Genetic diversity of *Flavobacterium psychrophilum* recovered from commercially raised rainbow trout and spawning Coho salmon

Yi-Chang Chen[1], Margaret Davis[1], Scott LaPatra[2], Ken Cain[3], Kevin Snekvik[1,4], and Douglas R. Call[1*]

[1]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA; [2]Clearspring Foods Inc., Buhl, ID; [3]Department of Fish and Wildlife Resources, University of Idaho, Moscow, ID; [4]Washington Animal Disease Diagnostic Laboratory, Pullman, WA.

Flavobacterium psychrophilum is a gram negative bacterium and it is the etiologic agent of bacterial cold water disease (BCWD) and rainbow trout fry syndrome (RTFS); both of these diseases impact salmonid aquaculture worldwide. There is experimental evidence that *F. psychrophilum* can be transmitted both vertically and horizontally, but is not clear which process contributes to repeated epizootics within hatchery facilities. As a first step to addressing this question, we developed a new protocol for DNA fingerprinting *F. psychrophilum* and examined the genetic diversity of strains recovered from a commercial rainbow trout facility and from spawning Coho salmon. *F. psychrophilum* isolates were collected from trout production facilities that experienced BCWD epizootics during the winter of 2006-07 and isolates from Coho salmon were collected from a hatchery located near Port Angeles, WA. Identification of *F. psychrophilum* was confirmed by colony morphology and a 16S rRNA PCR test. After testing a panel of potential restriction enzymes and developing a suitable pulsed-field gel electrophoresis protocol (PFGE), isolates were retrieved from glycerol stocks and PFGE profiles were determined using the SacI restriction enzyme. Band sharing statistics were calculated using Bionumerics software and results were visualized using an unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis. Between December 2006 and March 2007, fish were sampled from 7 distinct epizootic events at three hatchery facilities in southern Idaho. Ninety-three isolates were characterized by PFGE, which resulted in identification of 13 distinct haplotypes. Seven haplotypes were only observed one time. One haplotype was observed during three epizootic events at a single facility (20 isolates) whereas two other frequently occurring isolates were observed at two facilities (n=23, and n=18 isolates). One haplotype was observed at all three facilities during January. Average band sharing among the rainbow trout isolates was 87.7%. Coho isolates (n = 54) were collected on two occasions (November and December, 2007) and 19 haplotypes were detected. The majority of haplotypes (n=19) were observed during only one month and up to four isolates from a single fish represented distinct haplotypes. Average band sharing among Coho isolates was 69.5%. In conclusion, PFGE was successfully applied to study the clonality of *F. psychrophilum* from two salmonid hosts. Strains recovered from a commercial aquaculture facility were very clonal with only a 14% probability of detecting a new haplotype with each new bacterial isolate. Coho salmon harbored polyclonal infections and there was a 35% probability of detecting a novel haplotype with each new isolate tested from the Coho samples.