

RESEARCH COMMUNICATION

THE INDIRECT FLUORESCENT ANTIBODY TECHNIQUE FOR THE RAPID IDENTIFICATION OF STREPTOCOCCOSIS OF RAINBOW TROUT (*SALMO GAIRDNERI*)

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ABSTRACT

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The indirect fluorescent antibody technique has been used successfully for the rapid serological identification of the *Streptococcus* sp. responsible for streptococcosis of rainbow trout. This technique has been used to identify the *Streptococcus* sp. in pure cultures and smears made from experimentally infected and diseased fish.

INTRODUCTION

An unidentified species of *Streptococcus* has been isolated from fish in South Africa (Naudé, 1975; Roode, 1977; Boomker, Imes, Cameron, Naudé & Schoonbee, 1979; Bragg & Broere, 1986), in Australia (Munday & Copland, 1982), in Japan (Hishina, Sano & Morimoto, 1958) and in the USA (Plumb, Schachte, Gaines, Peltier & Carroll, 1974; Cook & Lofton, 1975). Streptococcosis is the most important disease of rainbow trout in South Africa and is the cause of extensive mortalities each summer (Bragg & Broere, 1986).

The *Streptococcus* sp. isolated from trout was susceptible to tetracycline, chloramphenicol and erythromycin (Bragg & Broere, 1986). However, it was found that levels of 75 mg oxytetracycline/kg of fish per day, added to the feed, had little effect on the outcome of the disease (Bragg & Broere, 1986). Similar results were obtained in Australia (Munday, personal communication 1986).

The difficulty of treating the disease has led to a search for a rapid and sensitive diagnostic test, to facilitate the detection and eradication of reservoirs and carriers of the pathogen before a major disease episode is established in the production ponds.

The indirect fluorescent antibody technique (IFAT) was used for the rapid serological identification of bacterial infections of fish (Busch, 1982; Bullock & Stuckey 1975; Klontz & Anderson, 1968; Ainsworth, Caply, Waterstreet & Munson, 1986).

This is a report of the use of IFAT for the rapid serological diagnosis of streptococcosis in rainbow trout.

MATERIALS AND METHODS

Bacteria

Streptococcus was isolated from diseased fish and identified as the species responsible for streptococcosis in rainbow trout by the methods described by Bragg & Broere (1986).

Antiserum production

Cultures of Isolate 7845 were incubated in nutrient broth for 24 h at 37 °C and sub-cultured on BTA plates. The purity was checked by visual examination of the colony morphology and by biochemical tests.

The nutrient broth culture was centrifuged for 10 min at 800 g and the pellet washed 3 times with phosphate buffered saline (PBS) at a pH of 7.4. The pellet was resuspended in PBS and the optical density was adjusted to 0.3 at 430 nm. The bacteria were inactivated by the addition of 10 ml of a 10 % formalin solution per 1 ml bacterial suspension and 0.5 ml aliquots were used to

intravenously inoculate 3 New Zealand white rabbits. The rabbits were re-inoculated on Day 5. On Day 9, the rabbits were inoculated intravenously with 1.0 ml aliquots of live bacteria and on Day 13 re-inoculated with 1.5 ml of live bacteria. A sample of blood was collected on Day 16 and the presence of antibodies was determined by the slide agglutination test. The rabbits were rested for 1 week and subsequently bled to death by cardiac puncture. The serum was collected and stored.

Slide agglutination test

Slide agglutination tests were performed by mixing equal volumes of rabbit serum with suspensions of Isolate 7845 and the other *Streptococcus* isolates, *S. faecalis*, *S. faecium* and *S. lactis*. A slide agglutination test was also performed by mixing serum collected before inoculation and the different species of *Streptococcus*.

IFAT on pure cultures of bacteria

The IFAT was carried out on pure cultures of Isolate 7845, *S. faecalis*, *S. faecium*, *S. lactis* and *Aeromonas hydrophila*. Smears of the bacteria were made on glass slides and air-dried. The slides were fixed in cold (-20 °C) acetone for 10 min and 0.02 ml of the rabbit serum was placed on them. The slides were incubated for 10 min at room temperature in a moist environment, then each washed 3 times with PBS for 10 min. A 0.02 ml sample of FITC-labelled, anti-rabbit IgG was added to each slide and incubated for 10 min in a moist environment at room temperature. The slides were washed well, air dried and examined.

