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Protein Isolation and Separation Techniques of *Pasteurella multocidavia* One- and Two-Dimen-Sional Gel Electrophoresis

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ABSTRACT

Introduction: Bacteria in the genus *Pasteurella* live as commensal parasites on the mucous membranes of vertebrates, particularly mammals and birds. *Pasteurella* species like *Pasteurella multocida* and *Pasteurella haemolytica* cause significant economic losses as a result of diseases caused by these two microorganisms.

Objective: This study compares protein isolation and analysis methods for effective one and two-dimensional gel electrophoresis of *Pasteurella multocida*proteins and a preliminary protein mapping and analysis of *P. multocida* serotype B.

Methodology: Protein samples were obtained by two isolation methods: homogenisation/sonication and detergent lysis. 1D SDS-PAGE methodology was optimized for protein loading, separating gel percentages and gel size. Detergent lysis was the preferred isolation method of total proteins.

Results: The optimum running condition for SDS-PAGE was determined to be 15 µg of protein loading run on a 12.5% separating gel with a gel size of 10cm x 10cm. Preliminary 1D SDS-PAGE analysis revealed 3 distinct protein bands (33 kDa, 39 kDa and >200 kDa), which could only be found specifically in serotype B samples. The samples were then separated by 2-DGE after optimization. The optimum running condition was 500µg of protein sample loaded using rehydration loading with immobilized pH gradient (IPG) strips pH 3–10 for the first-dimension isoelectric focusing (IEF) and 12.5% single percentage gel for the second-dimension SDS-PAGE. The protein map, which produced the most, spots were compared to *in silico* 2-DGE protein patterns of Pm70 in GELBANK database.

Conclusion: A total of 23 proteins were identified and protein of 33 kDA was identified in both 1D SDS-PAGE and 2-DGE. This is the first protein analysis and the first protein map of *P. multocida* serotype B ever produced.

Key Words: *Pasteurella multocida*, Homogenization, Sonication, Detergent lysis, Gel, Electrophoresis, Protein Isolation and Separation

INTRODUCTION

The genus Pasteurella is the type genus of the family Pasteurellaceae, which comprises the recognised genera of Actinobacillus, Haemophilus and Pasteurella.[1] The genus Pasteurella was established by Trevisan in 1887 and named after Louis Pasteur. The type species for the genus is *Pasteurella multocida*, which was previously named variously as *Pasteurella choleraegallinarum* and *Pasteurella gallicida*.

Bacteria in the genus Pasteurella are commensal parasites on mucous membranes of vertebrates, particularly mammals

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and birds. Many species are potential pathogens, acting opportunistically when the host's defenses are compromised. The genus Pasteurella contains species such as *Pasteurella multocida*, *Pasteurella pneumotropica, Pas-teurellahaemolytica, Pasteurella ureae, Pasteurella aerogenes and Pasteurella gallinarum*.[2,3] To date, most of the research on the pathogenesis and virulence factors of these bacteria has been focused on *Pasteurella multocida* and *Pasteurella haemolytica* due to the significant economic losses resulting from diseases caused by these two microorganisms.

The advent of recent techniques in molecular biology will continue to allow further understanding of the bacteria in the genus Pasteurella. The use of molecular approaches enables a stepwise dissection of their many virulence factors. An under-standing of the roles of the various virulence factors is important in the design and development of efficacious vaccines to protect animals against infection by these pathogenic bacteria.[4]

The proteomic approach to the characterization of *Pasteurella multocida* has not yet been performed. Therefore, this study is essential to develop the preliminary tools for studies of pathogenic and untypable strains of *Pasteurella multocida* that cannot be characterized by conventional serological typing. Thus, this study attempts to produce a preliminary comprehensive protein analysis of *Pasteurella multocida* by proteomic approach.

