# Chapter V

# Discussion

## 1. Bacteriological characteristics

As has been described by some investigators (3,4), P. multocida can occasionally be mistaken for other bacteria due to incorrect interpretation of gram stained smears or inadequate laboratory identification techniques. The results of bacteriological characteristics in table 9 which coincide with those have been described by Weaver et al (17) will be an aid in identification of P. multocida. These characteristics, however, can not differentiate between P. multocida isolated from human and animals.

Our results in table 8 indicate that P. multocida grows better on trypticase soy agar containing sheep blood. Nutrient agar plus 5% sheep blood is not suitable enough for the cultivation of P. multocida since human strains and some animal strains are rarely grown. In addition, human blood is unsatisfactory to use because it contains some inhibitors which interfere with the growth. The sizes of colonies vary with the capsular serotypes. Type A organism has the biggest colony whereas type B and E are the smallest. By examining under a light microscope, type A has thicker capsule than other serotypes whereas type B and E seem to be thinner. This observation is similar to the work of Jacques and Foiry (15) who

demonstrate by using an electron microscope that the capsules of type A are about three times thicker than those of type D. However, no evidence of capsular sizes of tyep B or E has been reported.

Biochemical characteristics of P. multocida human and animal strains shown in table 9 are identical except for the fermentation of some carbohydrates. The results herein show that xylose fermentation seems to correlate with serotypes. All serotype 1 as well as vaccine strain 8:A are xylose negative whereas all serotype 3, except one human strain, are xylose positive. Our finding correlates with that of Heddleston (49) who has demonstrated that serotype 3 ferments xylose but not dulcitol whereas serotype 1 has the vice versa reaction. In addition, we have shown that dulcitol fermentation can not be used to differentiate between serotype 1 and 3.

2. Serological typing by the Carter-Heddleston system

2.1 Capsular types

In table 10, we have shown that the production of capsular typing antisera A and D for the IHA test is unsuccessful. Early studies (8,51) have revealed that the difficulties in the production of these antisera are due to the inert materials in the capsules. The studies on the chemical nature of capsular type D are hitherto lacking.

However, it is known that the capsules of type A possess copious amounts of hyaluronic acid and possibly several other capsular polysaccharides (9,10,105). Pure hyaluronic acid is not antigenic. Therefore, capsular polysaccharides act as immunogens when associated with proteins or other antigens (106). Previous evidences have suggested that capsular hyaluronic acid interferes with antibody production and antigen antibody interaction (8). It has been shown in in vivo observation that rabbits inject repeatly with mucoid strains of P. multocida often fail to produce an antibody that is active in the IHA test (68). However, some investigators use hyaluronidase enzyme to overcome the action of hyaluronic acid (51,71,99,107) or change the antisera production to other animal species (51,108). Our study prefers to use the non-serological tests which have been known to be reliable for typing the P. multocida capsular type A and D (11,109,110,111).

On the contrary, high titers of antisera B and E have been obtained from the rabbits. This is due to the differences between the nature of capsular antigen B and E and those of type A and D. The capsules of type B and E contain protein, polysaccharide and LPS but no hyaluronic acid (12). Their immunogenicities are different. Their polysaccharides when coupled with proteins will become a good immunogen for antibody production and result in a high titer. Our results are

in agreement with those of Sawada et al (108) who demonstrated similar results of a high titer of antisera B and E.

It is noted that many kinds of animal red blood cells can be used for the sensitized phase in the IHA test (68). Our study prefers human red blood cells group O to sheep red blood cells since they contain no heterophile antigen which interfere with the IHA test. If animal red blood cells are used it is necessary to absorb the antisera with that animal red blood cells to get rid of the heterophile antibody before using them as typing antisera.

Hyaluronidase test has been demonstrated better on DSA than TSB. The explanation is DSA contains carbohydrate which promote the production of capsular polysaccharides and hyaluronic acid (8) whereas TSB contains no carbohydrate so that the reaction is dependent on the capsule production of the organism itself. This observation is in agreement with those of Rimler and Rhoades (51,111) but differs from the original work of Carter and Rundell (71) who demonstrated the test on TSB.

The performance in the acriflavine test is dependent on the growth of the organism. Carter and Subronto (11) have explained that the flocculation is possibly due to the capsular polysaccharide of type D strain which has physical characteristics resembling those

of the Vi antigen of Salmonella typhi strains. According to our results, we therefore recommend testing with a heavy growth of overnight culture, otherewise false negative reaction may be read.

Our results in table 11 show that P. multocida type B and E are not found in any isolates from human, pigs or poultry. These capsular serotypes are usually recovered from animals with epizootic hemorrhagic septicemia and chiefly found in cattle, bison and buffaloes (16). In Thailand, type B have been reported in water buffaloes and in swine but type E is not observed(83).

2.2 Somatic serotypes

The results of GDPT using somatic typing antisera in table 13 are different from those using reference typing antisera as shown in table 14. Only the results of prevalent serotypes of human and poultry isolates are similar. By using somatic typing antisera, more somatic serotypes especially cross-reaction of serotype 1 and 3 in pig strains have been evident. Each strain possesses the same major somatic antigen as typed by both antisera. There are several explanations for these differences. First, both antisera are produced in different animal species. Our antisera have been produced in rabbits whereas the references have been developed in chicken (73, 76, 112, 113). There may be species

differences in response between rabbits and chicken when a formalin-killed preparation of P. multocida is used as inoculum. The certain antigenic determinant may have been altered and cleaved by rabbits that respond differently than chicken (114). Second, the nature of heat-stable antigen is closely related to LPS-protein complex (73). The specificity of the antigen seems to reside in the LPS which is the major component of the type specificity (73,115). The LPS of each somatic serotype contains different amounts of glucose, 2-keto-3-deoxyoctonate, heptose and lipid (112), therefore, their common antigens may cause a cross-reaction between serotypes as demonstrated by Cary et al (114), and Brogden and Reber (75). In addition, our protein analysis on SDS-PAGE (Figure 8) has illustrated at least twelve common major protein bands among strains and the protein profiles of serotype 1 and 3 are indistinguishable. Therefore, cross-reaction among serotypes may be possible. Third, somatic typing antisera 1,3,12, and 13 used in this study have been prepared from the common strains among the isolates of human and animal (82). The antiserum type 4 has been prepared by immunizing with CU strain which possessed antigen 3 cross 4 (Table 12). However, we can not obtain only the antiserum type 4 because the titer of antiserum type 3 is too high to be absorbed.Thereby,all the isolates have been typed by both somatic typing antisera types 3

and 3x4. Any isolates that react with both typing antisera are serotype 3,4 whereas serotype 3 reacts only with antiserum type 3 even though they possess neither antigen 3 nor weak antigen 3 and 4. Finally, it has been proposed that comparison of P. multocida serotypes is unable to reliably correlate the results of the several methods used to serotype P. multocida (115.) This lack of correlation is due to the antigenic complexity of P. multocida in which only the major serotype is usually the same from test to test (116). According to our results, serotype 1,3 has serotype 3 as major serotype when typed with both antisera whereas serotype 1 is an additional serotype when somatic typing antisera were used.

