Aeromonas salmonicida - Epidemiology, whole genome sequencing, detection and in vivo imaging - DTU Orbit (11/01/2019)

Aeromonas salmonicida subsp. salmonicida is a bacterial fish pathogen that is the causative agent of furunculosis, a septicemic infection responsible for great losses in aquaculture around the world. In Denmark furunculosis was first seen in freshwater in the 1950s, though currently the infection causes problems in sea reared rainbow trout (Oncorhynchus mykiss) production. Outbreaks occur repeatedly during stressful conditions such as elevated temperatures, in spite of commercial vaccines being applied. Besides seemingly lacking adequate protection, the vaccines also produce undesirable side effects. Antibiotics are therefore used as treatment, which due to the possibility of developing resistance is neither a favorable nor sustainable solution. To complicate things further, it is possible that fish can be carriers of A. salmonicida and transfer the bacterium from freshwater to the sea where they develop septicemia when exposed to stressful sea-rearing conditions and high temperatures. By use of traditional bacteriological methods, continuous investigation of bacterial diagnostics on samples from different rainbow trout farms in Denmark was done, while studying the following three aspects of the concerns regarding A. salmonicida. First, we focused on investigation of the route of entry and initial dissemination of A. salmonicida in fish. This was done by tracing the bacterium using in vivo bioluminescence imaging. A Danish strain was transformed with a plasmid vector containing a green fluorescence protein gene and bacterial luciferase genes that served as fluorescent and bioluminescent reporters respectively. The transformed A. salmonicida was used in a series of immersion experiments where fish were followed over a 24-hour period. Results showed that probable main colonization sites of A. salmonicida were the gills and the dorsal and pectoral fins. This was followed by dissemination through internal organs. Although optimization and further immersion experiments are needed, our results indicated that this tool could be a valuable approach for visualizing A. salmonicida in fish. Focus was subsequently turned to finding a sensitive method for detecting A. salmonicida in infected and possible carrier fish. For this, a previously developed quantitative real-time polymerase chain reaction (real-time PCR) targeting the aopP gene located on A. salmonicida plasmid pAsal1 was assessed. The real-time PCR and bacterial culturing were employed for preliminary screening of A. salmonicida in 40 fish from Danish fresh- and seawater farms. A. salmonicida was detected by realtime PCR in freshwater farm fish showing no sign of disease, indicating possible presence of carrier fish. Out of five examined organs: spleen, kidney, intestine, gills and brain in each fish, A. salmonicida was most frequently detected in the spleen, brain and intestine, indicating that these three organs could play an important role in A. salmonicida infection. The real-time PCR exhibited highly sensitive detection of A. salmonicida as well as a high reproducibility and efficiency, though due to the fact that not all A. salmonicida seem to possess the target plasmid pAsal1, another sensitive detection method with a different and/or complementary target would need to be employed to be certain of avoiding false negatives. The final focal point of this thesis revolved around obtaining knowledge on genetic and virulence variation as well as epidemiology of the disease causing Danish A. salmonicida. Due to high homogeneity among the A. salmonicida subspecies population, standard molecular methods for bacterial typing cannot distinguish among A. salmonicida isolates. Whole genome sequencing was therefore applied on 99 Danish A. salmonicida isolated between years 1980 and 2014 from different geographical regions, one Scottish strain and the type strain NCIMB 1102. Sequences of the A. salmonicida were de novo assembled and then examined for presence of plasmids, virulence and iron acquisition proteins, and antibiotic resistance genes. The chromosome was also examined for single nucleotide polymorphisms that were aligned and subjected to Bayesian temporal tree reconstruction using the published genome of A. salmonicida A449 as reference. Main results revealed that there have been four major introductions of A. salmonicida into Denmark, A. salmonicida are highly homogenous with the exception of certain plasmids and virulence factors encoded on these plasmids, and nine A. salmonicida harbored several worldwide known genes encoding resistance against antibiotics. This study provided valuable information regarding the Danish disease causing A. salmonicida.

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