We have addressed the problems faced by researchers in low- and middle-income countries in the application of a proteomics approach to the studies of *Pasteurella multocida*. The main aims of the current study were the isolation of *Pasteurella multocida* proteins and the establishment of protein separation techniques via one- and two-dimensional gel electrophoresis. A preliminary protein mapping of *Pasteurella multocida* serotype B using SDS-PAGE and two-dimensional gel electrophoresis analysis was produced.

MATERIALS AND METHODS

Materials

Pasteurella multocida isolates used in this study were provided by the Veterinary Research Institute (VRI), Ipoh, Perak. Aeromonas hydrophila and *Staphylococcus aureus* used as controls in this study were obtained from Dr. Salmah Ismail's collections, Department of Molecular Medicine, Faculty of Medicine, University of Malaya. PageRuler™ protein ladder from Fermentas, Canada was used in this study to provide reference proteins of known molecular weights (10, 15, 20, 25, 30, 40, 50, 60, 70, 85, 100, 120, 150 and 200 kilo Daltons (kDa).

Solutions and Buffers

Extraction buffer was made by dissolving Urea (5.7 g), CHAPS (0.4 g), DTT (0.0028 g) and IPF buffer (200 µl) in 10ml of distilled water. Lysis solution was made by adding Urea (5.8 g), CHAPS (0.4 g) and Tris (0.0485 g) in 10ml of distilled water.

Characterization of Pasteurella multocida isolates

The cell morphologies of the *Pasteurella multocida* isolates were determined by Gram staining (Burke's method).[4] A series of standard biochemical tests (Table 1) were carried out on all the 6 isolates of *Pasteurella multocida*, in order to characterize them. Cultures were subcultured once on BHI blood agar, incubated at 37°C over-night. Pure cultures obtained were used in various characterization procedures.

Protein extraction

Homogenization/sonication

Extraction buffer and lysis solution were prepared as described above. 500 µl of a sample was added to an Eppendorf tube followed by 500 µl of lysis solution. Homogenization was carried out where the sample solution was mixed with the lysis solution by sucking it up and down slowly and consistently for about 20 times using a micropipette.

After homogenization, the tube was placed in the sonicator for 5 seconds using an ultrasonic cell disruptor (Branson Sonifier 450) and then the tube was immediately placed on ice for 2 minutes. The process was repeated twice more to breakdown the cell membrane of the bacteria and released the components of cells.These steps were repeated using 500µl of extraction buffer and deionized distilled water (ddH_2O) for the same sample in another two different Eppendorf tubes. This was an optimization step to decide which of the three solutions gave the best protein extraction result.

Detergent lysis using BugBuster® protein extraction reagent

Cells were harvested from the liquid culture by centrifugation at 20000–25000 xg for 10 minutes using a 1.5 ml tube. After that, the supernatant was decanted and the pellet was allowed to drain, removing as much liquid as possible. The cell pellet was re-suspended at room temperature by pipetting or gentle vortexing using 300µl Bug-Buster reagent. There are no adverse effects to using higher ratios volumes of Bug-Buster reagent.

The cell suspension was incubated on a shaking platform or rotating mixer at a slow setting for 10 to 20 minutes at room temperature. Insoluble cell debris was removed by centrifugation at 19000 xg for 20 minutes at 4°C. The supernatant was transferred to a fresh tube. The soluble clarified extract can be maintained on ice for short term storage (2 to 3 hours) or freeze at −20 °C until needed.

Protein quantification

The concentrations of all the bacterial protein samples extracted using homogenization/ sonication or detergent lysis methods were determined by using Bio-Rad Protein Assay (Bio-Rad Laboratories, U.S.A.), performed as per the manufacturer's instructions as the increase in absorption at 595 nm. In this study, a microtiter plate protocol was chosen instead of the standard protocol using spectrophotometer as this provides a relatively simple and fast method. The linear range of the micro-titer plate assay is 0.05 mg/ml to approximately 0.5 mg/ml.