Nevertheless, non-specific precipitin lines concave toward the antisera wells 1 and 3 have been observed by using the somatic typing antisera. These precipitin lines are derived from the residue of capsular components left after boiling (117). The capsule is a polysaccharide whose molecule is smaller than the molecule of heat-stable antigen, therefore, it migrates faster to the antibody well. In addition, these precipitin lines are usually found in mucoid strains of type A especially in pigs, but can not be detected in decapsulated "blue"colonies. The cell wall antigens are classified into three different kinds (77,78,79). $\beta$-Antigen is the part of capsular antigen, $\alpha$-antigen also resides in the capsule

and is antigenic whereas $\gamma$-antigen is responsible for the O-somotic antigen of Namioka and Murata when HCl treated cells are used in antigen preparation. It has been suggested that the $\alpha$-antigen is extremely difficult to separate and sufficient amounts probably remain in crude cell wall preparation (79).

In the present investigation we have extracted the heat-stable antigen from the selected colony on TSB which was subcultured at least three times for 24 hr incubation. The number of capsules would be less in this circumstance (115), however, some mucoid strains still possess an $\alpha$-antigen or a component of capsule left after extraction. Non-specific lines have only been observed when undiluted immune antisera 1 and 3 were used whereas this reaction has been eliminated by using diluted antisera.

Our data illustrate in table 9, 13 and 14 strongly suggest that vaccine strain 8:A prepared from local strain and two lyophilized strains 8:A isolated from ducks are Heddleston's type 1 and do not ferment xylose. Our results contrast to those of Brogden and Packer (115) who have demonstrated that the Namioka's 8:A serotype is usually Heddleston's type 3.In addition, Fujihara et al (118) have demonstrated that wild duck in Japan are Namioka's type 8:A and Heddleston's type 3 and ferment xylose. These different results may be due to the antigenic

complexity (115), the nature of antigen involved in each test and the different typing system (72,73,115).

2.3 P. multocida serotypes

The methods for serotyping the P. multocida isolates used in this study are the contemporary methods used in the United States (116). However, it is necessary to confirm its bacteriological characteristics before the serotyping is done. As has been mentioned, LPS is a major component of the heat-stable antigen. This LPS contains lipid A, 2-keto-3-deoxyoctonate, heptose and glucose which are similar to the LPS of other gram negative bacteria (112). Therefore, cross-reactivity can be observed if non-P. multocida strains are used.

The common P. multocida serotypes from isolates of human and animals in Thailand have been identified as type A:1,A:3 and A:3,4.However,untypable capsular or somatic serotypes are detected as well. It has been suggested that noncapsulated strains lack both capsular antigens and hyaluronic acid, therefore, they can not be typed by either the IHA test or the hyaluronidase test (115). In addition, the untypable strains still remain untypable even though they are passed into mice (119). This untypable strains should be further studied since additional serotypes may be encountered (51,76). The frequent occurance of serotype 1 and 3 are suggested to

call for an introduction of additional methods for their subdivision.

Our results in table 10 show that obtained reference strains type 6,8,13 and 15 are untypable. As has been examined by Jasmin method, these obtained strains possess only a few capsules therefore may lack both typing capsular antigens and components (115).

3. The analysis of protein patterns of P. multocida whole cell sonicated extracts by SDS-PAGE

Results of the SDS-PAGE described herein have revealed that the protein patterns of all strains show most bands indistinguishable in electrophoretic mobility and intensity as examined by naked eye. However some differences in the position of minor protein bands are seen. These differences seem to correlate with certain serotype such as B:2; A:3,4; somatic serotype 3,4 (Figure 8, lanes K, L, I, Q, O and R respectively) and between serotype D:3,12 and D:3,4,12 (Figure 8, lanes N and P). It is known that the two major somatic antigens exposed on the cell surface of P. multocida are LPS and proteins (73) which reside mostly in the outer membranes, and some locate in the inner membrane and cytoplasm (86,87). These proteins can be used for examining the similarities at the surface which is useful in the classification or clusters them into groups (120).

In the study by Barenkamp et al (121), the outer membrane proteins of Haemophilus influenzae type b have been prepared from the whole cell sonicated extracts and are classified into nine categories according to their reproducible and clearly resolvable differences in the outer membrane protein profiles. By the study of Krester and Ley (122), the protein of Agrobacterium sonicated extracts of twice centrifugations have been clustered into three groups by numerical analysis of electrophoretic protein patterns. Lee et al (97) by using the method of Barenkamp et al (121) have obviously demonstrated the different membrane proteins between CU strain and other nine P. multocida serotype 3,4 strains. Their results confirm those of ours which illustrate the different protein patterns of this serotype in figure 8, lanes I,Q,O and R respectively. However, the protein profiles of serotype A:1 and A:3 can not be differentiated. It is possible that the separation of whole cell sonicated extracts for 1 hr at 10,000 g is not suitable enough for the study on the differences among strains of certain serotypes.

It is difficult to conclude that the differences of the protein patterns among strains correlate with the origin of the isolates since only some variations in intensities of minor protein bands are examined between the common serotypes A:1 and A:3 (Figure 8, lanes B through H) and human reference strain A:12 (Figure 8, lane J).

