นิพนธ์ต้นฉบับ

Characteristics and Antimicrobial Susceptibilities of *Pasteurella (Moraxella) anatipestifer* Isolated from Ducks in Thailand

คุณสมบัติและความไวต่อยาต้านจุลชีพของเชื้อ *Pasteurella (Moraxella) anatipestifer* ที่แยกได้จากเป็ดในประเทศไทย

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บทคัดย่อ : **พรเพ็ญ พัฒนโสภณ, ทิพา ตันติเจริญยศ, และเทตซูโอะ โมโรซูมิ. 2534. คุณสมบัติและความไวต่อยาต้านจุลชีพของเชื้อ *Pasteurella (Moraxella) anatipestifer* ที่แยกได้จากเป็ดในประเทศไทย. วารสารวิจัยวิทยาศาสตร์การแพทย์ 5(1) : 55 - 61**

*ศึกษาเชื้อ **Pasteurella (Moraxella) anatipestifer** ที่ระบาดในเป็ด 20 ครั้ง ระหว่างเดือนตุลาคม 2531 ถึงเดือนกรกฎาคม 2532 เพื่อทราบถึงคุณสมบัติทางชีวเคมี ทางสรีรวิทยา ปฏิกิริยาต่อเอ็นซายม์ และความไวต่อยาต้านจุลชีพชนิดต่าง ๆ พบว่าคุณสมบัติทางชีวเคมีของเชื้อ 20 สเตรน ให้ผลบวกต่อน้ำตาลกลูโค้สและมอลโต้ส 18 สเตรน ให้ผลบวกต่อน้ำตาลฟรุ้คโต้ส 3 สเตรน และให้ผลบวกต่อน้ำตาลแมนโน้ส อะราบิโน้ส ทรีฮาโล้ส และซอบิทอล อย่างละ 1 สเตรน คุณสมบัติทางด้านสรีรวิทยาพบว่า ทุกสเตรนให้ผลบวกต่อ gelatinase และให้ผลลบต่อ urease สเตรนที่ให้ผลบวกต่อ indol test, Camp test, milk proteolysis และ coagulated serum proteolysis มี 2, 3, 5 และ 6 สเตรนตามลำดับ เชื้อทุกสเตรนมีความไวต่อยาเซฟาเร็คซิน เซฟาโลริดิน อีริโธรมัยซิน โอริแอนโดมัยซิน เตตร้าซัยคริน และ ไดเม็ทธิลคลอโรเตตร้าซัยคลิน และทุกสเตรนมีปฏิกิริยาต่อเอ็นซายม์ 7 ชนิด ได้แก่ phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, phosphatase acid และ phosphoamidase.*

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Abstract : **Pornpen Pathanasophon, Tipa Tanticharoenyos, and Tetsuo Morozumi. 1991. Characteristics and antimicrobial susceptibilities of *Pasteurella (Moraxella) anatipestifer* isolated from ducks in Thailand. Thai J Hlth Resch 5(1) : 55 - 61**

Twenty isolates of ***Pasteurella (Moraxella) anatipestifer*** *from twenty outbreaks of infectious serositis in ducks during October 1988 to July 1989 were examined on their biochemical, physiological characteristics, enzymatic reactions and antimicrobial susceptibilities. Eighteen isolates out of 20 isolates were glucose and maltose positive, 3 isolates were fructose positive and one of each was mannose, arabinose, trehalose and sorbitol positive from biochemical tests. Physiological characteristics of all isolates were gelatinase positive and urease negative, while 2/20, 3/20, 5/20 and 6/20 were positive to indole test, Camp test, milk proteolysis and coagulated serum proteolysis, respectively. All isolates were highly susceptible to cephalexin, cephaloridine, erythromycin, oleandomycin, tetracycline and dimethylchlorotetracycline. From APIZYM reactions, seven enzymes (phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, phosphatase acid and phosphoamidase) were detected from all isolates.*

Key words : *Pasteurella (Moraxella) anatipestifer; Characteristics, biochemical, physiological; Antimicrobial susceptibilities; Enzymatic reactions.*

INTRODUCTION

Pasteurella (Moraxella) anatipestifer causes a serious septicemic disease in avian species especially in ducklings. Since its first recognition in 1932 (Hendrickson and Hilbert, 1932), the disease has been reported in England (Asplin, 1955), Canada (Taylor, 1955), Australia (Rosenfeld, 1972), The Soviet Union (Avrorov *et al.*, 1964), The Natherland (Donder-Voet, 1962), Japan (Baba *et al.*, 1987), Bangladesh (Mustafa *et al.*, 1985) and Thailand (Mahitanan *et al.*, 1982). The organism's characteristics are described as gram negative, nonfermentative, nonmotile, nonspore-forming, nonhemolytic, negative with urease, indole and citrate tests. It can not grow in MacConkey agar or reduce nitrate (Smith, 1974). Taxonomy is still unsatisfactory, when low but significant degrees of DNA binding between selected strains of so-called *M. anatipestifer, Cytophaga marinoflava, Flavobacterium meningosepticum, F. odoratum* and *F. pectinovorum* were observed. On the basis of these findings the so-called *M. anatipestifer* is proposed to be Flavobacterium/Cytophaga group (family Cytophagaceae) (Piechulla *et al.*, 1986).

