Aeromonas liquefaciens Isolated from a Skin Rot Disease of Formosa Snakehead, Channa maculata (LACÉPÈDE), in Taiwan

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鱧魚腐膚病病原菌 *Aeromonas liquefaciens* 之分離

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1972年11月至1973年3月間，每於寒流來襲水溫驟變時，本省北部所養之鱧魚常發生疾病且有大量死亡之現象。病魚之體表常呈灰黑色，在灰黑色之病變部之周圍常呈淤血現象，解剖內臟觀察時腎臟與腸有或輕或重之淤血或發炎現象，其組織切片可見細菌之存在，且微血管內充滿紅血球及破裂出血。此病因其皮膚所呈之症狀而定名爲腐膚病 (skin rot)。可由病變部位及內臟分離出一種 Gram stain −, Cytochrome oxidase +, Glucose fermentative, 0/129 (Vibrio static agent) 一之端單鞭毛之短桿菌，依 Bergey's Manual 7th ed. 鑑定爲 *Aeromonas liquefaciens*。此菌以肌肉注射方式接種於泥鰍時，發現有很大的致死力，同時在接種部位可引起與鱧魚相同之病變，內臟亦有淤血或發炎現象。做藥片感受性試驗，發現其對 Tetracycline, Dihydrostreptomycin, Kanamycin 及 Colistine 有極高之感受性。

Introduction

A disease of Formosa snakehead, characterized externally by black rot areas and necrotic lesions of the skin on part of the body, has been occurred at Tao-Yuan in northern Taiwan from November 1972 to March 1973. As the symptoms of a black red, slime, necrotic areas extending through the skin and in some instances deepening into the muscle, the disease has been described as "skin rot" by the authors. The visceral organs, in gross appearence, were unchanged or in some instances the kidney appeared in color and softer in texture than the normal, and an inflammation of the intestine was observed in almost all the diseased fish. Generally fish die before the lesions become more extensive and destructive.

Some snakeheads from cultureing ponds showed the more severe superficial lesions and were subjected to a detailed bacteriological examination in an attempt to determine whether or not the disease was of bacterial origin, and if so, to isolate the causal agent.

Experimental Methods and Results

Description of isolates: A small pieces of tissue from skin lesions and viscera of each fish were cut and introduced into nutrient broth. The cultures isolated were examined and found

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morphologically and physiologically identical. The organism from 18 to 22 hrs nutrient broth cultures was gram-negative, short rod ($1-2\times0.65\ \mu$). It was actively motile by a single polar flagellum, non-sporing and non-capsulate. The colonies on nutrient agar plate were circular with an entire edge, convex, moist and pale brown or grayish white in color. No pigment was produced. On rabbit blood agar the colonies were hemolytic (β-hemolysis). Biochemical characteristics of the organism are given in Table 1.

All the media used for various purpose in this study contained 0.5% NaCl and cultures were all incubated at 28°C.

Table 1. Biochemical characteristics of *Aeromonas liquefaciens* isolated from diseased Formosa snakehead.

Test	Result
Hugh & Leifson test	+
Cytochrome oxidase	+
Catalase	+
H_2S production	−
Vibriostatic agent. (0/129)	−
Indole production	+
Methyl-red test	±
Voges-Proskauer test	+
Liquefaction of Gelatin (18°C)	+
2, 3-Butanediol production	+
Starch hydrolysis	+
Utilization of citrate	+
Utilization of malonate	±
Nitrate reduction	+
Hemolysis (rabbit blood)	β
Litmus milk	+
Acid from:	
Glucose	+
Lactose	−
L(+)-Arabinose	+
D-Cellobiose	+
Dextrin	+
Fructose	+
Galactose	+
Glycerol	+
Inositol	−
Maltose	+
D-Mannose	+
Raffinose	−
L-Rhamnose	−
Sucrose	+
L-Sorbose	−
Starch	−
Xylose	−

Table 2. Effects of *Aeromonas liquefaciens* isolated from diseased young Formosa snakehead on loach

Strains inoculated	Source of strain	No. of loaches	Time to die (hrs)						Temp. of water
			12	24	36	48	60	72	
TS-1	skin lession	3			1	2			19°C
TG-1	gill	2				1		1	19°C
TG-3	gill	2						2	19°C
TS-2	skin lesoin	2			1			1	16.3-17.6°C
TG-4	gill	1				1			16.3-17.6°C
TS-3	skin lesion	2			1	1			17.6°C
TI-1	intestine	2		2					16.5°C
TS-4	skin lesion	2			1	1			16.2-17.8°C
TS-5	skin lesion	2			1	1			16.2-17.8°C
TL-1	liver	2			2				16.3-17.6°C
TL-2	liver	2		1	1				16.2-17.8°C

Drug sensitivity in vitro of the strains were tested using Eiken Sensitivity discs. They were all highly sensitive to Tetracycline, Dihydrostreptomycin, Kanamycin and Colistine but resistant to Penicillin, Erythromycin, Oleaudomycin and Leucomycin.

