

The role of *rpoB* in the attenuation of *Flavobacterium psychrophilum* after passage with rifampicin

Karol S. Gliniewicz¹, Kenneth D. Cain², Kevin R. Snekvik^{1,2} and Douglas R. Call¹

¹Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA;

²Department of Fish and Wildlife Resources and the Aquaculture Research Institute, University of Idaho, Moscow, ID;

³Washington Animal Disease Diagnostic Laboratory, Pullman, WA

Flavobacterium psychrophilum is the etiologic agent of Coldwater disease (CWD) in rainbow trout and other salmonid species. Outbreaks of the disease usually occur with temperature ranging between 3-15°C and mortalities can reach up to 50% within a population. The pathogen is capable of vertical and horizontal transmission, can survive in fresh water up to several months, and is able to withstand disinfection. Presently there are no licensed vaccines against CWD and killed whole-cell vaccine or subunit vaccines showed only limited efficiency. Recently, a strain of *F. psychrophilum* (CSF259.93B17) was attenuated by passage on rifampicin plates. Exposure of this strain provided immunological protection to young rainbow trout against challenge with fully virulent strain of *F. psychrophilum*, CSF259.93. Rifampicin attenuated CSF259.93.B17 harbors a mutated *rpoB* gene as compared to its parental CSF259.93 strain. *RpoB* encodes a β subunit of DNA-dependent RNA polymerase, a natural target of rifampicin. Mutation of *rpoB* enables bacteria to grow in the presence of the antibiotic, but 2D gel electrophoresis and mass spectrometry demonstrated that the attenuated strain has a different protein profile with at least 10 proteins expressed differently as compared to parental CSF259-93 strain. We hypothesize that the mutated form of RNA polymerase is responsible for an abnormal transcriptional program leading to attenuation of the strain. Whether this process is due to interaction with multiple protein promoters or with a limited subset of transcriptional regulators has not been determined.