In this paper, the results of physiological, biochemical characteristics, antimicrobial sensitivity tests and enzymatic reactions of the bacterial isolates are reported. We hope that the given information will serve as a guideline for future references.

MATERIALS AND METHODS

Bacterial strains: *Pasteurella (Moraxella) anatipestifer (P. ana.)* was collected from 20 outbreaks in ducks during October 1988 to July 1989. The pure cultures were maintained at $-70°C$ in mist desciccans medium and tryptic soy agar (Difco) supplemented with 5% defibrinated sheep blood for future tests. Seven *P. ana.* reference strains which were kindly provided by Dr. KR Rhoades representing serotypes 1 to 7 (P – 1050, P – 1645, P – 1667, P – 1785, P – 1641, P – 2123 and P – 2361 respectively) were used for comparison.

Biochemical test: Fifteen carbohydrates (arabinose, dulcitol, fructose, glucose, inositol, lactose, mannitol, maltose, mannose, raffinose, rhamnose, sorbitol, sucrose, trehalose and xylose) 1% of each in CTA base (CTA medium "Nissui") with 5% horse serum v/v were each inoculated with the isolates. The tested cultures were incubated at 37°C and observed daily for 14 days. Growth was examined on MacConkey agar (Difco) and Simmons citrate agar (Difco) daily for three days. Indol and urease tests were carried out as

described by Kilian and Frederiksen (1981). Gelatin liquefaction and Camp test were done according to the methods of Kohn (1953) and Christie *et al.* (1944) respectively. Nitrate reduction test was done following the method of Daubner (1962) using tryptic soy broth base with 0.3% yeast extract as enrich medium. Other physiological tests included catalase, oxidase, coagulase tests. Coagulated serum proteolysis was performed in heat-treated tryptic soy agar (Difco) with 75% horse serum and 0.25% glucose. Milk proteolysis was also tested in tryptic soy agar (Difco) with 3% skim milk. After inoculation and incubation at 37°C the results were recorded daily over one week. Changing of coagulated serum to liquid state or clear zone around the inoculated spot was regarded as proteolysis positive.

Enzymatic activities were determined with the commercial APIZYM galleries (API System-La Balmeles Grottes 38390 Montalieu Vercieu, France). *P. ana.* isolates were cultured on tryptic soy agar (Difco) supplemented with 5% defibrinated sheep blood for 24 hr at 37°C in 5% CO_2 atmosphere, suspended in steriled 0.85% saline solution to McFarland No.5 turbidity. The suspensions were used for the determination of the enzymatic activities. The methods used were as described by the manufacturers.

Antimicrobial sensitivity tests: Disc method of antimicrobial sensitivity test was performed on Mueller-Hinton agar (MIC Sensitive test broth "Nissui" + 1.5% agar) supplemented with thiamine 5 ug/ml. The method was done as described by Bauer *et al.* (1966) with a slight modification. Twenty three antimicrobial drugs were tested. The tested plates were incubated at 37°C for 24 hr in 5% CO_2 incubator before inhibitory zones were measured.

RESULTS

Table 1 presents the results of physiological and biochemical properties of the isolated organisms. None of the twenty cultures grew on MacConkey agar, Simmons citrate agar and did not coagulate rabbit plasma or produced urease. Nitrates were not reduced to nitrites. They did not ferment the following substrates: dulcitol, inositol, lactose, mannitol, raffinose, rhamnose, sucrose and xylose. Most of the isolates (18/20) fermented glucose and maltose while all of them were positive to catalase, oxidase and gelatin liquefaction. Although 3/20 isolates gave strong positive reaction in Camp test, more than half of them (11/20) were weakly positive. Proteolytic enzyme could be produced in coagulated serum and skim milk from some isolates (6/20 and 5/20 respectively). Only two isolates were positive to indole test.

APIZYM reactions: The local isolated *P. ana.* performed nearly identical to the reference strains (Table 2). Seven enzymes (phosphatase alkaline, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, phosphatase acid and phosphomidase) were detected from all 20 isolates and 7 reference strains while negative to the following enzymes: lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. Only five enzymes (cystinearylamidase, trypsin, chymotrypsin, α-glucosidase and N-acetyl-β-glucosiminidase) varied among the strains tested.

