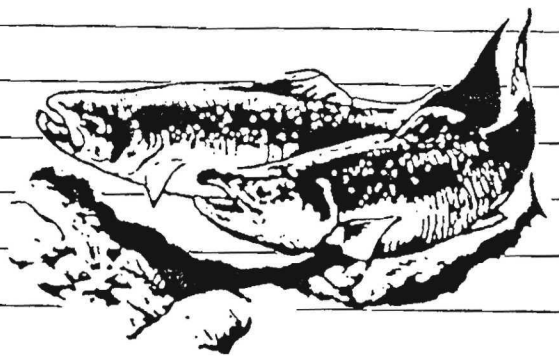
Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 44
DATE 1994



Characterization of *Cytophaga psychrophila* Obtained From Epizootics of Cold-water Disease Among Pacific Salmon

Cytophaga psychrophila, cause of bacterial cold-water disease, induces septicemic infection in salmonid fishes. Because *C. psychrophila* has a predilection for low water temperatures, the disease is especially a problem among cultured salmonids, such as Pacific salmon. The pathogen causes acute septicemia, in which massive numbers of bacteria are recovered from the kidney, spleen, heart, and peritoneum. The organism may also erode the caudal fin and dermis in advanced stages of disease. The etiologic agent is a gram-negative, yellow-pigmented bacterium that is strongly proteolytic and exhibits gliding motility but is relatively inert in most standard microbiological assays. The inert nature of this bacterium complicates accurate differentiation of this pathogen from other, closely-related yellow-pigmented bacteria. This study was conducted to assess epidemiological aspects of the disease and characterize the causative bacterium by phenotypic, serologic, and genetic means. The information could then be used to assist fish health biologists identify *C. psychrophila*.

Isolation of C. Psychrophila From Diseased Pacific Salmon

Chinook salmon (*Oncorhynchus tshawytscha*) from Mayr Brothers Cooperative Salmon Station (Aberdeen, Washington) and coho salmon (*O. kisutch*) at the Humptulips State Hatchery (Humptulips, Washington) and the Lewis River State Hatchery (Woodland, Washington) were studied. Although mortality was not evident when fish were sampled, *C. psychrophila* was readily obtained from most mucus and kidney samples that were processed. Bacterial prevalences attained 3.0×10^6 colony-forming units per gram of sample. Isolation from mucus produced slightly better results than assay of kidney; the pathogen was recovered from 96% of the samples of mucus and from 85% of the kidneys of fish at the Mayr Brothers hatchery. It was obtained from 72% of the mucus and 57% of the kidneys of salmon at the Humptulips State Hatchery and from 90% of the mucus and 85% of the kidney samples processed at Lewis River State Hatchery. Regardless of origin, bacteria identified as *C. psychrophila* were long (2.0–5.0 m), thin (0.5 m) gram-negative rods that produced bright yellow colonies with a thin spreading margin on agar used for yellow-pigmented microorganisms. These bacteria did not grow on standard microbiological media commonly used to cultivate most fastidious, gram-negative bacteria.

Cytophaga psychrophila Demonstrates Remarkable Structural and Serological Homology

Remarkable similarity was observed in protein whole cell lysates among *C. psychrophila*

electrophoresed on sodium-dodecyl-sulfate polyacrylamide gels. Protein electrophoregrams were similar regardless of whether the isolates were derived from mucus or kidney of individual fish or from different hatcheries. Serological analysis using polyclonal rabbit antiserum against four representative isolates with homologous and heterologous antigens also showed that these bacteria were antigenically homogeneous. There was little variation among agglutinin titers expressed between antisera and formalin-killed antigens, and all antisera reacted similarly in Western blot immunoassays with whole cell lysates. The homolgy observed in molecular structure and antigenic nature indicate that *C. psychrophila* is a potential candidate for vaccine development.

Genotypic Analysis Suggests Close Relationship Between *C. psychrophila*, *Flavobacterium odoratum*, and *Flexibacter flexilis*

Polymerase chain reactions are often used to study rDNA variation in eubacteria. Amplification products of the 16S–23S spacer region are often conserved between strains of bacteria but are highly variable between species. All isolates phenotypically identified as *C. psychrophila* produced a single rDNA spacer amplification product of about 240 BP suggesting that the organisms made up a single species. The comparison of the *C. psychrophila* rDNA sequence to other known prokaryotic ribosomal sequences denoted a close genetic relationship to *Flavobacterium odoratum* and *Flexibacter flexilis*.

Genetic ribotyping is also a powerful tool for the study of bacterial epidemiology. Variation is revealed by cleaving sites in and around the rRNA operon and detection of fragmentation patterns by hybridization with labeled probes. In contrast to the strain-to-strain uniformity observed using phenotypic and serotypic techniques, the *C. psychrophila* isolates elaborated varied ribotypes. Thus, ribotyping may offer selective advantages to further study epidemiological differences associated with the etiological agents causing epizootics of cold-water disease.

For further information contact:

Rocco C. Cipriano and W. Bane Schill
National Fish Health Research Laboratory
Box 700
Kearneysville, WV 25430
(304) 725-8461