasmids. The finding of this study will help to further investigate whether any of these plasmids are related to virulence mechanism (pathogenicity) of *P. multocida*. Therefore, the aim of this study is to detect the presence of plasmids in isolates of *P. multocida* serotype A, B, D and untypable (U).

**Key words:** Plasmid, *Pasteurella multocida*, profiling, serotype B, animal pathogen

## Introduction

The *Pasteurellaceae* family comprises the genera *Haemophilus*, *Actinobacillus* and *Pasteurella*), a group of Gram-negative, nonmotile organisms that includes many species pathogenic for birds, animals and man. *Pasteurella multocida* is an economically important bacterial pathogen of domestic animals. In Malaysia, *P. multocida* causes substantial economic losses by inducing diseases such as pneumonia of sheep and goats and acute haemorrhagic septicaemia (HS) in cattle and buffaloes (Chandrasekaran, 1988). There are five different serotypes of *P. multocida* (A, B, D, E and untypable) and with serotype B mainly associated with cattle (Carter 1984). Plasmids specifically in relation to isolation and characterization have been described in *P. multocida* (Hirsh *et al.* 1985; Hirsh *et al.* 1989; Ikeda and Hirsh 1990) but no one has reported the diversity of plasmids among the various serotypes of *P. multocida*. Therefore the goal of this study was to survey the presence, diversity and stability of plasmids among two hundred (200) animal isolates of *P. multocida* collected over 20 years from various sources at different time and locations in Malaysia. Many methods have been surveyed and developed in a laboratory for the isolation of bacterial plasmid DNA. These include the methods of Takahashi and Nagano (1984), Kado and Liu (1981), Birnboim and Doly (1979) and Sambrook, *et al.* (1989). It was found that the method of Sambrook, *et al.* (1989) was immediately useful for the visualization of a great many plasmids for screening *Pasteurella multocida* purposes. The other three methods failed to demonstrate the presence of plasmids in *P. multocida*. The size, number and profile of plasmids for each isolate were estimated by agarose gel electrophoresis.

## Materials and Methods

**Bacterial isolates and growth conditions:** A total of 200 *P. multocida* isolates of pure cultures were obtained from Veterinary. Diagnostic Laboratory, Petaling Jaya. Isolates included 45 serotype A (rabbits and chickens), 60 bovine of serotype B, 56 serotype D (deer, sheep, ovine and pig) and 39 untypable serotype (cattle, deer, sheep, chicken and pig), were subcultured once on BHI Blood Agar, incubated at 37°C overnight before amplified in BHI broth.

**Plasmid stability testing and rapid DNA isolations:** The stability of plasmids was tested as previously described (Lanka & Barth, 1981). The cultures were grown overnight at 37 C in BHI broth and were subinoculated into BHI agar before incubated at 37 C. This step was repeated until at least 20 times. After cultured, 20 colonies from each transfer of BHI broth and BHI agar were checked for the presence of plasmids. Plasmid DNA from *P.multocida* was prepared as described by Sambrook, *et al.* (1989). To ensure reproducibility all isolates were screened at least three times.

Table 1: Plasmids presence in *P. multocida*

| Serotype | Total isolates | No. of positive isolates (%) | Estimated size of plasmid DNA | |
|---|---|---|---|---|
| | | | (Megadalton) | (Kilobase) |
| A | 45 | 0 (0%) | - | - |
| B | 60 | 60 (100%) | 2.6, 2.0 | 3.9, 3.0 |
| D | 56 | 3 (1.5%) | 3.6, 3.5 | 5.5, 5.3 |
| | | | 2.6, 2.2 | 3.9, 3.3 |
| U | 39 | 11 (28%) | 3.6, 3.5, 2.4 | 5.5, 5.3, 3.6 |
| | | | 2.3, 1.7, 1.5 | 3.5, 2.6, 2.3 |

U, untypable.