Table 8 Physiological characteristics of P.multocida

| Characteristics / Types | Colony features on TSB | Morphology [a] |
|---|---|---|
| Type A | Whitish, opaque, mucoid, smooth, entire edge, convex, 2-2.5 mm in diameter. Non-hemolytic but produced greenish discoloration at the origin of inoculum sites. | Gram-negative coccobacilli with capsules, arranged in singly. |
| Type B | Greyish, off-white, semitransparent smooth, entire edge, convex, 0.5-1 mm in diameter. Non-hemolytic. | Gram-negative, pleomorphic, coccobacilli or short rod with capsules, arranged in singly |
| Type D | Same as type A but smaller, about 1-2 mm in diameter, Non-hemolytic. | Gram-negative, pleomorphic, coccobacilli, rod or occasionary short or long filaments were seen, arranged in singly or chain |
| Type E | Same as type B but whiter, not as mucoid as type A and D. Non-hemolytic. | Gram-negative coccobacilli with capsules, arranged in singly |

a = by Gram staining

Table 9 Biochemical characteristics of *P. multocida*

| Tests \ Sources | Reference strains (15+4) [a] | Vaccine strains (3)[b] | Human strains (17) | Pig strains (23) | Poultry strains (29) | total (91) | per cent positive |
|---|---|---|---|---|---|---|---|
| 1.Hemolysis on TSB | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.Growth on : | | | | | | | |
| MacConkey | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SS | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.Capsules | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 4.Motility | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5.Fermentative /oxidative | F | F | F | F | F | 91 | 100 |
| 6.Gas from glucose | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7.Acid from glucose | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| mannitol | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| lactose | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10%lactose | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| sucrose | 19 | 3 | 17 | 20 | 29 | 88 | 96.70 |
| maltose | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 9 (continued)

| Tests \ Sources | Reference strains (15+4) [a] | Vaccine strains (3)[b] | Human strains (17) | Pig strains (23) | Poultry strains (29) | total (91) | per cent positive |
|---|---|---|---|---|---|---|---|
| mannose | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| arabinose | 3 | 0 | 5 | 1 | 24 | 33 | 36.26 |
| dulcitol | 2 | 0 | 0 | 0 | 0 | 2 | 2.20 |
| xylose* | 14 | 2 | 11 | 23 | 3 | 53 | 58.24 |
| 8. Catalase | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 9. Oxidase | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 10. Simmon citrate | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11. Urea Christensen's | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12. Nitrate reduction | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 13. Gas from nitrate | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14. Indole | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 15. TSI | A/A | A/A | A/A | A/A | A/A | 91 | 100 |
| 16. MR | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17. VP | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18. Gelatin hydrolysis | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 9 (continued)

| Tests \ Sources | Reference strains (15+ 4)[a] | Vaccine strains (3)[b] | Human strains (17) | Pig strains (23) | Poultry strains (29) | total (91) | per cent positive |
|---|---|---|---|---|---|---|---|
| 19.Growth at | | | | | | | |
| 25 C | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 37 C | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 42 C | 18 | 3 | 13 | 22 | 29 | 91 | 93.40 |
| 20.Esculin hydrolysis | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21.Lysine decarboxylase | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22.Ornithine decarboxylase | 19 | 3 | 17 | 23 | 29 | 91 | 100 |
| 23.Arginine dihydrolase | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24.Nutrient broth, | | | | | | | |
| 0% NaCl | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6% NaCl | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

a = 4 capsular types : A,B,D, and E; 15 somatic types = 1-16 except type 4.

b = 3 vaccine strains : CU, 8 : A, and 6 : B.

* = all serotype 1 are xylose negative

Table 10 Results of capsular serotyping of P. multocida reference and vaccine strains as determined by IHA and non-serological tests.

| Cultures of | IHA test* using capsular antisera [a] of | | Non-serogical tests | | Results |
|---|---|---|---|---|---|
| | Type B | Type E | Hyaluro--nidase | Acriflavine | |
| **Type A(P-1059) | - [b] | - | + [c] | - [d] | A |
| **Type B(M-1404) | 8192 | - | - | - | B |
| **Type D(P-3881) | - | - | - | + | D |
| **Type E(P-1235) | - | 8192 | - | - | E |
| Type 1 (X-73) | - | - | + | - | A |
| Type 2 (M-1404) | 4096 | - | - | - | B |
| Type 3 (P-1059) | - | - | + | - | A |
| Type 5 (P-1702) | - | - | + | - | A |
| Type 6 (P-2192) | - | - | - | - | untypable |
| Type 7 (P-1997) | - | 16 | - | - | E |
| Type 8 (P-1581) | - | - | - | - | untypable |
| Type 9 (P-2095) | - | - | + | - | A |
| Type 10 (P-2100) | - | - | + | - | A |
| Type 11 (P-903) | - | - | - | + | D |
| Type 12 (P-1573) | - | - | + | - | A |
| Type 13 (P-1591) | - | - | - | - | untypable |
| Type 14 (P-2225) | - | - | + | - | A |
| Type 15 (P-2237) | - | - | - | - | untypable |
| Type 16 (P-2723) | - | - | + | - | A |
| CU | - | - | + | - | A |
| 8 : A | - | - | + | - | A |
| 6 : B | 4096 | - | - | - | B |

a = Titers are expressed as the reciprocals

b = No hemagglutination at dilution 1:2

c = positive

d = negative

* = Type A and D are negative

** = Strains used for standardization of the methods

Table 11 Results of capsular serotypes of P. multocida from human, pig, and poultry strains.

| Number of findings ( )[a] / Types | Human | Pig | Poultry | Total |
|---|---|---|---|---|
| A | 11(64.71%) | 19(82.61%) | 25(86.22%) | 55(79.71%) |
| B | - | - | - | - |
| D | 5(29.41%) | 4(17.39%) | - | 9(13.04%) |
| E | - | - | - | - |
| Untypable | 1(5.88%) | - | 4(13.78%) | 5(7.25%) |
| Total number of strains | 17 | 23 | 29 | 69 |

( )[a] = per cent of findings

Table 12 Results of somatic serotyping of P. multocida reference and vaccine strains as determined by GDPT.

