

Chapter II

Materials

1. Bacterial cultures and serotypes

Ninety-one strains of P. multocida (Table 5) used in the studies of bacterial characteristics and serotypes had been obtained from various sources. There were included four reference strains of Carter's capsular serotypes A,B,D, and E; fifteen reference strains of Heddleston's somatic serotypes 1-16, except type 4; three vaccine strains 8:A, CU and 6:B; seventeen strains were isolated from patients and fifty-two strains were isolated from animals. All the isolates were stabbed into the stock culture media and stored at room temperature. They were also stored in skim milk and frozen at -85 C.

2. Organism for production of typing antisera

The antigens for production of typing antisera were prepared from the lyophilized strains of P. multocida capsular serotypes A,B,D, and E and somatic serotype 1,3, 3x4, 12, and 13. They were streaked on TSB and incubated overnight at 37 C. The isolated colonies were subcultured on TSB and incubated at 37 C. After 18 hrs of incubation, the typical colonies of type A,B,D, and E were picked up and transferred to 5 ml of BHI broth and incubated for 6

Table 5 *P. multocida* strains used in the studies of bacterial characteristics and serotypes

Strains	Sources
1. Type A (P-1059)	Reference strain
2. Type B (M-1404)	-----"
3. Type D (P-3881)	-----"
4. Type E (P-1235)	-----"
5. Type 1 (X-73)	-----"
6. Type 2 (M-1404)	-----"
7. Type 3 (P-1059)	-----"
8. Type 5 (P-1702)	-----"
9. Type 6 (P-2192)	-----"
10. Type 7 (P-1997)	-----"
11. Type 8 (P-1581)	-----"
12. Type 9 (P-2095)	-----"
13. Type 10 (P-2100)	-----"
14. Type 11 (P-903)	-----"
15. Type 12 (P-1573)	-----"
16. Type 13 (P-1591)	-----"
17. Type 14 (P-2225)	-----"
18. Type 15 (P-2237)	-----"
19. Type 16 (P-2723)	-----"

Table 5 (Continued)

Strains	Sources
<u>Vaccine Strains</u>	
20. 8 : A	Fowl cholera vaccine
21. 6 : B	Hemorrhagic septicemia vaccine
22. CU	Fowl cholera vaccine
23. PATH-2	Human strain (blood)
24. PATH-3	Human strain (Pus)
25. RAJVIDHI-1	Human strain (sputum)
26. RAJVIDHI-2	----- " -----
27. TECH-1	----- " -----
28. TECH-2	----- " -----
29. TECH-176/31	----- " -----
30. TECH-177/31	----- " -----
31. TECH-6	----- " -----
32. TECH-965/31	----- " -----
33. TECH-7	----- " -----
34. RAMA-1	Human strain (pus)
35. RAMA-2	Human strain (sputum)
36. RAMA-414	Human strain (blood)
37. RAMA-271	Human strain (sputum)
38. AC-01	Human strain (blood)
39. AS-01	Human strain (blood)

Table 5 (Continued)

Strains	Sources
40. PM 1002	Pig strain
41. PM 1018	----- " -----
42. PM 1019	----- " -----
43. PM 1020	----- " -----
44. PM 1021	----- " -----
45. PM 1022	----- " -----
46. PM 1026	----- " -----
47. PM 1028	----- " -----
48. PM 1029	----- " -----
49. PM 1030	----- " -----
50. PM 1032	----- " -----
51. PM 1033	----- " -----
52. PM 1034	----- " -----
53. PM 1035	----- " -----
54. PM 1036	----- " -----
55. PM 1037	----- " -----
56. PM 1038	----- " -----
57. PM 1040	----- " -----
58. PM 1045	----- " -----
59. PM 1046	----- " -----
60. PM 1052	----- " -----
61. PM 1058	----- " -----
62. PM 1062	----- " -----



Table 5 (Continued)