Antimicrobial sensitivity test: All strains were sensitive to cephalexin, cephaloridine, erythromycin, oleandomycin, tetracycline and dimethylchlorotetracycline. Sensitivity and resistant patterns of the isolates and the standard strains were more or less similar (Table 3).

Table 1 *Culture and biochemical characteristics of the twenty isolates from ducks in comparison to the reference strains (Rhoades).*

Test	Local isolates	Rhoades
Growth on Simmons citrate	0	0
Growth on MacConkey	0	0
Catalase test	20	7
Oxidase test	20	7
Gelatin liquefaction	20	6
Coagulase test	0	0
Coagulated serum proteolysis	6	4
Milk proteolysis	5	4
Nitrate reduction	0	0
Indol production	2	1
Urease production	0	3
Camp test	3*	6
Production of acid from carbohydrates		
Arabinose	1	0
Dulcitol	0	0
Fructose	3	0
Glucose	18	3
Inositol	0	0
Lactose	0	0
Mannitol	0	0
Maltose	18	3
Mannose	1	1
Raffinose	0	0
Rhamnose	0	0
Sorbitol	1	0
Sucrose	0	0
Trehalose	1	0
Xylose	0	0
No. tested (N)	20	7

*11 isolates were weakly positive

Table 2 *APIZYM reactions of twenty isolates from ducks and reference strains*

Strain	n	Number of strains positive																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Thai	20	0	20	20	20	0	20	20	19	13	19	20	20	0	0	0	18	0	2	0	0
Rhoades	7	0	7	7	7	0	7	7	7	2	5	7	7	0	0	0	7	0	0	0	0
	27	0	27	27	27	0	27	27	26	15	24	27	27	0	0	0	25	0	2	0	0

API System - La Balme Les Grottes 38390 Montalieu (France)

1: negative control, 2: phosphatase alkaline, 3: esterase (C4), 4: esterase lipase (C8), 5: lipase (C14), 6: leucine arylamidase, 7: valine arylamidase, 8: cystine arylamidase, 9: trypsin, 10: chymotrypsin, 11: phosphatase acid, 12: phosphoamidase, 13: α-galactosidase, 14: β-galactosidase, 15: β-glucuronidase, 16: α-glucosidase, 17: β-glucosidase, 18: N-acetyl-β-glucosaminidase, 19: α-mannosidase, 20: α-fucosidase

Table 3 *Antimicrobial sensitivity tests of twenty isolates from ducks and reference strains*

Strain	Number tested	No. of strain sensitive to antimicrobial disc																						
		Aminobenzylpenicillin	Bacitracin	Cephalexin	Cephaloridine	Chloramphenical	Erythromycin	Fradiomycin	Gentamycin	Kanamycin	Lincomycin	Nalidixic acid	Neomycin	Oleandomycin	Oxytetracycline	Penicillin	Polymyxin B	Spiramycin	Streptomycin	Sulfamonomethoxin	Sulfadimethoxin	Sulfamethoxazole & Trimethoprim	Tetracycline	Dimethylchlorotetracycline
Thai	20	19	3	20	20	17	20	–	–	–	16	15	18	20	15	18	–	15	1	1	–	18	20	20
Rhoades	7	7	1	7	7	7	7	–	–	–	7	5	6	7	7	6	–	7	1	–	1	7	7	7

DISCUSSION

The physiological and biochemical characteristics of 20 field isolates were typical for *P. ana.* The variations among the strains were proteolytic enzyme production, gelatin liquefaction, indole or urease production, and Camp test. Urease produced-strain was not available in this study. Most of the cultures were positive with Camp test but different in degree of the reactions. Only 6 field isolates and one reference strain yielded negative results. Strains of *P. ana.* which yielded strong Camp positive were those which gave coagulated serum proteolysis positive. *Staphylococcus aureus* strain used seems to influence the degree of the Camp reaction. Most of the isolates fermented both glucose and maltose readily whereas those reported by Bangun *et al.* (1981) did with a low frequency. The different outcome may be depended on the different techniques used.

Antimicrobial sensitivity test: *P. ana.* was highly susceptible to the antimicrobial drugs normally used in the therapy of gram positive bacterial infection such as penicillins, cephalosporins, macrolide group, etc. It also yielded resistance to antimicrobial agents which were used in treating gram negative bacterial infections, such as aminoglycosides (excepted neomycin) and polypeptides. It was also resisted to sulfonamides.

From our experience API system was the rapid, reliable and most convenient for identifying *P. ana.* when compared to the conventional methods. However the cost of the test need to be condidered.

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