Phenotypic and Genetic Predictors of Pathogenicity and Virulence in Flavobacterium psychrophilum

Flavobacterium psychrophilum causes bacterial cold-water disease (BCWD) in farmed rainbow trout (Oncorhynchus mykiss), with the multilocus sequence typing (MLST) clonal complex (CC) CC-ST10 accounting for the majority of outbreaks globally. The development of alternative strategies to antibiotic treatment of BCWD using bacteriophage-based control of F. psychrophilum, or virulence factors as targets for therapy, requires knowledge of the phage-sensitivity of outbreak strains and of universal traits contributing to their pathogenicity. To examine the association between virulence and both genetic (MLST sequence type (ST) and PCR-serotype) and phenotypic characteristics (adherence, antibiotic resistance, colony spreading motility, hemolytic and proteolytic activity), the median lethal dose (LD₅₀) of 26 geographically disparate F. psychrophilum isolates was determined in rainbow trout. Furthermore, the in vitro sensitivity of the isolates against five bacteriophages was determined by the efficiency of plating (EOP). The tested F. psychrophilum isolates were mainly represented by CC-ST10 genotypes (22 out of 26) and showed up to 3-log differences in LD₅₀ (8.9 x 10⁵ to 3.1 x 10⁶ CFU). No association between MLST ST and virulence was found because of a high variation in LD₅₀ within STs. All identified serotypes (0, 1, and 2) were pathogenic, but ten most virulent isolates belonged to serotype 1 or 2. Isolates of high (LD₅₀ <10⁵ CFU), moderate (LD₅₀ = 10⁵-10⁶ CFU), and weak (LD₅₀ > 10⁶ CFU) virulence were similar in phenotypic characteristics in vitro. However, the only non-virulent CC-ST10 isolate was deficient in spreading motility and proteolytic activity, indicating that the characteristics are required for pathogenicity in F. psychrophilum. Univariate correlation studies found only non-significant associations between LD₅₀ and the measured phenotypic characteristics, and the multivariable analysis did neither reveal any significant predictors of virulence. The majority of isolates (16 out of 26) were sensitive to at least four bacteriophages, with up to a 6-log variation in the EOP. Most CC-ST10 isolates (16 out of 22) were sensitive to the examined phages, including 5 out of the 7 most virulent isolates represented by prevalent and antibiotic-resistant STs. Our findings suggest that control of BCWD using lytic phages or interventions targeting shared characteristics of pathogenic F. psychrophilum strains should be further explored.