Pathogenicity for loach: In preparation of inoculation colonies were scraped from the surface of nutrient agar plates after 18-22 hrs incubation at 28°C and suspened in sterile saline (0.8% NaCl) in various concentrations. Two or three loaches were inoculated by intramuscular injection with a dose of 2 mg of bacteria per 100 g fish body weight. The site of inoculation became hemorrhagic, gradually swelled up and finally became necrotic and many hemorrhagic spots appeared in various parts of the body surface. Usually the loach died before the tissue of the inoculation site became markedly necrotic and other conspicuous external symptoms appeared. Almost all of the fish died within 3 days after injection (Table 2).

It was found in examination of moribund specimens that the intestine became inflamed and the spleens and kidneys were often affected, too.

Discussion

The organism isolated from diseased young Formosa snakehead is one kind of gram-negative, short rod bacteria. It is motile by means of a single polar flagellum. Test of glucose fermentation results in producing acid and gas, and the test of cytochrome oxidase is postive. It is not sensitive to the vibriostatic agent (0/129). These morphological and biochemical characteristics suggest that the bacterium belongs to the *Aeromonas*.

Although the symptoms of the disease are similar to those of "tail rot" of silver carp[1,2] and "red sore" of trout[3], which are both known to be caused by *Aeromonas*, the marked symptoms are formation of eroded, sometimes blood-colored areas and extensive necrotic areas on the surface of the body. Therefore, it is described as "skin rot" by the authors.

Bergey's Manual of Determinative Bacterilolgy[4] (7th. ed. 1957) lists the three motile sp., *Aeromonas liquefaciens*, *A. punctata* and *A. hydrophila*. However opinion is divided among investigators regarding the taxonomy of the motile species of genus *Aeromonas*. Ewing[5], Eddy[6~8],

Snieszko[9,10], Schubert[11~13] and Kou[14,15] expressed the opinion that bacteria causing most of the outbreaks of infectious dropsy and hemorrhagic septicemia of warm water fish, red mouth of trout and ayu, red sore disease of pike, red fin disease of eel, and red leg disease of frogs should be classsified as type species. Therefore *A. liquefaciens* will be here adopted.

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Referances

1. Conroy, D. A. (1963). Studies on the application of Kanamycin to the control and treatment of some bacterial diseases of fish. J. App. Bact., **26,** 182–192.
2. Conroy, D. A. (1964). Tail rot in fish. Nature., **201,** 732–733.
3. Griffin, P. J. (1954). The nature of bacteria pathogenic to fish. Trans. Amer. Fish. Soc., **83,** 241–253.
4. Breed, R. S., E. G. M. Murray and N. R. Smith (1957). Bergey's Manual of Determination Bacteriology 7th ed. William and Wilkins Company.
5. Ewing, W. H., R. Hugh and J. G. Johson (1961). Studies on the Aeromonas group. U. S. Dept. of Health, Education and Welfare, Communicable Diseases Center, Atlanta, Gergia, U. S. A. pp. 37
6. Eddy, B. P. (1960). Cephalotrichous, fermentative gram-negative bacteria: the genus Aeromonas. J. Appl. Bact., **23,** 216–249.
7. Eddy, B. P. (1962). Further studies on Aeromonas-I. Additional strains and supplementary biochemical test. J. Appl. Bact., **25,** 137–146.
8. Eddy, B. P. and K. P. Carpenter (1964). Further studies on Aeromonas-II. Taxonomy of Aeromonas and C_{27} strains. J. Appl. Bact., **27,** 96–109.
9. Snieszko, S. F. and G. L. Bullock (1962). Fresh water fishes diseases caused by bacteria belonging to the genera Aeromonas and Pseudomonas. U. S. Fish and Wildlife Service. Fishery Leaflet 459 (Revised), pp. 7.
10. Snieszko, F. (1954). Research on fish diseases: a review of progress during the past 10 years. Trans. Amer. Fish. Soc., **83,** 219–349.
11. Schubert, R. H. W. (1967). The taxonomy and nomenclature of the Genus Aeromonas Kluyver and van Niel 1936-1. Suggestions on the taxonomy and nomenclature of the aerogenic Aeromonas species. Intl. J. Syst. Bact. **17,** 23–37.
12. Schubert, R. H. W. (1967). The taxonomy and nomenclature of the genus Aeromonas Kluyver and van Niel 1963-II. Suggestions ou the taxonomy and nomenclature of the anaerogenic aeromonads. Ibid., **3,** 273–279.
13. Schubert, R. H. W. (1968). The taxonomy and nomenclature of the genus Aeromonas Kluyver and van Niel 1936-III. Suggestions on the definition of the genus Aermonas Klyver and van Niel 1936. Ibid., **18,** 1–7.
14. Kou, G. H. (1972). Studies on the Fish Pathogen, Aeromonas liquefaciens-I. Classification and Serological type. (in Chinese). Aquiculture. **2(1)** 22–33.
15. Kou, G. H. (1972). Studies on the occurrence and biochemical properties of virulent and avirulent strains of freshwater fish pathogen, *Aeromonas liquefaciens* (in Chinese). This Bull. **1(2)** 8–13.