Protein Separation Techniques

One-dimensional protein separation via SDS-PAGE

The one-dimensional protein separation was conducted via the SDS-PAGE method.[5]

SDS-PAGE with 10cm x 10cm gels (Dual Vertical Mini Gel Unit)

SDS-PAGE was carried out for both the protein samples obtained from protein extraction by homogenization/sonication, and by detergent lysis. Protein samples were chosen randomly from both extraction methods to run the SDS-PAGE. PMA4963, PMB202 and PMU9577, which were extracted by homogenization and sonication, were chosen for SDS-PAGE using 12.5% separating gel and 5% stacking gel. PMA2006, PMA4963, PMB202 and PMB713, which were extracted by detergent lysis using Bug-Buster® protein extraction reagent, were chosen for SDS-PAGE using 12.5%, 15.0%, 18.0% separating gels and 5% stacking gel. The reason to run SDS-PAGE for protein samples extracted with two different methods was to optimize the protein extraction method for *Pasteurella multocida*and to compare which protein extraction method provides the better result.

SDS-PAGE with 20cm × 20cm gels

SDS-PAGE for 20cm × 20cm gels was performed by 1st BASE Laboratories (Selangor, Malaysia). All the SDS-PAGE procedures were similar to SDS-PAGE procedures by using dual vertical mini gel unit. However, the differences were that protein samples were extracted by detergent lysis, PMA2006, PMA4963, PMB202 and PMB713, were used and the SDS-PAGE was carried out using only 12.5% separating gel with the gel size of 20cm × 20cm. The SDS-PAGE was carried out using the same protein concentrations as the dual vertical mini gel unit. Staining and destaining processes were the same as the dual vertical mini gel unit.

Two-dimensional protein separation via 2-DGE

The 2-DGE methods used in this study was adapted from Görg et al.[6,7] The 2-DGE equipments used, Ettan™ IPGphor Isoelectric Focusing System, EttanDALT twelve Large Format Vertical System, Ettan™ DALTsix gel castor and glass plates, were from Amersham Biosciences, Sweden.

RESULTS

Identification of Pasteurella multocida isolates

Before carrying out any further studies, all the isolates first confirmed as *Pasteurella multocida* by Gram staining, colony morphology and a series of biochemical tests (Table 1). All the isolates were found to be Gram-negative bacteria and when examined microscopically, it showed the characteristic coccobacillus or short rod; cells occur singly, in pairs and occasionally as chains of filaments, thus confirming the 6 isolates to be of the genus *Pasteurella*(Figure 1a,b & c).

Comparison of SDS-PAGE electrophoretic profiles

Protein samples of PMA4963, PMB202 and PMU9577 were first extracted by the homogenization/sonication method using lysis solution, extraction buffer and ddH_2O. These three extraction solutions were used for each sample to determine which solution gave the best extraction performance. After protein samples had been homogenized and sonicated, SDS-PAGE was performed on these samples with 12.5% separating gel and 5% stacking gel using 10cm x 10cm gel of Dual Vertical Mini Gel Unit and stained using Coomassie Brilliant Blue R-250.

Based on the result shown in Figure 2, the overall visualization of protein bands was not very clear. Some of the protein bands can be seen on the upper part of the gels, these were protein bands with higher molecular weights while protein bands with lower molecular weights could not be seen clearly. Blotches were seen on the lanes of PMA4963, PMB202 and PMU9577 extracted using extraction buffer (Figure 2a Lane 2, 5 and 8). None of the bands of protein marker on lane M could be visualized.

Gel Percentages: 12.5%, 15.0% and 18.0% separating gels

Based on the results shown in Figure 2b–d, a relationship could be observed, where protein bands detected will accumulate on the upper part of the gel when gel percentage increased. For the SDS-PAGE result of 12.5% gel, proteins bands can be seen at the upper, middle and near the end of the gel. However, when gel percentage was increased to 15.0% and 18.0% using the same protein samples, all the protein bands obtained in 15.0% and 18.0% gels were the same as protein bands obtained from 12.5% gel. However, all the protein bands obtained from 15.0% gel can be seen at the upper and middle part of the gel while all the protein bands obtained from 18.0% gel can only be seen on the upper

part of the gel. 12.5% is the lowest acrylamide concentration in this study, which allowed protein bands to be seen throughout the gel including near the dye front. However, when the gel percentage increased, protein bands were concentrated on the upper part of the gel.

