

---

## Abstract

Rainbow trout is a highly valuable aquaculture species due to its rich nutritional profile, including high-quality proteins, unsaturated fatty acids, vitamins, and minerals. It is the second most widely farmed fish species worldwide, and its immune system that adapted to both freshwater and marine environments, represents an important model from both evolutionary and economic perspectives. However, intensive aquaculture has led to the emergence of numerous diseases causing major economic losses. One of them is furunculosis, caused by the opportunistic pathogen *Aeromonas salmonicida*, which is difficult to study due to its unstable genome and high pathogenic flexibility, substantially linked to plasmid content.

In this study we addressed the early infection mechanisms of *A. salmonicida* in rainbow trout. To uncover the mechanisms underlying distinct immunity profiles, we analyzed early infection stages at the whole-fish level using high-throughput workflows and systems biology tools to study both host and pathogen, as one interdependent system. We assumed that the host (fish) serves as a niche for the pathogen (bacterium) and their interactions are dynamic process through which *A. salmonicida* adapts to new environments. Using bacterial strains that displayed different levels of virulence despite nearly identical genotypes, we compared bacterial genomes of high and low virulent isolates to a reference strain.

In the first part the whole genome analyses demonstrated that even though we have one bacteria species, the genomic composition varied in sequence which affects the virulence towards the host. The unique genes for certain isolate and the genes having lower consensus were found also in a group of genes participating in infection process. In the second part the transcriptomic study identified several factors significantly contributing to infection on both sides — pathogen (virulence factors) and host (receptors responding to bacterial factors). The type III secretion system was identified as a key determinant of infection progression, although focusing on a single virulence molecule proved misleading. Transcriptomic analysis revealed that the process to induce infection is very complex and it is a huge interconnected network of pathways such as arginine pathway or iron and flagella regulation. Both known and previously uncharacterized genes were described to be involved in the early stages of infection. Particular emphasis was placed on virulence-associated molecular patterns (VAMPs), which play a crucial role in enabling the host immune system to distinguish self from non-self components of bacterial origin, thereby shaping the course of infection and disease development.

In the last part of this thesis the fish cellular responses were investigated showing cytotoxicity effect on erythrocytes, bacteria limited growth in culture with head kidney leukocytes known being first sentinels of pathogen and also respiratory burst study in myeloid and lymphoid

---

---

fractions during bacteria stimulation. The latter demonstrated that myeloid fraction is mainly responsible to bacteria first recognition and clearance but there is a small subset of lymphoid cells that are also able to perform respiratory burst. Finally, molecular study measuring expression of TLR receptors during stimulation show that it is dependent on the leukocyte cell type, time after exposure and TLR receptor ligands they recognize. The study shows how complex defense mechanism the pathogen has to face during infection and explains the reasons for such flexibility of the species.

In summary, this study provides a comprehensive characterization of the virulence factors of *Aeromonas salmonicida*, offering new insights into its interaction mechanisms with the rainbow trout host.

Jeanne Stuelens