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A Serological Study on *Pseudomonas anguilliseptica* Isolated from Diseased Eels in Taiwan*1

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Four strains of *Pseudomonas anguilliseptica* isolated from pond-cultured Japanese eels, *Anguilla japonica*, in Taiwan were compared with Japanese and Scottish representative strains. From the results of agglutination and precipitation with rabbit antisera, and pathogenicity tests for Japanese eels, the three of Taiwanese isolates proved identical to Japanese K antigen-possessing type (K^+ type) of *P. anguilliseptica*. The other Taiwanese strain seemed to be intermediate type between K^+ and K^- types.

Red spot disease of pond-cultured eels, *Anguilla japonica* and *A. anguilla*, has occurred in Japan (WAKABAYASHI and EGUSA, 1972; JO *et al.*, 1975), Taiwan (KUO and KOU, 1978), and Scotland (NAKAI and MUROGA, 1982; STEWART *et al.*, 1983) so far. The previous works (NAKAI *et al.*, 1981, 1982) on serological properties of the causative agent of the disease, *Pseudomonas anguilliseptica*, established that the strains isolated from diseased Japanese and European eels in Japan shared a heat-stable antigen (O antigen), but they were divided into two serotypes on the basis of the presence of a heat-labile K antigen, which behaved as agglutination-inhibitor against the rabbit O antiserum. Of the two serotypes, only the K^+ type was confirmed virulent by injection experiments in eels. The strains of *P. anguilliseptica* isolated from European eels in Scotland were found identical to Japanese K antigen-lacking type (K^- type) (NAKAI and MUROGA, 1982).

In the present study, the strains of *P. anguilliseptica* isolated from Japanese eels in Taiwan were compared serologically with the Japanese and Scottish strains, and found identical to K^+ type of Japanese strains.

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Materials and Methods

Four strains of *P. anguilliseptica* (810313-3K, 810313-9K, 810314-4K1, and 810314-6L) isolated from cultured Japanese eels, *A. japonica*, at Lukang in Taiwan in 1981 were used in this study. Three Japanese strains, ET-7601 (K^+ type), NCMB 1949 (K^+ type), and ET-2 (K^- type), and one Scottish strain RiCl (K^- type) were employed as reference. Each strain was cultured on nutrient agar at 20°C for 2 days.

Rabbit antisera were prepared with formalinized cells (A antigen) of 810313-9K, 810314-4K1, ET-7601, and NCMB 1949, and with 121°C-30 min heated cells (B antigen) of ET-7601 by the same method as described before (NAKAI *et al.*, 1981). The agglutinating titers of those antisera were measured against each A and B antigen using microtiter plates, and the antisera were absorbed with antigens to confirm the antigenic identity among the strains. For precipitin analysis, the antigens prepared from heated extracts (at 100°C or 121°C for 30 min) of sonicated cells were reacted with antisera on veronal-buffered 0.8% agarose gel by Ouchterlony double diffusion method.

Pathogenicity to Japanese eels was tested by intramuscular injection of each culture with a dose of 5×10^9 CFU (colony forming unit) per 100 g fish weight. The injected fish were kept at 20°C

and observed for 3 weeks. The dead fish were examined bacteriologically.

Results and Discussion

Prior to serological tests, biochemical properties of Taiwanese strains were confirmed consistent with those of Japanese strains.

Agglutinin titers of two rabbit anti-ET-7601 sera against A and B antigens of 8 strains are shown in Table 1. It was indicated that the all strains shared heat-stable antigen(s) (O antigen) by the results that the all B antigens agglutinated with both antisera at titers of 64–128. While all A antigens agglutinated with anti-ET-7601(A) serum at titers ranging from 256 to 2048, A antigens of three Taiwanese strains (810314-4K1, 810314-6L, and 810313-3K) as well as Japanese K^+ strains (ET-7601 and NCMB 1949) showed the inagglutinability (titer: <4 or 4) to anti-ET-7601(B) serum. This means that these 3 Taiwanese strains possess K antigen.

Table 1. Agglutinating reactions of *P. anguilliseptica* strains against rabbit anti-ET-7601 sera

Agglutinogen	Agglutinin titers of	
	Anti-ET-7601 (A) serum	Anti-ET-7601 (B) serum
ET-7601 (A)	512	<4
NCMB 1949 (A)	256	<4
810314-4K1 (A)	512	<4
810314-6L (A)	512	<4
810313-3K (A)	1024	4
810313-9K (A)	2048	64
ET-2 (A)	1024	256
RiCl (A)	1024	256
ET-7601 (B)	128	128
NCMB 1949 (B)	128	128
810314-4K1 (B)	128	128
810314-6L (B)	128	128
810313-3K (B)	64	128
810313-9K (B)	64	128
ET-2 (B)	128	128
RiCl (B)	128	128

(A): formalinized cells
(B): 121°C–30 min heated cells

The results of absorption tests using anti-ET-7601(A) serum are shown in Table 2. Agglutinating antibodies were completely absorbed by each A antigen of ET-7601, 810314-4K1, and 810313-9K. On the contrary, absorption with K^- strains (ET-2 and RiCl) retained the agglutinins for the strains except 810313-9K. Similar results occurred when anti-NCMB 1949(A) and anti-810314-4K1(A) sera were tested. Thus, three Taiwanese strains (810313-3K, 810314-4K1, and 810314-6L) and Japanese K^+ strains were indistinguishable in the antigenicity. The other Taiwanese strain (810313-9K) seemed to be an intermediate type, because this strain showed lesser O-agglutination inhibition (Table 1) and did not agglutinate with K specific antiserum (Table 2), but absorbed K antibody in the anti-ET-7601 (A) serum (Table 2).

