SHORT COMMUNICATIONS

The Rapid Serological Diagnosis of Fish Furunculosis Caused by 'Smooth' and 'Rough' Strains of *Aeromonas salmonicida*

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INTRODUCTION

Furunculosis, an acute or sub-acute contagious disease primarily affecting *Salmonidae*, is caused by one prime specific aetiological agent, the bacterium *Aeromonas salmonicida*. First reported from Germany in 1894, the disease is now widespread and, with the possible exception of Australia and New Zealand, is a cause of fish-losses wherever salmonid culture is practised. It was first confirmed in the U.K. in 1911 and subsequently made notifiable by the Diseases of Fish Act, 1937.

Furunculosis can be controlled by chemotherapy if instituted promptly; consequently, rapid identification of *A. salmonicida* is essential. For this, the slide agglutination test may be used providing the strains under examination are in the 'smooth' phase, that is, do not auto-agglutinate. Unfortunately, the majority of isolates of *A. salmonicida* from diseased fish are 'rough', that is, auto-agglutinating (Mackie *et al.*, 1930; Karlsson, 1964). To overcome auto-agglutination Duff (1939) suspended *A. salmonicida* in 0.05 % NaCl and Karlsson (1964) used ultrasonics to disperse auto-agglutinated cells, before testing.

During a detailed study of *A. salmonicida* using whole-cell and passive haemagglutination (PHA) serological techniques, we have shown that the method of Duff (1939), although adequate to permit examination of some 'rough' strains, is ineffectual with those that auto-agglutinate strongly. This observation confirms the earlier report by Williamson (1928) of auto-agglutination of *A. salmonicida* in both 0.1 % NaCl and distilled water. This communication describes a technique which allows rapid serological examination of both 'smooth' and 'rough' strains of *A. salmonicida*.

METHODS

Organisms. Thirty strains of *A. salmonicida* (12 'smooth' and 18 'rough') were examined and, to test specificity, 6 representative strains of each of the following bacteria pathogenic to fish were included: *A. hydrophila*; *Vibrio anguillarum*; *Pseudomonas fluorescens* (see Table 1).

Antisera. Five 'smooth' and 1 'rough' strain of *A. salmonicida* were used to prepare antisera in female New Zealand white rabbits previously tested for pre-immune agglutinating activity for *A. salmonicida*. Each rabbit was injected intravenously with 1 ml of increasing concentrations of bacterial suspensions, twice weekly for 3 weeks.

Cells for injections were sedimented from 72 h cultures in tryptone soya broth (Oxoid), washed with 0.9 % saline, re-centrifuged, suspended in 20 times their volume of absolute alcohol, and heated at 45 °C for 30 min. To remove fimbriae, and to destroy heat-labile

Table 1. *Details of organisms used*

Taxon	Strain number	Source or donor
<i>Aeromonas salmonicida</i>	1109	National Collection of Marine Bacteria (NCMB)
	10402	National Collection of Type Cultures (NCTC)
	14174	American Type Culture Collection (ATCC)
	7	Dr T. Kimura, Japan
	13	Dr O. Ojala, Finland
	14	Dr G. L. Bullock, U.S.A.
	15	Dr T. P. T. Evelyn, Canada
	16, 17, 18	Mr D. P. Anderson, U.S.A.
	72, 80	Dysentery Reference Laboratory (DRL)
	99	Mr D. A. Conroy, U.K.
	1, 10, 12, 26, 27, 37, 86, 98, 119, 144	<i>Salmo trutta</i> , U.K.
	114, 115, 135	<i>S. gairdneri</i> , U.K.
	100, 131	<i>S. salar</i> , U.K.
	9	<i>Blicca bjoerkna</i> , U.K.
	121	<i>Tinca tinca</i> , U.K.
<i>A. hydrophila</i>	7810, 8049	NCTC
	73	NCMB
	9234	National Collection of Industrial Bacteria
	64, 71	DRL
<i>Vibrio anguillarum</i>	6, 571	NCMB
	19, 20	Dr T. Haastein, Norway
	32	<i>Anguilla anguilla</i> , U.K.
	34	<i>S. gairdneri</i> , U.K.
<i>Pseudomonas fluorescens</i>	8251, 9494, 10038	NCTC
	96, 106	<i>S. salar</i> , Iceland and U.K.
	52	<i>Silurus glanis</i> , U.K.

antigens, the cells were centrifuged, re-suspended in saline, heated at 100 °C for 30 min, re-centrifuged and, finally, re-suspended in saline.