| Culture extracts | Reference typing antisera | Somatic typing antisera (type 1,3,3x4,12,13) |
|---|---|---|
| Type 1 (X-73) | 1 | 1 |
| Type 2 (M-1404) | 2 | -[b] |
| Type 3 (P-1059) | 3 | 3[c] |
| Type 5 (P-1702) | 5 | - |
| Type 6 (P-2192) | 6 | - |
| Type 7 (P-1997) | 7 | - |
| Type 8 (P-1581) | ND[a] | - |
| Type 9 (P-2095) | 9 | - |
| Type 10 (P-2100) | 10 | - |
| Type 11 (P-903) | 11 | - |
| Type 12 (P-1573) | 12 | 12 |
| Type 13 (P-1591) | 13 | 13 |
| Type 14 (P-2225) | 14 | - |
| Type 15 (P-2237) | ND[a] | - |
| Type 16 (P-2723) | 16 | - |
| Type A (P-1059) | 3 | 3[c] |
| Type B (M-1404) | 2 | - |
| Type D (P-3881) | 3,12 | - |
| Type E (P-1235) | 2 | - |
| CU | 3,4 | 3,4[d] |
| 8 : A | 1 | 1 |
| 6 : B | 2 | - |

a = not done

b = negative result

c = also positive with antiserum type 3x4

d = also positive with antiserum type 3

Table 13 Results of somatic serotyping of P. multocida from human, pig, and poultry strains using reference typing antisera.

| Number of findings ( )[a] / Serotypes | Human | Pig | Poultry | Total |
|---|---|---|---|---|
| 1 | 6(35.30%) | - | 27(93.10%) | 33(47.82%) |
| 3 | 3(17.64%) | 14(60.88%) | - | 17(24.64%) |
| 1,3 | - | - | - | - |
| 3,4 | 4(23.54%) | 6(26.08%) | 2(6.90%) | 12(17.39%) |
| 1,3,4 | - | - | - | - |
| 12 | - | - | - | - |
| 3,12 | - | - | - | - |
| 1,3,12 | - | - | - | - |
| 3,4,12 | 2(11.76%) | - | - | 2(2.90 %) |
| 4,7,12 | - | 3(13.04%) | - | 3(4.35 %) |
| Untypable | 2(11.76%) | - | - | 2(2.90%) |
| Total number of strains | 17 | 23 | 29 | 69 |

( )[a] = per cent of findings

Table 14 Results of somatic serotyping of P. multocida from human, pig, and poultry strains using immune typing antisera.

| Number of findings ( )[a] / Serotypes | Human | Pig | Poultry | Total |
|---|---|---|---|---|
| 1 | 6(35.30%) | - | 26(89.65%) | 32(46.38%) |
| 3 | 2(11.76%) | 6(26.08%) | 1(3.45%) | 9(13.04%) |
| 1,3 | 2(11.76%) | 11(47.84%) | 1(3.45%) | 14(20.29%) |
| 3,4 | 4(23.54%) | 2(8.69%) | - | 6(8.69 %) |
| 1,3,4 | - | 1(4.35%) | 1(3.45%) | 2(2.90%) |
| 12 | - | 1(4.35%) | - | 1(1.45%) |
| 3,12 | 1(5.88%) | - | - | 1(1.45%) |
| 1,3,12 | 1(5.88%) | 2(8.69%) | - | 3(4.35%) |
| 3,4,12 | - | - | - | - |
| 4,7,12 | - | - | - | - |
| Untypable | 1(5.88 %) | - | - | 1(1.45%) |
| Total number of strains | 17 | 23 | 29 | 69 |

( )[a] = per cent of findings

Table 15 Results of P. multocida serotypes from human, pig, and poultry strains in Thailand, using reference typing antisera in GDPT.

| Number of findings ( )[a] / Serotypes | Human | Pig | Poultry | Total |
|---|---|---|---|---|
| A:1 | 5(29.44%) | - | 25(86.20%) | 30(43.48%) |
| A:3 | 3(17.64%) | 14(60.88%) | - | 17(24.64%) |
| A:1,3 | - | - | - | - |
| A:3,4 | 3(17.64%) | 5(21.74%) | - | 8(11.58%) |
| A:1,3,4 | - | - | - | - |
| D:1 | 1(5.88%) | - | - | 1(1.45%) |
| D:3 | - | - | - | - |
| D:12 | - | - | - | - |
| D:1,3 | - | - | - | - |
| D:3,4 | - | 1(4.35%) | - | 1(1.45%) |
| D:3,12 | - | - | - | - |
| D:1,3,12 | - | - | - | - |
| D:3,4,12 | 2(11.76%) | - | - | 2(2.90%) |
| D:4,7,12 | - | 3(13.03%) | - | 3(4.35%) |
| D:untypable | 2(11.76%) | - | - | 2(2.90%) |
| Untypable : 1 | - | - | 2(6.90%) | 2(2.90%) |
| Untypable : 3 | - | - | - | - |
| Untypable : 3,4 | 1(5.88%) | - | 2(6.90%) | 3(4.35%) |
| Untypable : 1,3,4 | - | - | - | - |
| Total number of strains | 17 | 23 | 29 | 69 |

( )[a] = per cent of findings

Table 16 Results of P. multocida serotypes from human, pig, and poultry strains in Thailand, using immune typing antisera in GDPT.

| Number of findings ( )[a] / Serotypes | Human | Pig | Poultry | Total |
|---|---|---|---|---|
| A:1 | 5(29.44%) | - | 24(82.75%) | 29(42.02%) |
| A:3 | 2(11.76%) | 5(21.74%) | - | 7(10.14%) |
| A:1,3 | 1(5.88%) | 10(43.48%) | 1(3.45%) | 12(17.39%) |
| A:3,4 | 3(17.64%) | 2(8.69%) | - | 5(7.25%) |
| A:1,3,4 | - | 1(4.35%) | - | 1(1.45%) |
| D:1 | 1(5.88%) | - | - | 1(1.45%) |
| D:3 | - | 1(4.35%) | - | 1(1.45%) |
| D:12 | - | 1(4.35%) | - | 1(1.45%) |
| D:1,3 | 1(5.88%) | 1(4.35%) | - | 2(2.90%) |
| D:3,4 | - | - | - | - |
| D:3,12 | 1(5.88%) | - | - | 1(1.45%) |
| D:1,3,12 | 1(5.88%) | 2(8.69%) | - | 3(4.35%) |
| D:3,4,12 | - | - | - | - |
| D:4,7,12 | - | - | - | - |
| D:untypable | 1(5.88%) | - | - | 1(1.45%) |
| Untypable : 1 | - | - | 2(6.90%) | 2(2.90%) |
| Untypable : 3 | - | - | 1(3.45%) | 1(1.45%) |
| Untypable : 3,4 | 1(5.88%) | - | - | 1(1.45%) |
| Untypable : 1,3,4 | - | - | 1(3.45%) | 1(1.45%) |
| Total number of strains | 17 | 23 | 29 | 69 |