Table 2: Plasmid DNA analysis of *P. multocida* isolates

| Serotype (total no.) | No. of positive plasmid isolates (%) | DNA profiling | Estimated size of plasmid (kilobase) | RE Analysis (REA) |
|---|---|---|---|---|
| A (45) | 0 (0%) | no plasmid | - | - |
| B (60) | 60 (100%) | identical | 3.0, 5.5 | identical |
| D (56) | 3 (1.5%) | identical | 3.6, 6.5 | non identical |
| U (39) | 11 (28%) | non identical | 2.6, 3.6 | non identical |
| | | & identical | 3.6, 6.5 | & identical |

U, untypable.

**Agarose gel electrophoresis**: Plasmid DNA was resolved by electrophoresis in submerged horizontal agarose slab gel (0.7%) in tris-borate buffer (TBE). The required amount of agarose (Sigma Chemical Co., U.S.A.) was dissolved in tris-borate buffer (pH 8.3) by boiling. The homogeneous molten agarose solution was cooled at 50°C before casting. An aliquot of extracted DNA (15 to 30 $\mu$l) was mixed on a square of parafilm M (American Can Co., U.S.A.) with a small (5 to 10 $\mu$l) volume of gel loading buffer. The DNA- dye mixture was then loaded into samples wells and the tank was carefully filled with tris-borate buffer (pH 8.3) to completely submerge the gel by about 1 mm. Electrophoresis was stopped when the loading buffer was 10 to 20 mm from the anode end of the gel. Gels were stained in ehtidium bromide (0.5 $\mu$l /ml) solution for 30 minutes. The DNA-ethidium bromide complex in the gels were visualized on a 302 nm UV transilluminator. Photography was carried out with a polaroid MP-4 Land camera fitted with a yellow filter and Polaroid Type 665 (black and white) Land films. The exposure times varied from 60 to 90 seconds. Gloves were worn at all times when handling solutions and agarose slab gels containing ethidium bromide, as it is a powerful mutagen and carcinogen.

**Plasmid DNA profiling**: Plasmid sizes were determined by comparison with plasmids of known molecular sizes (1.8, 2.0, 2.6, 3.4, 3.7, 4.8 and 35.5 Mda) from *E. coli* V517 (Macrina *et al.* 1978). A standard curve was obtained by plotting in molecular weights of the standard marker versus their mobility on a semi-log graph paper (Aiij and Borst 1972). The approximate sizes of the unknown DNA plasmids were then determined from the standard curve.

## Results

The presence of plasmids and their molecular weight in the respective *P. multocida* isolates are presented by Table 1. Plasmids were detected in 74 of 200 (37%) *P. multocida* isolates (Fig. 1- 9). The breakdown for the respective serotypes was as follows: 100% were observed for serotype B, 28% for untypable isolates and 1.5% for serotype D. No plasmids could be detected for serotype A (Fig. 1). The plasmid profile analysis divided the 74 plasmid-containing isolates into seven 105 categories, plasmid-type-1, plasmid-type-2, plasmid-type-3, plasmid-type-4, plasmid-type- 5, plasmid-type-6 and plasmid-type-7 with two plasmids of molecular size ranging from 1.5 to 3.6 Mda (Table 2). The most common plasmid-type-3 contained plasmids of size 2.0 and 2.6 Mda (Figs. 2-4). All isolates from this group were collected from 60 of bovine from an outbreak of haemorrhagic septicaemia at different time (year) and 110 locations. In serotype D, isolates PMD10 and

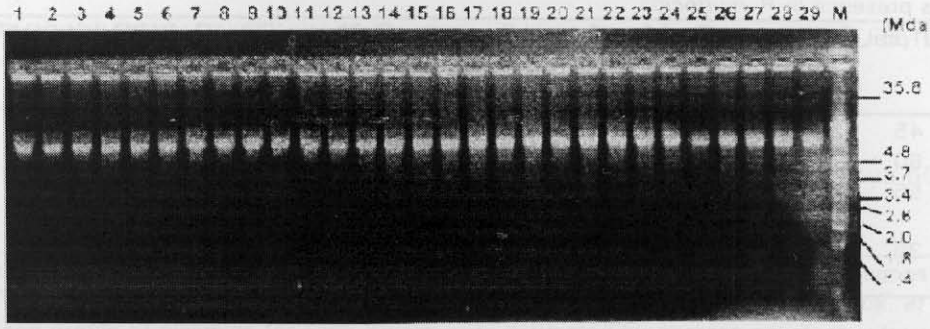


Fig. 1: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from representative isolates of *P. multocida* serotype A. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1-29) correspond to the isolate numbers (PMA1-PMA29).

