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Serotyping of *Photobacterium damsela* subsp. *piscicida* isolates during the epizootic of pasteurellosis using yellowtail antiserum

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INTRODUCTION

Photobacterium damsela subsp. *piscicida* (=*Pasteurella piscicida*) (Gauthier *et al.*[1]) is the causative bacterium of "pseudotuberculosis" of the young cultured yellowtail, *Seriola quinqueradiata*. Various investigations have been made on the development of vaccines against this disease (Kawakami *et al.* [2]) although a commercially available vaccine has not yet been developed in Japan. Recently an antigenic type analysis of the pathogen suggested the existence of three serotypes among the isolates (Kawahara *et al.* [3]). The antigenic differences must affect the vaccine development. In this study, during the epizootic of the disease, antigenic differences of the isolates were determined using the yellowtail antiserum.

MATERIALS AND METHODS

During the epizootic of pasteurellosis in six aquaculture areas, Usuki, Kamiura, Saeki, Tsurumi, Yonouzu and Kamae in Oita Prefecture, Japan, in May to October, 1998, twenty-five strains of *Ph. damsela piscicida* were isolated from diseased yellowtail cultured in a net-pen. The isolates were cultured in brain-heart infusion broth (Difco) containing 2 % sodium chloride at 25℃ for 48 h with shaking. Formalin-killed cells (FKC) were prepared by adding 0.5% formalin to the culture and incubating for 24 h at 4 ℃. Three yellowtail antisera against the FKC of typical serotype strains (types A, B and C) [3] were raised as mentioned below. FKC was adjusted to 2 mg/m*l* in 10 mM phosphate buffered saline (PBS) at pH 7.0, and emulsified with Freund's incomplete adjuvant (Difco) at a volume ratio of 1:1. One hundred microliters of the emulsion was intraperitoneally injected into the hatchery-produced yellowtail weighing about 25g. At 2 and 3 weeks after the first injection, 100 μ *l* of the FKC suspension without adjuvant was intraperitoneally injected as a booster into the fish. One week after the last injection, the fish were bled from the heart, and antisera were obtained and stored at -20℃. Absorbed yellowtail antisera were prepared by adding 500 mg of FKC to 1 m*l* of the antisera. The mixture was incubated at 25℃ for 2 h. FKC was removed by centrifugation and the supernatant fluids were used for serotyping of the twenty-five isolates. Twenty-five microliters of two-fold serial dilutions of the yellowtail sera, absorbed or non-absorbed, were mixed with the same volume of the isolate FKC suspension of 2 mg/m*l* in PBS in each well of the microtiter plates. The plates were incubated at room temperature for 2 h and at 4℃ for 16 h, and then agglutination was observed.

RESULTS AND DISCUSSION

Based on three serotype isolates possessing common and distinctive antigens, [3] serotyping of the twenty-five isolates were determined using absorbed and unabsorbed yellowtail antisera. When the antisera were absorbed with homologous antigens, the antibodies were completely eliminated. However, each antiserum was not completely eliminated by a cross-reaction against the heterologous antigens (Tables 1-3). The

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Table 1 Cross-absorption test using yellowtail anti-type A serum

Absorbed with	Formalin-killed cells		
	type A	type B	type C
type A	<2*	<2	<2
type B	4	<2	<2
type C	5	3	<2
unabsorbed	11	12	10

*Agglutinating antibody titer(log_2).

Table 2 Cross-absorption test using yellowtail anti-type B serum

Absorbed with	Formalin-killed cells		
	type A	type B	type C
type A	<2*	6	5
type B	<2	<2	<2
type C	6	4	<2
unabsorbed	11	12	10

*Agglutinating antibody titer (log_2).

Table 3 Cross-absorption test using yellowtail anti-type C serum

Absorbed with	Formalin-killed cells		
	type A	type B	type C
type A	<2*	4	5
type B	<2	<2	<2
type C	<2	<2	<2
unabsorbed	11	12	10

*Agglutinating antibody titer (log_2).

respective numbers of the isolates identifying serotypes A, B and C were 9, 11 and 5. The numbers of serotyping isolates for each month during the epizootic of pasteurellosis in Oita Prefecture in May to October, 1998, are shown in Fig. 1. The serotype A isolates were found in May to August, and the serotype B and C isolates were found in June to September or October. Fig. 2 shows the number of serotyping isolates in the six aquaculture areas. At Usuki, the serotype B isolate was only detected, and at Tsurumi, the serotype A isolate was also only detected. At Kamiura, the serotype B and C isolates were detected. At the other three aquaculture areas, Saiki, Yonouzu and

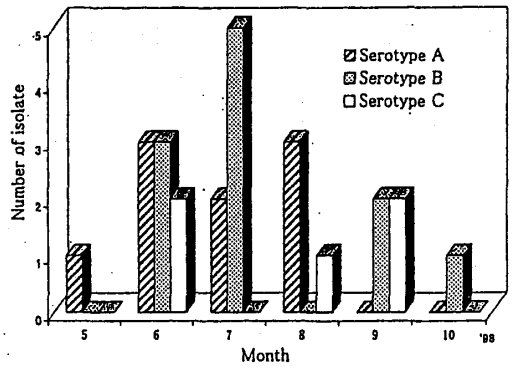

Fig. 1 Number of three serotypes, A, B and C, *Photobacterium damsela* subsp. *piscicida* strains isolated in Oita Prefecture in May to October, 1998.

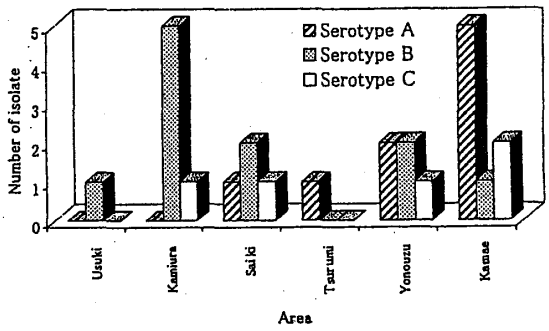

Fig. 2 Number of three serotypes, A, B and C, *Photobacterium damsela* subsp. *piscicida* strains isolated in six aquaculture areas in Oita Prefecture in 1998.

Kamae, the three serotype isolates were detected. These results indicate that the serotype of the isolates must affect vaccine the development. An examination of the effectiveness of the monovalent and polyvalent vaccines prepared from the three type strains against pasteurellosis for yellowtail is necessary.

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