( )[a] = per cent of findings

TABLE 17 Protein concentrations of whole cell sonicated extracts of P. multocida selected strains used for SDS-PAGE

| Strains | Protein concentrations mg% |
|---|---|
| 1. TECH - 177/31 | 286.47 |
| 2. Type 1 (x-73) | 559.89 |
| 3. TECH-965/31 | 572.92 |
| 4. PM 1020 | 690.10 |
| 5. V-2 | 768.23 |
| 6. CU | 911.46 |
| 7. Type 12 (P-1573) | 1080.73 |
| 8. Type 3 (P-1059) | 1223.96 |
| 9. TECH - 2 | 1289.06 |
| 10. Type B (M-1404) | 1367.19 |
| 11. 6:B | 1380.21 |
| 12. PM 3011 | 1380.21 |
| 13. Type D (P-3881) | 1458.33 |
| 14. 8:A | 1510.42 |
| 15. PM 1002 | 1549.48 |
| 16. Type E (P-1235) | 1588.54 |
| 17. AC-01 | 1718.75 |

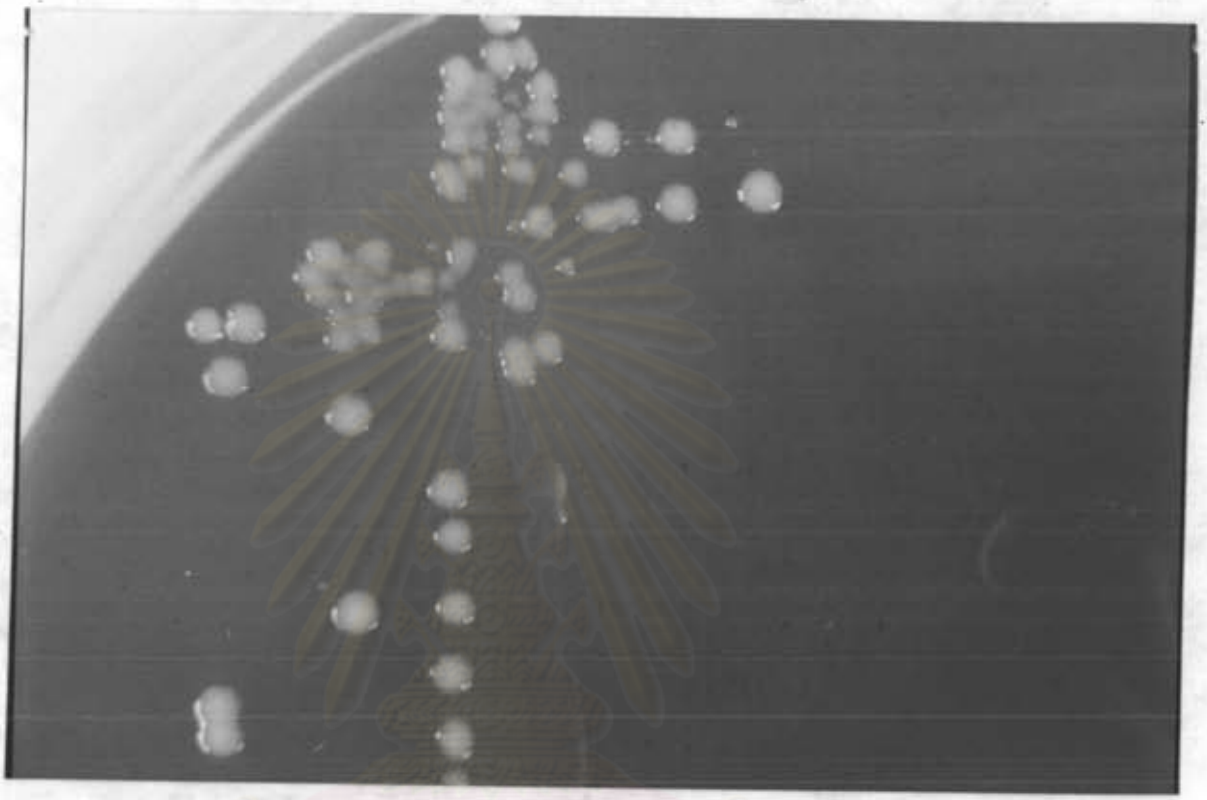

Figure 2 Colony feature of P. multocida type A reference strain on TSB

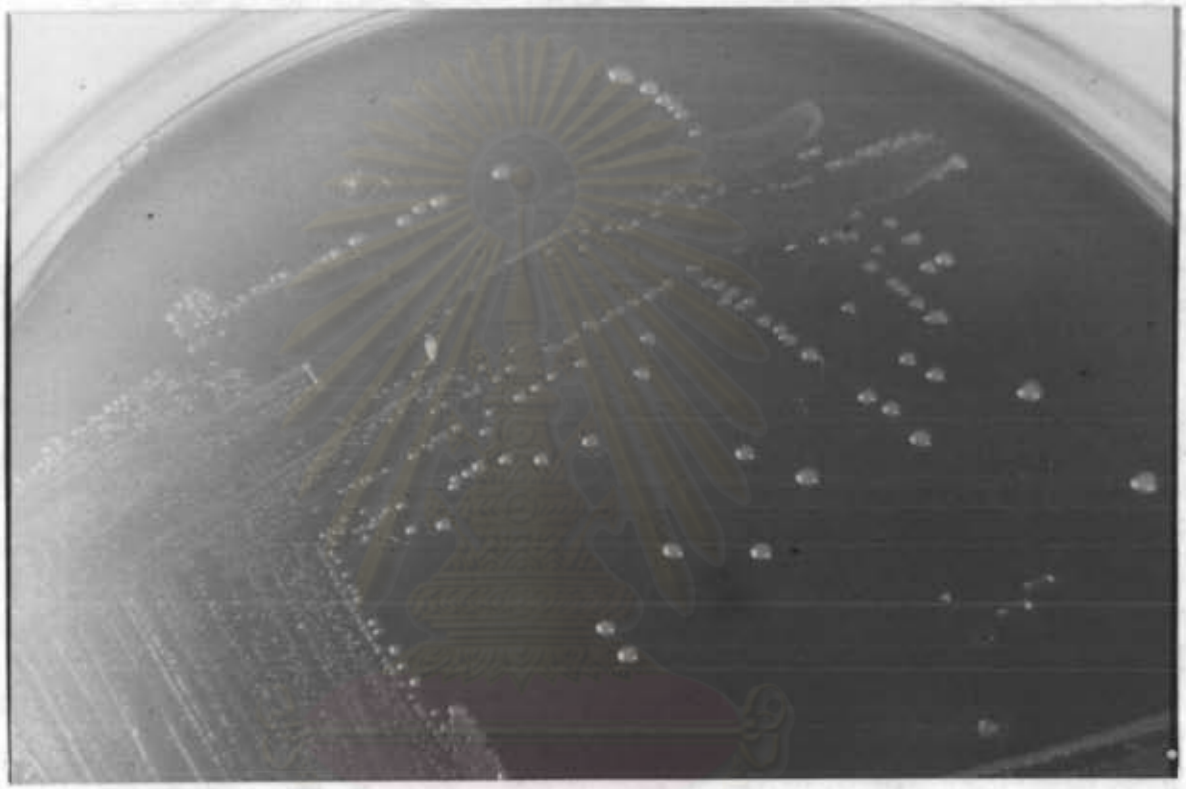

Figure 3 Colony feature of P. multocida type B reference strain on TSB

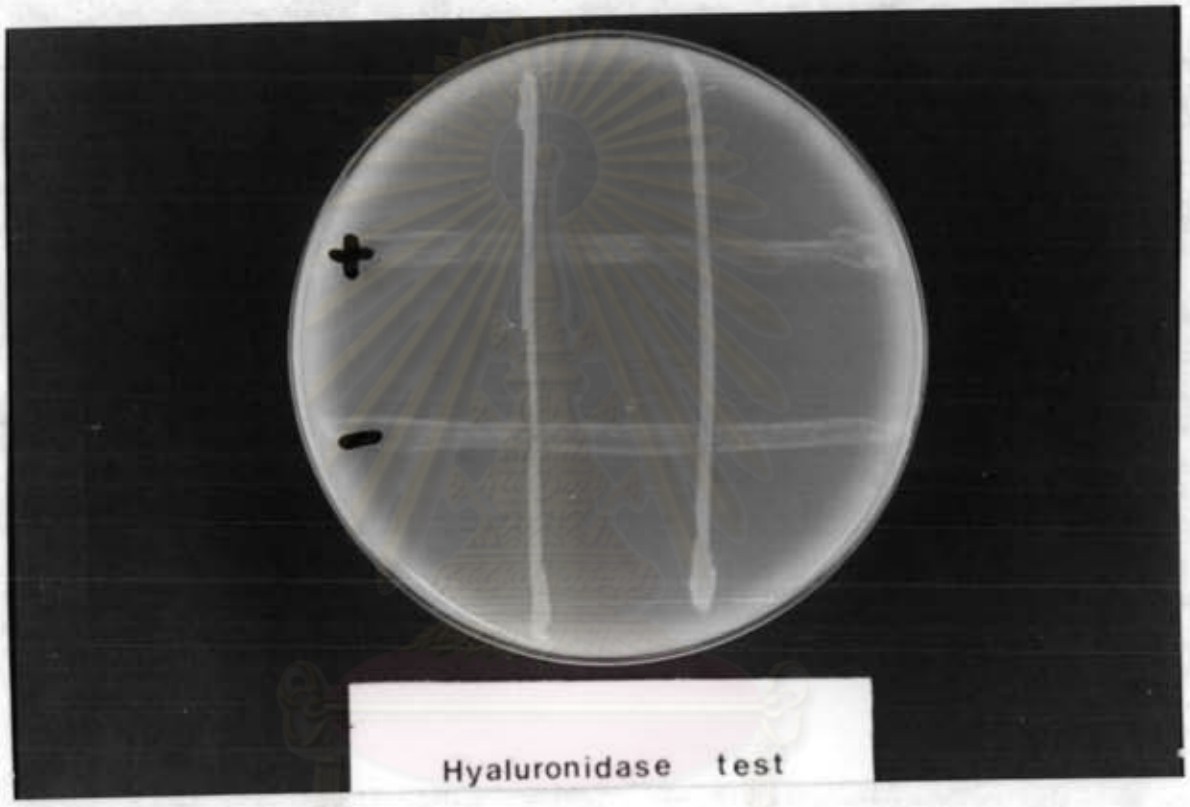


Figure 4 Hyaluronidase test

P. multocida type A shows the diminution of growth at the intersection of the two organisms (+) whereas other types show no reaction (-).