Based on the results, 12.5% was chosen to be the best gel percentage as protein bands were seen throughout the gel and they were well distanced between each other, allowing better gel analysis as compared to 15.0% and 18.0%.

Comparison of 2-DGE electrophoretic profiles

In preparing the second-dimension gels, single percentage gels were used and not gradient gels as single percentage gels offer better resolution. In this study, homogeneous gels containing 15.0% and 12.5% total acrylamide were used for 2-DGE. Stacking gels are not necessary for 2-DGE.

Based on the results shown in previously, no distinct possible protein spots were noted in PMB4 and PMB202 on 15.0% second-dimension gels. Bands of protein marker were not clearly seen for both the gels of PMB4 and PMB202. This could be due to many reasons, the samples loaded (100 µg) might be insufficient or insufficient sample entered the IPG strip due to poor sample solubilization. Another possibility was samples might contain impurities that prevent the first-dimension focusing. Many horizontal streaking was seen on the upper part of PMB4 and slight streaking were observed in PMB202 while lower part of the gels for both the samples were relatively clean without any protein spots detected. The bands for protein marker were not well separated and could not be easily visualized.

On the other hand, PMB202 protein sample ran on 12.5% gels produced better visualization of gels and many distinct possible protein spots were detected on the gels as compared to 15.0% gel. In this attempt, various protein amounts ranging from 200 µg to 500 µg were used in order to obtain gel with the highest number of possible protein spots detected. For gel with 200 µg of PMB202, 6 possible protein spots were detected, 10 possible protein spots were detected for 300 µg of PMB202, 20 possible protein spots were detected for 400 µg of PMB202 and 32 possible protein spots were detected for 500 µg of PMB202. Horizontal streakingwere observed in gels loaded with 200 µg, 300 µg and 400 µg of PMB202. With the increase in protein loading concentration, the number of possible protein spots detected was also increased. This showed that loading concentration is a very important optimization factor in order to obtain reproducible results of total protein distribution. However, due to limited time factor and relatively tedious 2-DGE procedures, a short of protein loading amounts were chosen, that is 200µg to 500µg.

Based on the results, 12.5% second-dimension gel loaded with 500 µg of PMB202 were chosen to be the best running conditions in *Pasteurella multocida*. Thus, it is to be presented here a preliminary protein map of *Pasteurella multocida* serotype B strain 202 (PMB202) using the above-mentioned running conditions.

Preliminary 2-DGE analysis

A total of 32 possible protein spots were detected on the preliminary protein map of 12.5% second-dimension gel loaded with 500 µg of PMB202 (figure 2f shown in red arrows). After comparing with Pm70 in silico 2-DGE protein patterns using GELBANK database, 23 proteins were identified (Figure 2f), number in red] while the remaining 9 spots were not protein spots. The proteins identified, their names, theoretical mass and pI are shown in Table 2.

Based on the genome of Pm70, a total of 2014 ORFs, hence there will be 2014 theoretical molecular weight and pI in the theoretical proteome of Pm70. However, 1891 ORFs fall within the pI range of 3 to 10 and molecular weight of between 10 kDa and 200 kDa, which is the range of pH gradient and protein marker used in this study respectively. Within the 1891 ORFs, 13 proteins and 10 unknown proteins were obtained as used in the Pm70 genome annotation. One of the proteins identified, Psd (phosphatidylserine decarboxylase) possessed theoretical molecular weight of 33.4kDa. This is in consistent with the previous SDS-PAGE study, in which a distinct 33kDa protein band was detected by SDS-PAGE in PMB202 and PMB713 but did not appear in PMA2006 and PMA4963.