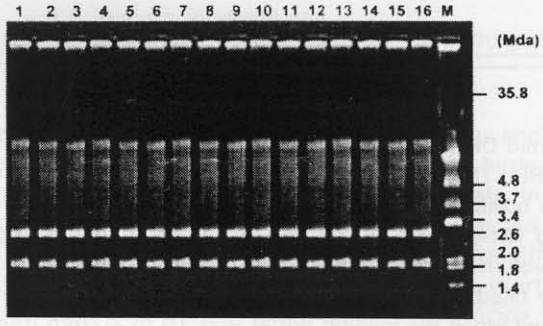


Fig. 2: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from *P. multocida* serotype B. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1 – 16) correspond to the isolate numbers (PMB1-PMB16).

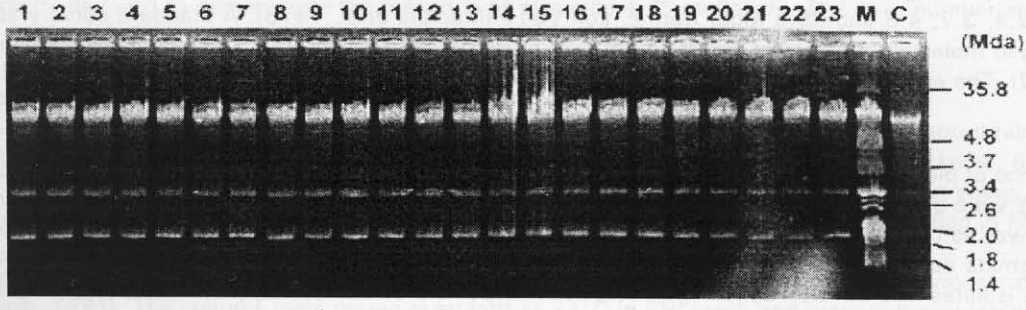


Fig. 3: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from *P. multocida* serotype B. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1-23) correspond to the isolate numbers (PMB17-PMB39). Lane C, plasmidless strain of *P. multocida* serotype A, PMA1

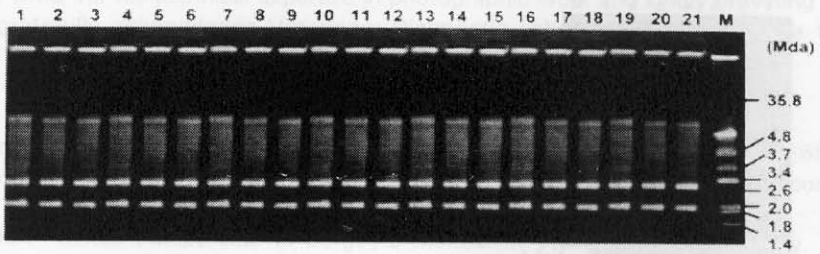


Fig. 4: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from *P. multocida* serotype B. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1-21) correspond to the isolate numbers (PMB40-PMB60).

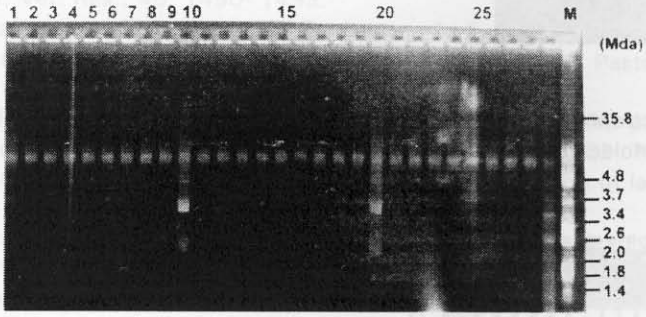


Fig. 5: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from *P. multocida* serotype D. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1-29) correspond to the isolate numbers (PMD1-PMD29).