Figure 5 Acriflavine test

P. multocida type D shows a heavy flocculant precipitate (+) whereas other types show no reaction (-).

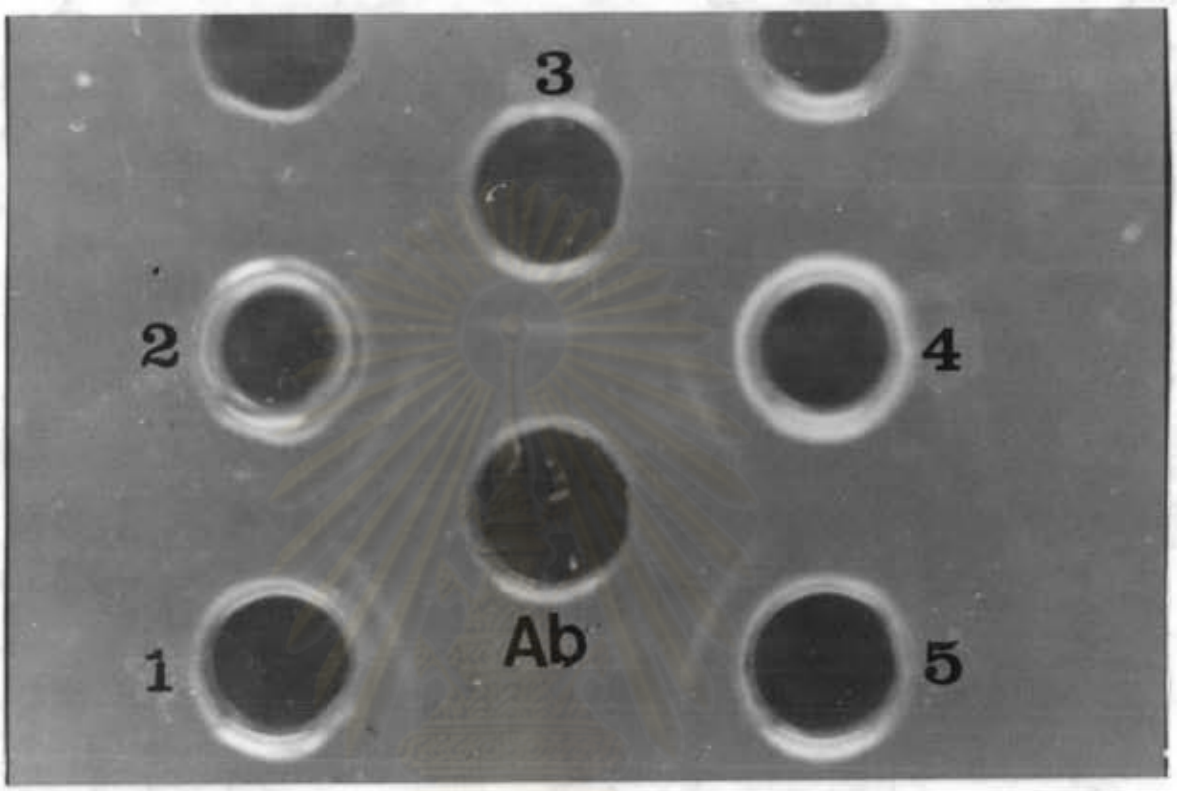


Figure 6 Somatic serotyping by GDPT.

Reference antiserum (Ab) is placed in the center well. Antigens (1,2,3,4,5) are placed in the outer wells.

It is noted that the precipitin lines are in between the antigen-antibody wells and nearer to the antigen wells.

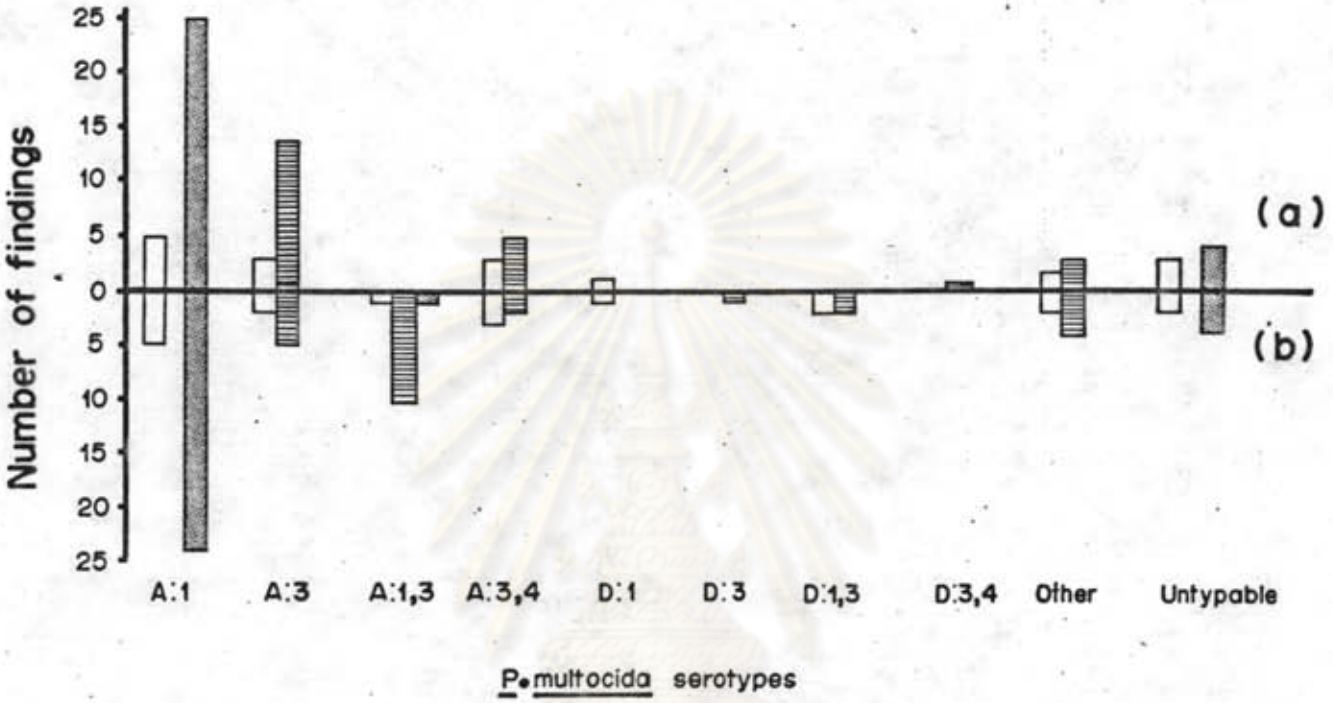


Figure 7 Distribution of P. multocida serotypes from human, pig and poultry strains in Thailand.
(a). By using reference typing antisera in GDPT.
(b). By using immune typing antisera in GDPT.