DISCUSSION

The results of biochemical characterization of the *Pasteurella multocida* isolates were in accordance with those reported by various researchers.[8–11] All of the isolates possessed similar biochemical profiles in all serotypes. They were found to be non-hemolytic, produced indole, hydrogen sulphide gas, and reduced nitrates to nitrites. Positive results were also observed for oxidase and catalase reaction and negative results were obtained for ONPG test, MRVP, urease and citrate utilization test.

SDS PAGE profile results support the data reported by Ireland et al. (1991) in which they found that the protein profiles of sonicated isolates were complex.[12]There were several factors contributed to the poor visualization result. Proteases may be liberated upon cell disruption and proteolysis greatly complicates SDS-PAGE visualization. Cells should be disrupted in such a way to minimize proteolysis and other mode of protein degradation at as low a temperature as possible and with a minimum of heat generation.[13] The composition of the solutions used was particularly critical for protein solubilization. For both the lysis solution and extraction buffer, they contained urea. Urea can cause protein modifications if

the samples were heated. Hence, with the heating generated by sonication process, elevated temperature caused urea to hydrolyze to isocyanate, which modifies protein by carbamylation.[6,7,14]

Protein samples were also obtained by detergent lysis using BugBuster™ protein extraction reagent and SDS-PAGE was first performed on these samples with 12.5% separating gel and 5% stacking gel using 10cm x 10cm gel of Dual Vertical Mini Gel Unit and stained using Coomassie Brilliant Blue R-250.[15] Based on the result shown in Figure 2b, a large number of protein bands were detected by SDS-PAGE. The overall visualization of protein bands produced were far better than the SDS-PAGE result of protein samples extracted by homogenization and sonication with a large number of protein bands detected clearly and they were nicely oriented. The band intensity of PMA2006 and PMA4963 on lane 1 and 2 respectively were lesser than PMB202 and PMB713 respectively on lane 3 and 4. Protein bands of PMB202 provide the best visualization effect. No blotches were observed in the gel. All the known molecular weight fragments of protein marker were clearly seen on the gel.

Based on the results shown in Figure 2b,e, protein bands on 20cm x 20cm gel were seen to be well distanced where protein bands tend to be smaller, well orientated and produced relatively straight bands as compared to protein bands on 10cm x 10cm gel where the protein bands were broader and some bands were curved. The protein bands on 20cm x 20cm gel was well separated where distance between the bands are wider, providing better gel analysis as compared to protein bands on 10cm x 10cm gel which were more concentrated and bands were close to each other. This shows that smaller gel size can contribute in the limitation of SDS-PAGE analysis as chances in visualizing overlap protein bands are much higher as compared to bigger gel size, which is able to separate proteins even better and the possibility in obtaining false negative or false positive results can be minimized.[14,16,17]

Due to the significant economic losses caused by *Pasteurella multocida* serotype B to Malaysia, two samples of *Pasteurella multocida* serotype B, PMB 4 and PMB202, were separated using 15.0% second-dimension gel with 100µg loading amount. For 12.5% second-dimension gel, only PMB202 was used. Four loading amount of 200 µg, 300 µg, 400 µg and 500 µg were tried out at 12.5% second-dimension gels. Only one sample of PMB202 was used for 12.5% second-dimension gels in order to produce a preliminary protein map of the strain PMB202 isolated from hemorrhagicsepticemia (HS) had been studied extensively by Salmah and coworkers. In which they had produced a number of recombinant clones containing inserts, and clone ABA392 was produced with the specific aim of cloning unique DNA sequences related to pathogenesis of HS.[18]

The horizontal streaking observed were also due to many possible reasons. These possibilities were samples not completely solubilized prior to application, samples were poorly soluble in rehydration solution, interfering substances and especially non-protein impurities in the samples could interfere with IEF causing horizontal streaking in the final 2-D results.[19]Moreover, It is suggested that the 33kDa protein band detected by SDS-PAGE was most likely be phosphatidylserine decarboxylase. Phosphatidylserine decarboxylase plays a pivotal role in the synthesis of phospholipid by the mitochondria.[20]