IFAT on experimentally infected fish

Fish were experimentally infected by being dipped into a 1:100 dilution of Isolate 7845. Control fish were dipped into a 1:100 dilution of nutrient broth. Both the infected and control fish were kept in separate tanks until symptoms, such as exophthalmia, could be observed in the infected fish. The fish were killed and smears of the spleen, liver and kidney were prepared. Samples were also removed for the isolation and identification of the bacteria, according to the method described by Bragg & Broere (1986). The slides were processed according to the method described above.

IFAT on diseased fish from the field

Samples of fish showing the symptoms described earlier (Bragg & Broere, 1986) were received from the field. Samples of the liver and spleen were removed and processed as for the experimentally infected fish. Attempts were also made to isolate and identify the bacteria according to the method previously described (Bragg & Broere, 1986).

RESULTS

Slide agglutination test

The slide agglutination test was positive when samples of the rabbit serum was mixed with Isolate 7845 (Fig 1). Similar results were obtained when the rabbit serum was mixed with any of the other *Streptococcus* isolates. This test was negative when samples of the rabbit serum were mixed with the *S. lactis*, *S. faecalis* and *S. faecium*. No agglutination was found when samples of the serum collected before inoculation were mixed with any of the bacteria.

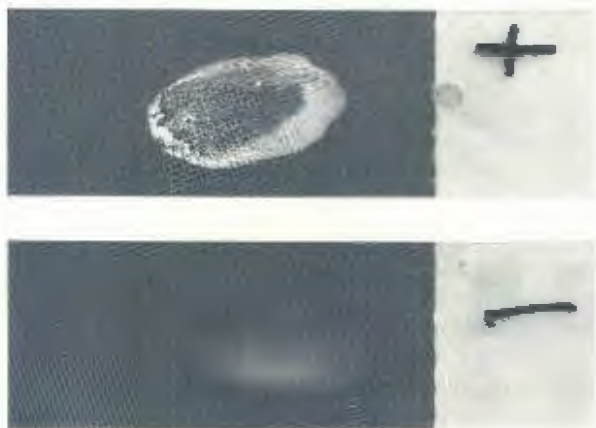


FIG. 1 Slide agglutination test performed using serum of rabbits inoculated with Isolate 7845. The positive agglutination was obtained when Isolate 7845 was mixed with rabbit serum; negative wells when the rabbit serum was mixed with *Streptococcus faecalis*.

IFAT on pure cultures of Isolate 7845

Specific fluorescence was seen when samples of Isolate 7845 were treated with immune serum and conjugated anti-rabbit (Fig. 2 & 3). Only slight, non-specific fluorescence, described as very bright and irregular, was seen when the IFAT was carried out on samples of *S. faecalis*, *S. faecium*, *S. lactis* and *A. hydrophila*.

IFAT on experimentally infected fish

Experimentally infected fish showed a darkening of the skin and exophthalmia 3 days post-inoculation. Positive fluorescence was found on the smears of the spleen, liver and kidney of infected fish, while the smears of the control fish showed only non-specific fluorescence. A *Streptococcus* sp., biochemically indistinguishable from Isolate 7845, was isolated from the experimentally infected fish. No *Streptococcus* could be isolated from the control fish.

IFAT on diseased fish from the field

The IFAT was carried out on 25 diseased trout. Positive fluorescence was detected in smears from the spleen, liver and kidney of 14 of the fish, and a biochemically similar *Streptococcus* was isolated from 12 of them. The remaining 9 cases showed no fluorescence, nor could *Streptococcus* be isolated from these cases.

DISCUSSION

The slide agglutination test was used successfully to demonstrate the presence of specific antibodies in the serum of inoculated rabbits. From the negative results