Following satisfactory trial bleedings, rabbits were exsanguinated and sera separated aseptically.

Test medium. All strains for subsequent serological study, whether as pure or mixed cultures, were grown for 24, 48 and 72 h at 22 °C on tryptone soya agar, a primary isolation media for *A. salmonicida*.

Serological test. A loopful of bacterial colony was transferred to a small glass tube (50 × 5 mm) containing approximately 0.5 ml 0.9 % NaCl adjusted to pH 11 with NaOH, mixed and, if necessary, dispersed with a Pasteur pipette. The tube was then heated in a water bath at 90–95 °C for 30 min or, alternatively, boiled for 15 min. After heating, the suspended material was sedimented by centrifugation and the supernatant removed to a fresh tube containing approximately 0.04 ml of packed, fresh commercial (Wellcome Ltd) or formalized (Csizmas, 1960) sheep red blood cells (previously washed twice with 0.9 % NaCl) and placed in a water bath at 37 °C for 15 min. The sensitized cells were then sedimented, washed once in 0.9 % NaCl, sedimented and re-suspended in approximately 0.25 ml 0.9 % NaCl. Finally, one drop of sheep cells was mixed with one drop of antiserum on a clean white tile and observed for haemagglutination. Negative controls consisted of: (i) one drop of antiserum and one drop of unsensitized cells; (ii) one drop of 0.9 % NaCl and one drop of sensitized cells.

RESULTS AND DISCUSSION

Preliminary results demonstrated strong cross-reactions between all six antisera and each of the six antigens (titres 1:1280 to 1:40960). Subsequent tests were therefore performed with one antiserum diluted to a homologous titre of 1:250. This antiserum agglutinated sheep red blood cells sensitized with all recently isolated strains of *A. salmonicida*, the reaction being evident within 30 s. However, occasional old laboratory strains maintained as agar cultures were not reactive in this or the PHA test. One such organism, *A. salmonicida* ATCC14174, maintained in this laboratory for a number of years as an agar-slope culture, failed to react, whereas a new lyophilized culture of this strain, obtained from the American Type Culture Collection, reacted strongly with all antisera in both tests. Furthermore, passage of this strain, maintained as an agar culture, through rainbow trout (*Salmo gairdneri*) failed to restore its reactivity. Hence, old laboratory agar cultures of *A. salmonicida* may not be suitable for serological studies or for vaccine production, even following fish-passage. Both recently isolated non-pigmented varieties of *A. salmonicida* (strains 7 and 9) reacted strongly in the present method, while the other (strain NCMB1109), maintained as an agar culture, did not.

Besides the method described being applicable to the identification of both 'smooth' and 'rough' strains of *A. salmonicida* of recent origin, it also allows examination of both 24-h-old cultures, when growth is slight and the characteristic brown pigment absent, and also mixed cultures, provided *A. salmonicida* predominates, thus yielding sufficient antigen for adequate sensitization of the sheep red blood cells.

No cross-reactions were observed with any of the other bacteria pathogenic to fish tested (see Table 1). Nor, under the test conditions used, was there any interference by rabbit anti-sheep haemolysins or agglutinins. Although both fresh and formalized sheep red blood cells gave satisfactory results which were easy to read, the former produced a more contrasting reaction.

Finally, the present method has now been in use as a routine diagnostic procedure for over 12 months and all strains of *A. salmonicida* isolated from diseased fish have reacted strongly.

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