Detergent lysis was the preferred isolation method of total proteins in *Pasteurella multocida*. Protein separation of *Pasteurella multocida* can be done on one- and two-dimensional gel electrophoresis. For one-dimensional gel electrophoresis (SDS-PAGE), the optimum running condition was 15 µg of protein loading amount run on 12.5% separating gel with the gel size of 10cm × 10cm. For two-dimensional gel electrophoresis, the optimum running condition was 500µg of protein sample loaded using rehydration loading with immobilized pH gradient (IPG) strips pH 3 to 10 for the first-dimension isoelectric focusing (IEF) and 12.5% single percentage gel for the second-dimension SDS-PAGE. However, further optimization study for one-dimensional and two-dimensional gel electrophoresis in *Pasteurella multocida* needs to be done in order to obtain the best possible results. Preliminary 2-DGE analysis identified 23 proteins of *Pasteurella multocida* serotype B (PMB202) and protein of 33 kDa (33.4 kDa in 2-DGE) was identified in both SDS-PAGE and 2-DGE. However, further studies have to be done in order to confirm this finding.

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Table 1: Different biochemical tests for *Pasteurella multocida* isolates.

Biochemical tests	Isolate (+ positive test; - negative test)					
	PMA 2006	PMA 4963	PMB 4	PMB 202	PMB 713	PMU 9577
Acid from glucose	+	+	+	+	+	+
Catalase reaction	+	+	+	+	+	+
Citrate utilisation	-	-	-	-	-	-
Haemolysis	-	-	-	-	-	-
H_2S production	+	+	+	+	+	+
Indole production	+	+	+	+	+	+
Mac Conkey	-	-	-	-	-	-
Motility	-	-	-	-	-	-
MRVP	-	-	-	-	-	-
Nitrate production	+	+	+	+	+	+
ONPG	-	-	-	-	-	-
Oxidase reaction	+	+	+	+	+	+
Urease production	-	-	-	-	-	-

Table 2: Proteins identified based on the *in silico* 2-DGE protein reference map of Pm70.

Spot no.	Protein identified	Protein name [1]	Theoretical mass (kDa)	Theoretical pI
1	DsbD	Thioldisulphide interchange protein	65.7	6.1
2	DppA	Dipeptide transport protein	59.3	6.4
3	Unknown	-	90.9	6.9
4	DmsA	Anaerobic dimethyl sulfoxide reductase, chain A	94.2	7.5
5	SpoT	Guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase	79.8	7.7
6	GmhA	Phosphoheptose4 isomerase	21.4	6.0
7	CcmE	Cytochrome-c biosynthesis	19.9	6.2
8	Unknown	-	28.8	6.6
9	HsdA	Type I site-specific deoxyribonuclease	48.9	6.1
10	MurE	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	54.1	6.3
11	HflX	GTP-binding protein	50.1	6.5
12	WbjC	o-antigen locus	43.1	6.7
13	Unknown	-	45.5	6.7
14	Unknown	-	50.4	6.8
15	Unknown	-	46.9	6.9
16	Unknown	-	46.4	7.2
17	Psd	Phosphatidylserine decarboxylase	33.4	7.2
18	Unknown	-	30.4	7.2
19	Unknown	-	28.2	7.7
20	ModB	Molybdenum transport protein	26.5	7.7
21	Unknown	-	27.7	5.2
22	Unknown	-	16.6	5.5
23	DsbA	Thiolsulphide interchange protein	23.2	6.9