Human Pig Poultry

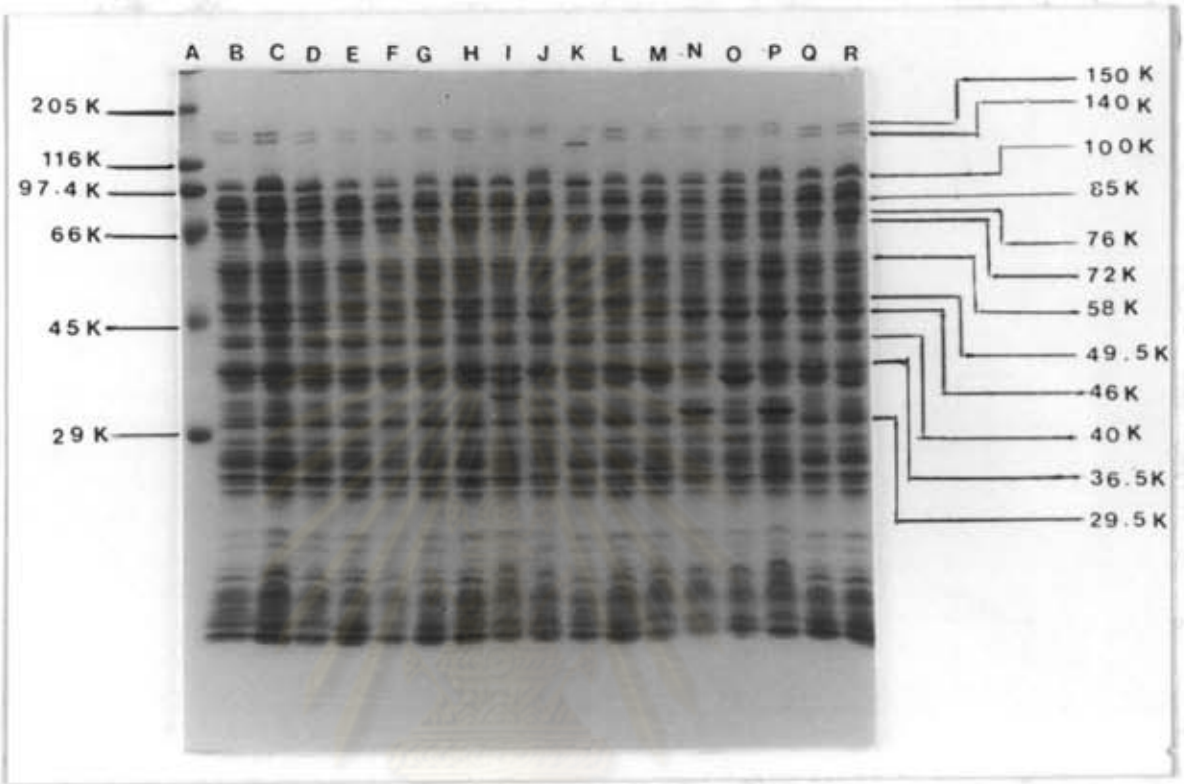


Figure 8 SDS-PAGE resulted in protein patterns from differnt strains of P. multocida

The lanes represent the following strains : A, molecular weight markers; B, type 1 (A:1); C, V-2 (A:1); D, TECH-177/31 (A:1); E, type 3 (A:3); F, PM 1020 (A:3);G, TECH-965/31(A:3); H, 8:A (A:1); I, CU (A:3,4); J, type12 (A:12); K, type B (B:2); L, 6:B (B:2); M, type E (E:2); N,type D (D:3,12), O,PM 1002 (D:3,4); P,TECH-2 (D:3,4,12); Q,AC-01 (A: 3,4); and R, PM 3011 (untypable : 3, 4). The numbers on the left are the MWs of the markers and on the right are the MWs of the unknowns.

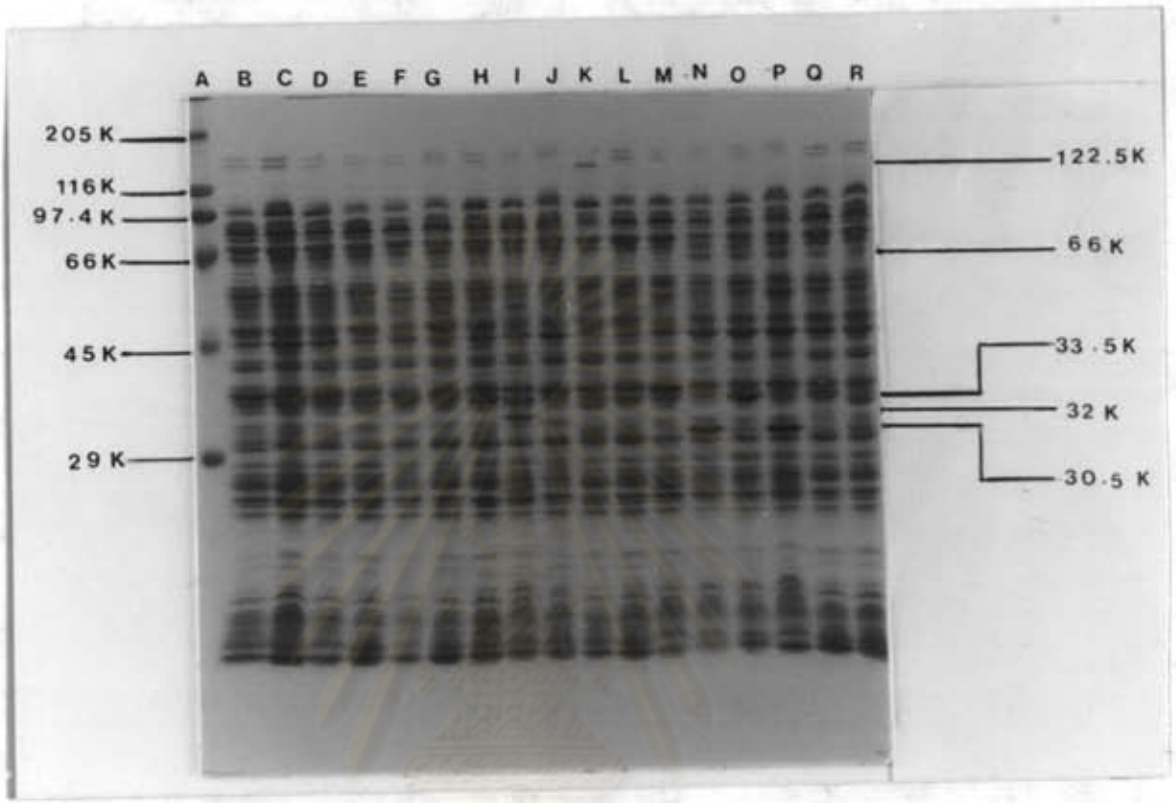


Figure 9 SDS-PAGE protein profiles of whole cell sonicated extracts showing specific dense bands of *P. multocida*

The lanes represent the following strains : A, molecular weight markers; B, type 1 (A:1); C, V-2 (A:1); D, TECH-177/31 (A:1); E, type 3 (A:3); F, PM 1020 (A:3); G,TECH-965/31 (A:3); H, 8:A (A:1); I,CU (A=3,4); J,type 12 (A:12); K, type B (B:2); L, 6:B (B:2); M, type E (E:2); N, type D (D:3,12); O, PM 1002 (D:3,4) : P,TECH-2 (D:3,4,12); Q,AC-01 (A:3,4); and R, PM 3011 (untypable : 3,4). The numbers on the left are the MWs of the markers and on the right are the MWs of the unknowns.