Figures 1 and 2 show the results of precipitation tests in agarose gel. In Fig. 1, in order to test the specificity of K antigen, rabbit anti-ET-7601 (A), anti-NCMB 1949(A), anti-810313-9K(A), and anti-810314-4K1(A) sera, which were absorbed with A antigen of K^- strain (ET-2), were poured in each trough, and 100°C-30 min heated antigens of 8 strains were added in wells. Five strains (ET-7601, NCMB 1949, 810314-4K1, 810314-6L, and 810313-3K) developed one continuous pre-

Table 2. Antigenic analysis of *P. anguilliseptica* strains by absorbed anti-ET-7601 (A) serum

Agglutinogen	Agglutinin titers of anti-ET-7601 (A) serum absorbed with				
	ET-7601 (A)	810314-4K1 (A)	810313-9K (A)	ET-2 (A)	RiCl (A)
ET-7601 (A)	0	0	0	256	512
NCMB 1949 (A)	0	0	0	128	128
810314-4K1 (A)	0	0	0	128	512
810314-6L (A)	0	0	0	256	256
810313-3K (A)	0	0	0	256	256
810313-9K (A)	0	0	0	0	0

(A): formalinized cells

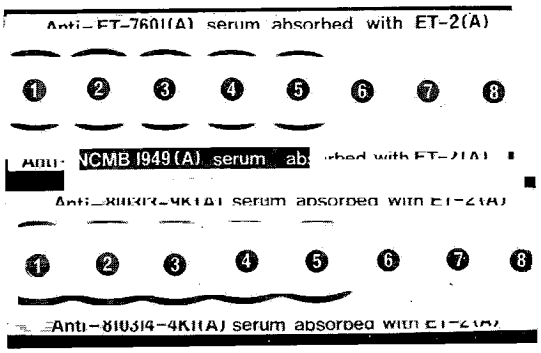

Fig. 1. Immunodiffusion analysis of *P. anguilliseptica* K antigen. Each antiserum in trough was absorbed with ET-2 (A) antigen, and each 100°C-30 min heated extract of sonicated cells was added in wells: 1 (ET-7601), 2 (NCMB 1949), 3 (810314-4K1), 4 (810314-6L), 5 (810313-3K), 6 (ET-2), 7 (RiCl), 8 (810313-9K).

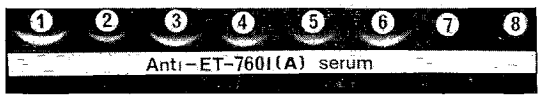

Fig. 2. Immunodiffusion analysis of *P. anguilliseptica* O antigen. Each 121°C–30 min heated extract of sonicated cells in wells (see Fig. 1) was reacted with anti-ET-7601 (A) serum.

Table 3. Pathogenicity of *P. anguilliseptica* strains to Japanese eels by intramuscular injection

Strain	Number of fish died/tested	Time to death
ET-7601	3/3	5–6 days
NCMB 1949	3/3	5–7
810314-4K1	3/3	6–7
810314-6L	3/3	5–6
810313-3K	3/3	13–17
810313-9K	0/3	—
ET-2	0/3	—
RiCl	0/3	—

Fish were injected with 5×10^6 CFU/100 g fish weight at 20°C of water temperature.

cipitin line against any of the tested sera, but 810313-9K formed a line weakly. These results indicate that K antigen between Japanese and 3 Taiwanese strains are identical. In Fig. 2, 121°C-30 min heated antigens of 8 strains were reacted with unabsorbed anti-ET-7601(A) serum to compare the homogeneity of O angiten. One continuous line developed clearly in 6 strains including 3 Taiwanese ones, but weakly in strains RiCl and 810313-9K. Thus, at least, 3 Taiwanese strains were identical with Japanese ones in serological points. This seems reasonable because mutual transportation of glass and marketable sized eels occurred frequently between Taiwan and Japan.

The results of pathogenicity tests to Japanese eels are shown in Table 3. Three of Taiwanese strains, 810314-4K1, 810314-6L, and 810313-3K, were pathogenic to eels as well as Japanese K^+ strains, ET-7601 and NCMB 1949, but the others were not. Therefore, the correlation between the presence of K antigen and the pathogenicity was also demonstrated in Taiwanese strains.

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