[1]Protein names are as used in the Pm70 genome annotation

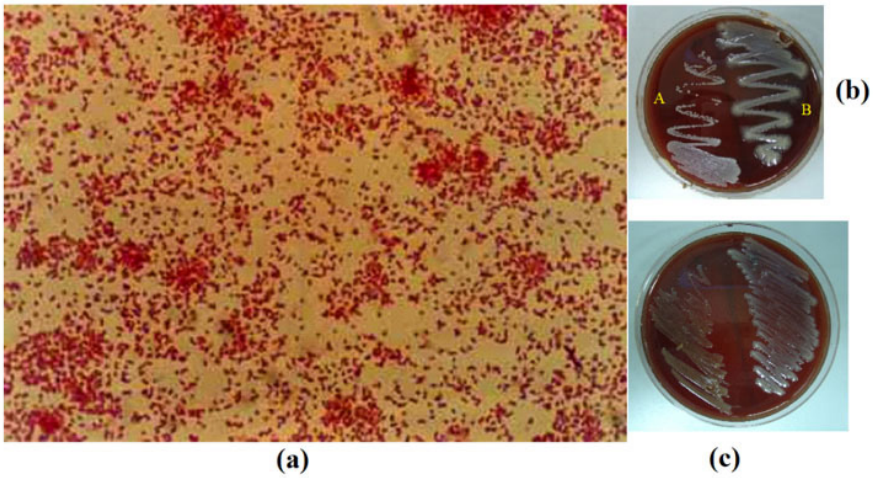

Figure 1: *Pasteurella multocida* Gram staining and colony morphology. **(a)**Gram-stained smear of *Pasteurella multocida*(1000x) appears as small bipolar Gram-negative short rod**. (b)** Petri dish showing the growth of smooth *Pasteurella multocida* colonies (Side A) and growth of watery mucoid colonies (Side B) on BHI blood (7%) agar medium. **(c)** Petri dish showing the growth of rough *Pasteurella multocida* colonies on BHI blood (7%) agar medium.