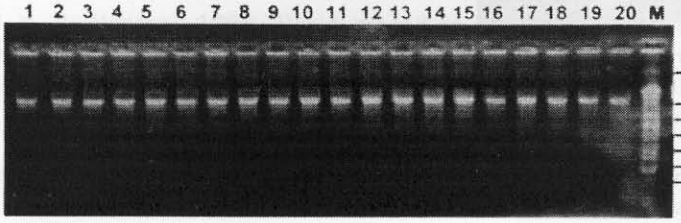


Fig. 6: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from a representative plasmidless isolates of *P. multocida* serotype D (Lane 1-20). Lane M, *E. coli* V517 molecular size marker.

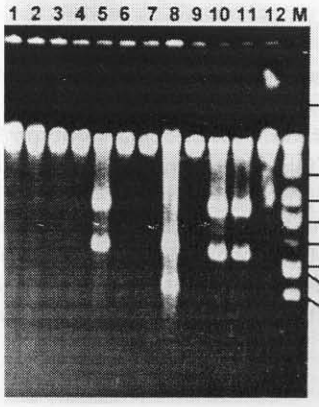


Fig. 7: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from untypable isolates of *P. multocida*. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1-12) correspond to the isolate numbers (PMU1 -PMU12).

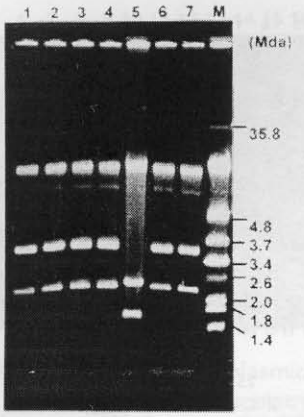


Fig. 8: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from untypable isolates of *P. multocida*. Lane M, *E. coli* V517 molecular size marker. Lane numbers (1-7) correspond to the isolate numbers (PMU13- PMU19).

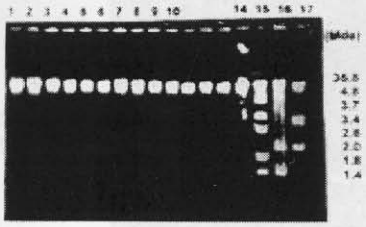

Fig. 9: Agarose gel (0.7%) electrophoresis of plasmid DNA extracted from a representative untypable plasmidless isolates of *P. multocida* (Lane 1-14). Lane 15, *E. coli* V517 molecular size marker .Lane 16 and 17, are plasmid-containing isolates of PMU8 and PMU10 were used as a plasmid control.

PMD20 had similar profile of plasmid-type- 4 with molecular sizes of 2.3 and 3.5 Mda whereas, isolate PMD29 was found to harbor plasmid-type-5 with molecular sizes of 2.6 and 3.5 Mda (Fig. 5 and 6). The remaining isolates (untypable) showed four different profiles of plasmid-type-1 (PMU8), plasmid-type-2 (PMU17), plasmid-type-6 (PMU5, PMU10 and PMU11) and plasmid- type-7 (PMU13 – PMU19) with molecular sizes ranging from 1.5 to 3.6 Mda (Figs. 7-9).

## Discussion

It should be noted that each time of the plasmid isolation carried out, all the serotype A isolates which are mainly from rabbits and chickens are plasmid-free. Approximately one-third of the *P. multocida* isolates in this study harbored plasmids, ranging in size from 1.5 to 3.6 Mda. *P. multocida* serotype B which was specifically cause a haemorrhagic septicaemia disease in cattle and buffalo, had common plasmids in the range of 2.0 and 2.6 Mda although all of this isolates were from different geographical
area and time or year of isolation. The 2 isolates, PMD10 and PMD20 were isolated from deer in Perak at the year of 1990 and goat in Selangor area in 1988, respectively were found to be similar in plasmid size. The plasmid-containing isolates appeared to have maintained their plasmid quite stable. Further investigation has to be carried out to characterize all these plasmids where, restriction enzymes analysis is currently progressively being carried out.

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