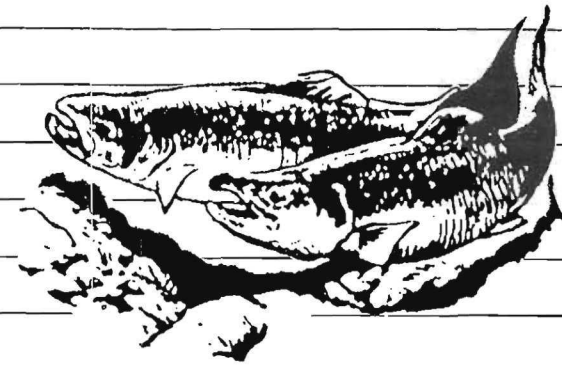
Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
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Fish Antisera Does Not Neutralize Bacterial Chondroitinase

Commercial and recreational fishes such as salmon, trout, catfish, bass, and baitfish are all susceptible to columnaris disease, caused by the bacterium *Cytophaga columnaris* (a.k.a. *Flexibacter columnaris*). Infection typically occurs during warm weather when water temperatures are elevated, but epizootics have occurred among Pacific salmon when water temperatures were slightly above freezing. Infection spreads rapidly causing acute mortality and symptomatically appears as a body and tail rot. Fins commonly erode trailing strings of yellowish material (i.e., bacterial masses) that remain attached to the fish. Many of the details related to this progressive pathology are yet unknown, but investigators speculate that degradative bacterial enzymes are involved. One such enzyme, chondroitin AC lyase or chondroitinase breaks down chondroitin sulfates ($ChSO_4$) composing intracellular matrices and cartilage. Strong activity occurs in vitro and the enzyme may, wholly or in part, be responsible for cartilage degradation resulting in fin rot. Conclusive evidence, however, does not exist to state that chondroitinase is a virulence factor of columnaris disease.

Rapid Method Used To Study Chondroitinase

Prerequisite to studying the kinetics and possible role of chondroitinase in the pathogenesis of columnaris disease, a rapid assay was developed that yields quantitative data (i.e., μg $ChSO_4$ degraded/mL/h) in less than 3 h. The assay consists of incubating $ChSO_4$ (1 part), a standardized concentration of *C. columnaris* cells (1.5 parts), and a low nutrient basal medium (7.5 parts) at room temperature for 2.5 h. At 30-min intervals the concentration of $ChSO_4$ that has not been degraded is determined by addition of an acidic albumin solution. The optical density of the reaction vessel is determined and compared to a standard curve. This assay has proven valuable for evaluating chondroitinase activity among individual isolates of *C. columnaris* and correlating enzyme kinetics to in vivo pathogenicity. Reaction kinetics of the enzyme vary among bacterial isolates, and preliminary data suggests that isolates with high degradative rates are more virulent in fish.

Antibody Effect on Enzyme Secretion

The presence of antibody produced against a specific bacterial pathogen can be detected in fish serum by an agglutination assay. Serial dilutions of serum (containing antibody) are reacted with a standardized, fixed bacterial suspension (antigen). If antibody against the bacteria is present, then a binding reaction results in the formation of an antibody–antigen matrix that agglutinates or precipitates and is visualized within a reaction vessel. Some bacteria, such as *C. columnaris*, form self-aggregates, and agglutination of cells can

produce a false-positive reaction in the absence of antibody. In an attempt to eliminate false reactions and increase our ability to detect anti-*C. columnaris* antibody in fish serum (indicating prior exposure to the pathogen), the chondroitinase assay was used to assess the effect of antibody (bound to the bacterial cell) on the bacteria's ability to secrete chondroitinase. If, in the presence of antibody, *C. columnaris* cannot secrete chondroitinase because of hinderance of bound antibody, then the absence or decrease of chondroitinase activity can indicate the presence of antibody.

Thus, the test is essentially a neutralization assay.

Quick Neutralization Assay Fails

Cell suspensions of *C. columnaris* were incubated for 1 h with and without antiserum. The antiserum used was previously produced by injecting formalin-treated bacterial cells into brook trout (*Salvelinus fontinalis*) and striped bass (*Morone saxatilus*). The cell–serum suspension was mixed with other components of the reaction (ie., $ChSO_4$ and basal medium) and the concentration of undegraded $ChSO_4$ determined at 30-min intervals for 2.5 h. Neither brook trout (Fig. 1) nor striped bass (Fig. 2) antisera inhibited the secretion and activity of chondroitinase produced by *C. columnaris*.

These results suggest that the enzyme is not sterically hindered by the fish antibodies bound to the bacterial cell surface. Perhaps the antigenic sites on which the antibodies were produced are localized in specific regions on the cell surface or are distributed over the cell surface in such a way as to leave large channels. The end result is the free passage of secreted chondroitinase. The assay, therefore, can be used as a tool to study any possible role of chondroitinase in the pathogenesis of columnaris disease and as a diagnostic tool to differentiate *C. columnaris* from a related yellow-pigmented bacteria, *C. psychrophila*, but cannot not be used to detect specific antibody in fish serum.

For further information contact:

Jeffrey D. Teska or Rocco C. Cipriano
National Fish Health Research Laboratory
Box 700
Kearneysville, WV 25430
(304) 725-8461

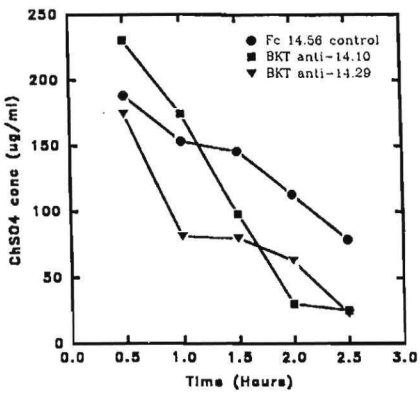

Fig. 1. Kinetics of chondroitinase produced by *Cytophaga columnaris* (Fc 14.56) in the absence and presence of brook trout (BKT) antisera against two isolates of *C. columnaris* (14.10 and 14.29).

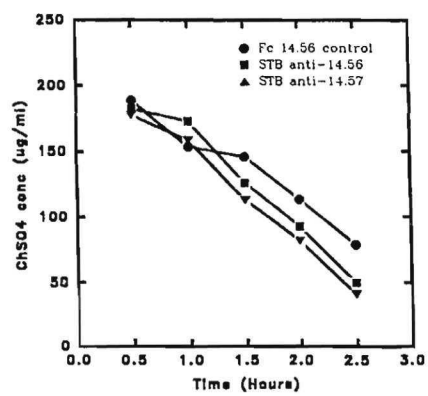

Fig. 2. Kinetics of chondroitinase produced by *Cytophaga columnaris* (Fc 14.56) in the absence and presence of striped bass (STB) antisera against two isolates of *C. columnaris* (14.56 and 14.57).