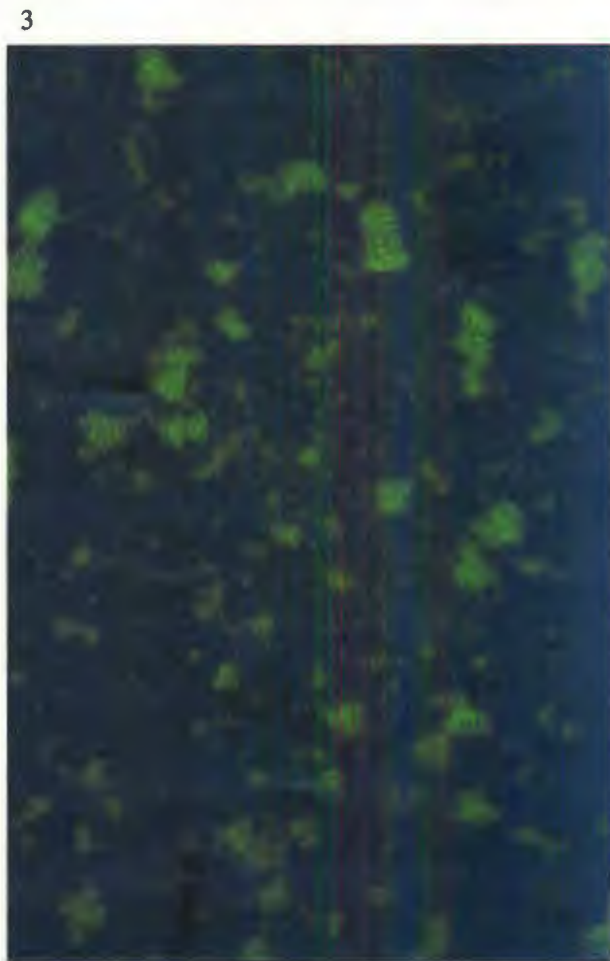
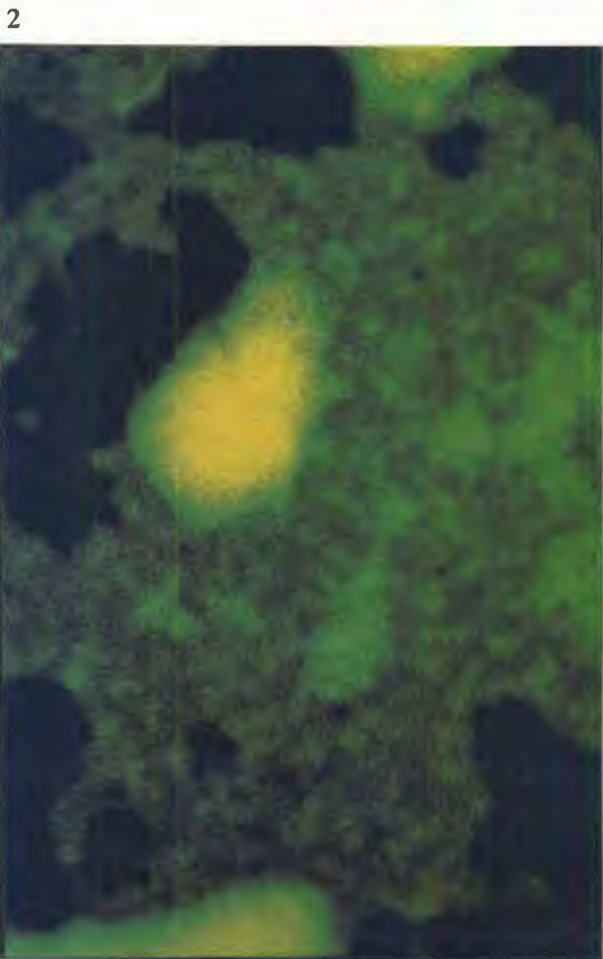


FIG. 2 & 3 Fluorescence obtained when pure cultures of Isolate 7845 were stained by the IFAT using serum from rabbit inoculated with Isolate 7845 and overlaid with FITC labelled anti-rabbit.

obtained when the slide agglutination test was performed on other D group *Streptococcus* spp., it can be stated that the antibodies in the rabbit serum were specific for Isolate 7845.

The IFAT on pure cultures of Isolate 7845 showed clear fluorescence and the morphology of the bacteria can clearly be seen in Fig 2 & 3.

The IFAT was used successfully to detect the presence of Isolate 7845 in the organs of experimentally infected fish. Non-specific fluorescence, seen as very bright, irregular areas of fluorescence, occurred in both infected and non-infected fish. The fact that bacteria biochemically identical to Isolate 7845 could also be isolated from the experimentally infected fish proves that there were bacteria present in the experimentally infected fish, and confirms the results of the IFAT.

Streptococcosis was detected in 14 cases with the IFAT and only 12 cases by isolation and identification techniques. The IFAT proved to be a sensitive and rapid technique for the diagnosis of streptococcosis in rainbow trout.

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