Strains	Sources
63. PM 2002	Poultry strain
64. PM 2003	----- " -----
65. PM 2004	----- " -----
66. LUNG-1	----- " -----
67. LUNG-2	----- " -----
68. V-1	----- " -----
69. V-2	----- " -----
70. LIVER-15	----- " -----
71. LIVER-25	----- " -----
72. FC (3)	----- " -----
73. FC (4)	----- " -----
74. PM 3004	----- " -----
75. PM 3005	----- " -----
76. PM 3006	----- " -----
77. PM 3007	----- " -----
78. PM 3008	----- " -----
79. PM 3010	----- " -----
80. PM 3011	----- " -----
81. PM 3012	----- " -----
82. PM 3014	----- " -----
83. PM 3015	----- " -----
84. PM 3016	----- " -----
85. DUCK-egg	----- " -----

Table 5 (Continued)

Strains	Sources
86. DUCK-1	Poultry strain
87. DUCK-3	----- " -----
88. V-3	----- " -----
89. V-4	----- " -----
90. FC (1)	----- " -----
91. FC (2)	----- " -----

Strains No.1-19, Dr.K.R.Rhoades, NADC, Iowa, USA; No.20-21, Department of Livestock Development, Ministry of Agriculture and Cooperative; No.22, W.T.Derieux, Division of Livestock-Poultry Health College of Agriculture Sciences, Clemson University, USA; No.23-24, Mrs.Surang Dejsirilert, Division of Pathology, Department of Medical Sciences, Ministry of Public Health; No.25-26, Miss Nareekul Surapat, Rajavithi General Hospital; No.27-33, Asso. Prof. Churairatana Nilakul; Department of Clinical Microbiology, Faculty of Medical Technology, Mahidol University; No.34-37, Asso.Prof. Malai Vorachit, Ramathibodi Hospital; No.38-39; Dr.Anan Chongthaleong, Chulalongkorn Hospital; No.40-65 and 74-84, Dr.Tien Cheusiri, AHTSO; No.66-73 and 85-91, Dr.Kriengsak Saitanu, Division of Microbiology, Faculty of Veterinary Sciences, Chulalongkorn University.

hrs at 37 C. These young cultures were heavily seeded on DSA and incubated for 18 hrs at 37 C. The cultures were harvested and washed twice with 0.85% sodium chloride solution. The cells were diluted in 0.3% formalinized saline and adjusted to an absorbance of 1.00 in the spectrophotometer at wavelength 540 nm which was equivalent to 3.0×10^9 cells per milliliter (98). The antigens were stored in the refrigerator. The somatic antigens were prepared in the same way except that they had been subcultured on TSB at least six times in order to make them lose their capsules. The antigens were mixed with an equal volume of complete or incomplete Freund's adjuvants. These antigens were used for production of typing somatic antisera. They were also stored in the refrigerator until used.

3. Organisms for the analysis of protein patterns by SDS-PAGE

Seventeen strains of *P. multocida* were used in the analysis of protein patterns by SDS-PAGE. They were selected from human and animal strains of common serotypes mostly found in this study, i.e., serotypes A:1; A:3; A:3,4; and D:3. The vaccine strains and some reference strains were also selected. The seventeen strains were as follows:

Four human strains; TECH-177/31 (A:1), TECH-965/31(A:3), AC-01(A:3,4) and TECH-2 (D:3,4,12)

Two pig strains; PM 1020 (A:3) and PM 1002 (D:3,4)

Two poultry strains; V-2 (A:1) and PM 3011 (untypable : 3,4)

Three vaccine strains; 8:A (A:1), CU (A:3,4) and 6:B (B:2)

Six reference strains; type B(B:2), type D (D:3,12) type E(E:2), type 1 (A:1), type 3 (A:3), and type 12 (A:12)

These strains were stored in skimmilk and frozen at -85 C.

4. Staphylococcus aureus producing hyaluronidase strains

Two strains of S. aureus producing hyaluronidase enzyme were used in the hyaluronidase test. The ability of S. aureus to produce hyaluronidase enzyme was tested according to the method of Carter and Rundell (71). Both S. aureus strains gave the accurate results with the reference strains of P. multocida type A (positive) and type D (negative).



