IDENTIFICATION OF ERYSIPELOTHRIX INSIDIOSA WITH FLUORESCENT ANTIBODY

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Fluorescein isothiocyanate was prepared from fluorescein amine, fraction II, by the method of J. L. Riggs (Masters Thesis, University of Kansas, 1957). The serum used was commercial anti-swine erysipelas serum (Anchor Serum Company, South St. Joseph, Missouri) prepared by hyperimmunizing horses. The globulin fraction of the serum was precipitated by half saturation with ammonium sulfate and purified by dialyzing against physiological saline. The amount of protein in the globulin was determined and adjusted to 2.5 per cent with saline. The solution was buffered with phosphate to pH 9 and conjugated by adding the powdered fluorescein isothiocyanate to the globulin solution at the rate of 1 mg of dye to 20 mg of protein. Following conjugation, the excess dye was removed by dialysis against buffered saline. The labeled protein was diluted to 1 per cent and absorbed twice with swine kidney tissue powder.

Two cultures of E. insidiosa from swine and one culture from a turkey were used as well as several other genera in establishing specificity of the conjugated globulin. Heat fixation of the smears proved to be the method of choice although several other fixatives (ethyl alcohol, acetone, 10 per cent formalin, Hanks formalin) were also adequate. The slides were stained, washed, and mounted by the method described by Coons and Kaplan (J. Exptl. Med., 91, 1, 1950). Labeled antibodies prepared against guinea pig complement for use in testing the complement combining ability of the system were furnished by Dr. R. A. Goldwasser.

A 0.05 per cent solution of the conjugated protein was the lowest concentration that would stain E. insidiosa without visible diminution in staining (figure 1). Nonspecific staining was adequately removed by the tissue powder absorptions. The following controls served to establish specificity of the staining:

1. E. insidiosa, with hyperimmune antisera, blocked staining when the organisms were subsequently mixed with labeled antisera.

2. Normal sera conjugates did not stain the organism.

3. Staining was inhibited by dilution of conjugates in unlabeled antiserum.

Figure 1. Lowest concentration to stain Erysipelothrix insidiosa without visible diminution; 0.05 per cent conjugated protein solution.
4. Staining was inhibited by dilution of conjugate in antigen suspension.

5. Labeled sera did not stain the following: *Listeria monocytogenes*, *Salmonella choleraesuis*, *Bacillus subtilis*, *Corynebacterium renale*, and *Corynebacterium xerosis*.

It was found that guinea pig complement was not bound by the horse serum-*E. insidiosa* system when labeled antibodies prepared against the complement were used as the mechanism to demonstrate binding.

Further studies in progress will evaluate the indirect staining technique to determine the significant titers in animals infected with this organism.