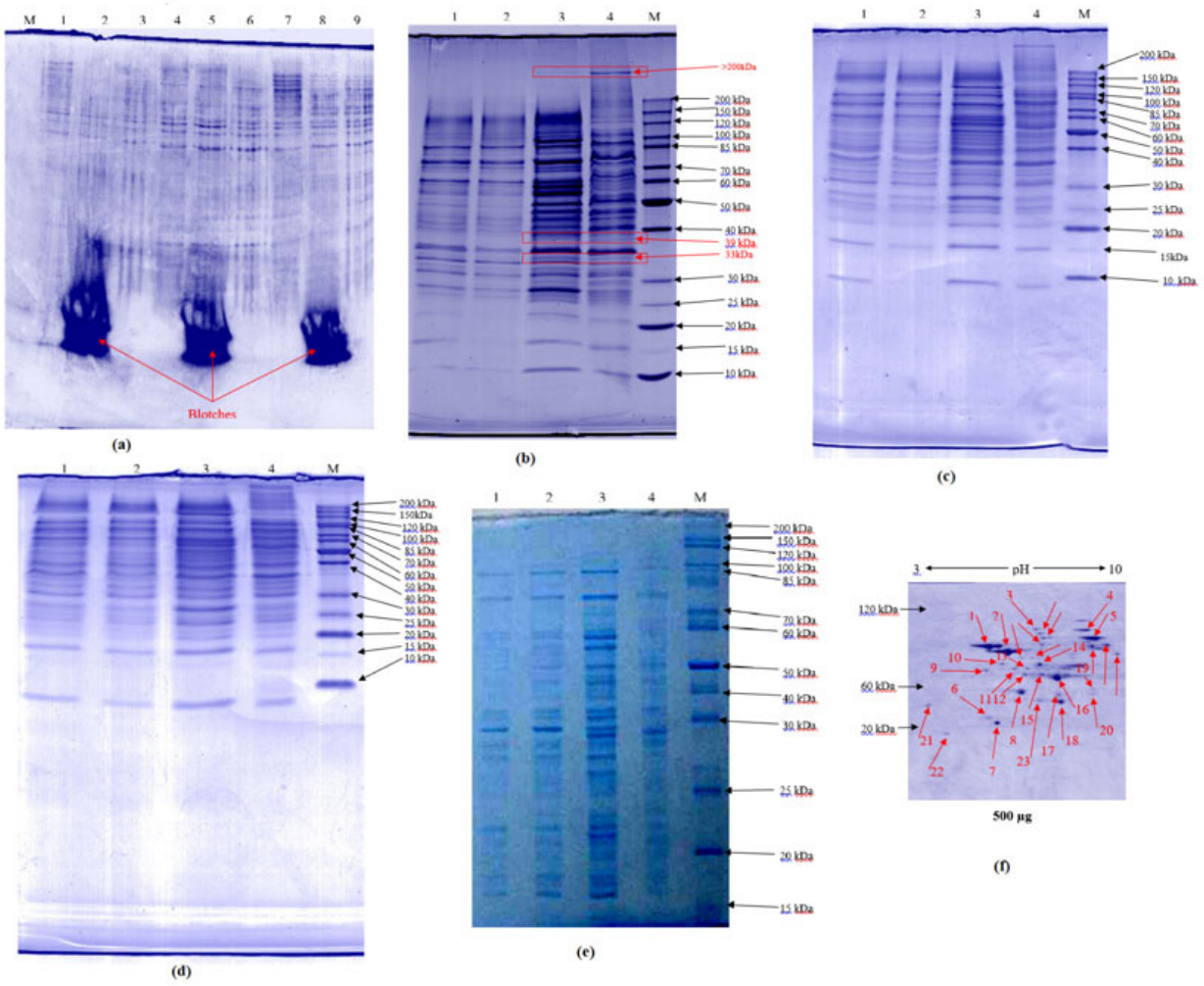

Figure 2: 1D and 2D SDS-PAGE separation of *Pasteurella multocida* proteins. (a) SDS-PAGE (12.5% separating gel and 5% stacking gel) with protein samples extracted by homogenisation and sonication. Blotches were detected at lane 2, 5 and 8.
Lane Key: M: PageRuler™ protein ladder; 1: PMA 4963 in lysis solution; 2: PMB 202 in lysis solution; 3: PMA 4963 in ddH_2O; 4: PMA 4963 in ddH_2O; 5: PMB 202 in extraction buffer; 6: PMB 202 in ddH_2O; 7: PMU 9577 in lysis solution; 8: PMU 9577 in extraction buffer; 9: PMU 9577 in ddH_2O. All loaded at 10µg.
b) SDS-PAGE (12.5% separating gel and 5% stacking gel) with protein samples extracted by detergent lysis using BugBuster® protein extraction reagent. 3 distinct protein bands were detected, at 33 kDa, >200 kDa.
Lane Key: 1: PMA 2006; 2: PMA 4963; 3: PMB 202; 4: PMB 713; M: PageRuler™ protein ladder. All samples loaded at 15µg.
c) SDS-PAGE (15.0% separating gel and 5% stacking gel) with protein samples extracted by detergent lysis using BugBuster® protein extraction reagent. Bands were seen concentrated at the upper part of the gel.
d) SDS-PAGE (18.0% separating gel and 5% stacking gel) with protein samples extracted by detergent lysis using BugBuster® protein extraction reagent. Bands were seen concentrated at the upper part of the gel.
e) SDS-PAGE (12.5% separating gel and 5% stacking gel) with protein samples extracted by detergent lysis using BugBuster® protein extraction reagent. Protein bands were seen to be well distanced where protein bands tend to be smaller, well orientated and produced relatively straight bands. However, lower resolution of gel was obtained.
f) 2-DGE of PMB 202 extracted by detergent lysis using BugBuster® protein extraction reagent. Proteins were separated in the first dimension IEF using a pH 3-10 IPG strip and in the second dimension SDS-PAGE by 12.5% gel. The red arrows showed the possible protein spots while number 1-23 in (d) showed proteins identified using GELBANK database.