5. Animals

The immune antisera were produced in young adult rabbits weighing about 2 kgs. Two rabbits per each serotype were used. They were tested to be free from pasteurella antibodies by the IHA and GDPT tests. The methods were described in chapter III, 2.1.4 and 2.3.3.2 respectively. Any rabbits which produced pasteurella antibodies were excluded from this study.

6. Reference typing antisera

Reference typing antisera of Heddleston serotypes 1-16, except type 8 and 15, were received from National Animal Disease Control, Iowa, USA.

7. Media

Aesculin

Bacto agar

Bacto beef extract

Bacto gelatin

Bacto peptone

Bacto bile salt

Brain heart infusion broth (Difco)

Brilliant green

Bromcresol purple

Bromthymol blue
Cystein hydrochloride
Dextrose
D-arabinose
D-dulcitol
D-xylose
Lactose
Maltose
Mannitol
Mannose
Neutral red
Phenol red
Proteose peptone No.3
Soluble starch
Stock culture media
Sucrose
Tryptone
Trypticase soy agar
Urea
Yeast extract

8. Chemicals

8.1 For bacteriology

Absolute alcohol

Acetone

Acriflavine neutral (Sigma)
Amyl alcohol
Ammonium dihydrogen phosphate
Conc. HCl.
Crystal violet
Copper sulfate
Disodium phosphate
Ethyl alcohol
Ferric ammonium citrate
Ferric citrate
Hydrogen peroxide
Monoammonium phosphate
Monopotassium phosphate
Magnesium sulfate
Mercuric chloride
Methyl red
 α - Naphthol
p-Dimethylaminobenzaldehyde
Potassium nitrate
Sodium chloride
Sodium citrate
Sodium thiocyanate
Sodium thiosulfate
Sodium carbonate
Sodium hydroxide
Soft paraffin and liquid paraffin

Safranin

Serum

Tetra-methylphenylene-diamine dihydrochloride

8.2 For serotyping

Agarose (Sigma)

Ethylene diamine tetra-acetate (EDTA)

Complete and incomplete Freund's adjuvants

Human blood group O (citrate-phosphate-dextrose as anticoagulant)

Formaldehyde

Sodium azide

8.3 For SDS-PAGE

Acrylamide (Sigma)

Ammonium persulfate (Merck)

Bromophenol blue

Cello-seal compound (LKB)

Coomassie blue G 250 (Fluka)

glacial acetic acid (Merck)

glycerol

glycine (Sigma)

2-Mercaptoethanol

Methanol

N,N-methylenebis acrylamide (Sigma)

Phenol reagent

Sodium dodecyl sulfate (Merck)

Sodium potassium tartrate

TEMED (Sigma)

Tris (Sigma)

9. Glasswares

Beakers

Erlenmeyer flasks

Funnels

Glass slides

84 x 94 mm glass plates for GDPT

16x18 cm glass plates for SDS-PAGE

Measuring cylinders

Measuring pipettes

Pasteur pipettes

Petri dishes

Syringes

Test tubes

10. Instruments

Analytical balance (Mettler H35AR)

Autoclave

Automatic refrigerated centrifuge model 20 PR -

52D; Hitachi Koki Co., Ltd., Japan,

Deep freezer -85 C

Hot air oven

Incubators

LKB telescopic well punchers and template

LKB 2001-001 vertical electrophoresis unit

LKB 2219 multitemp II thermostatic circulator

LKB 2301 macrodrive 1 power supply

LKB 2003 slab gel dryer

Microscope

pH meter

Sonicate Handy Sonic Model UR-20P, Tomy, Seiko
Co., Ltd., Tokyo, Japan.

Spectrophotometer (Bausch & Lomb)

Table-top centrifuges (Labofuge, Hettich)

Vacuum pump

Waterbaths

11. Others

Automatic pipettes with tips

Disposable needles gauges 18G; 21G and 26G

Graph papers

U bottom microtiter plates

Whatman filter paper No.1