Current research on *Flavobacterium psychrophilum*

Inger Dalsgaard, Maya M.M. Henriksen, Rói H. Christiansen & Lone Madsen

National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Denmark

Correspondence: inda@vet.dtu.dk

Abstract

Rainbow trout fry syndrome (RTFS) has caused serious losses in the production of rainbow trout in Denmark since the mid 1980s and is still one of the most important bacterial infections in Danish freshwater farms [1]. For the last 15 years, florfenicol has been the drug of choice for control of RTFS, and there is a risk that florfenicol will become less effective in the future. A total of 387 strains of *Flavobacterium psychrophilum* isolated in the period 1994 - 1998 were tested with the antimicrobial agents used in Denmark using an agar dilution method [2]. Forty nine strains from the period 1988 - 1992 and 60 strains from the years 2006 - 2009 have been tested by the same method to evaluate whether the resistance pattern had changed during the period from 1988 - 2009.

Oxytetracycline has been used for treating RTFS since the first recognised outbreak in 1985 in Denmark. As treatment efficacy dropped in the early nineties, the use was ceased resulting in a lower level of MIC values of oxytetracycline. Varying resistance patterns in *F. psychrophilum* to the licensed drugs oxolinic acid and trimethoprim/sulfadiazine were seen during the whole period. An unexpected high level of MIC values for both oxolinic acid and trimethoprim/sulfadiazine was found in strains isolated from the years 2006-2009, despite these antimicrobials had not been used to treat RTFS. A possible explanation might be that the drugs are used for treatment of other bacterial diseases.
At present, RTFS can be controlled by florfenicol. A higher mean MIC level was found during the last period compared to the two earlier periods, which indicates that resistance problems might occur in the future. The results investigating antimicrobial resistance in 500 strains of *F. psychrophilum* show that resistance patterns can change over time.

Alternatives to antimicrobial treatments of *F. psychrophilum* are evaluated in different projects, among them the potential use of bacteriophages to control infections with *F. psychrophilum.* In order to examine the possibilities for phage control of the host, and the development of phage resistant strains, phage-host interactions were studied in three growth experiments with the pathogenic *F. psychrophilum* strain 950106-1/1 and various host specific phages [3]. After 16 hours of incubation phages were added in 3 different concentrations (low, medium and high) as single phages or as phage-cocktails consisting of 3 and 10 phages. Phage production and growth of the host was examined using OD_{525} measurements and flow cytometry, and resistant host strains were isolated for characterization of phage susceptibility patterns and physiological fingerprinting. Phage effects were highly dependent on initial phage concentration. At the highest phage concentration, the phages were able to completely control the host population and prevent growth of phage resistant strains, whereas the low phage concentrations did not show any controlling effect. Addition of phages in cocktails had a more significant effect than when added as individual phages. A host range study of the isolated phage-resistant strains showed several unique patterns of susceptibility to the 22 phages used, indicating different resistant

strains developed during incubation. Laboratory results show that high phage concentration and phage cocktails are more efficient than a single phage in controlling the growth of *F. psychrophilum*.

To optimize the health of fry, knowledge is gained on the influence of diet types with or without probiotics on the microbiota in the intestine. The project OPTIFISH is focusing on how organic vs. non-organic diet types as well as diets with or without probiotics affect the intestine, the intestinal microbiota and survival rates of rainbow trout fed these different diets following exposure to pathogens, e.g. *F. psychrophilum*. Traditional bacteriology as well as molecular methods (e.g. 16S rRNA PCR combined with next generation sequencing) are used in the study. The outcome of the project shall be a more robust and healthy fry, which will lead to a higher production in not only organic but also conventional aquaculture.

Another project is focused on examining gene expression in rainbow trout related to infection with *F. psychrophilum* including antibody production assessed by ELISA on serum samples. The overall purpose is to be able to optimize vaccination or immune-stimulation. Using hydrogen peroxide as a pre-treatment stressor, fry were challenged by immersion in *F. psychrophilum.* Tissue samples for q-PCR were taken four times through the course of eight days and blood samples after 50 days for ELISA. The goal was to examine the adaptive immune response to infection and elucidate whether pre-treatment with hydrogen peroxide alters the response. A tendency towards less *F. psychrophilum* specific antibody was seen for the stressed/infected group in ELISA, while the gills of infected fish showed an inflammatory response but little or no adaptive response to pathogen exposure.

A previous study revealed that results of serological and genetic analyses of strains of *F. psychrophilum* originating from a disease outbreak on a farm and from wild fish caught downstream of the farm showed more variation in the bacteria isolated from the wild fish compared to bacteria isolated from diseased cultured fish.
Methods used for characterization of *F. psychrophilum* have been biotyping, serotyping, plasmid profiling and ribotyping [1,4]. Previous characterization of other Danish strains showed a high degree of similarity using these methods, which might indicate the use of other genotypic typing methods with a higher level of discrimination power. The use of pulsed-field gel electrophoresis (PFGE) to examine the diversity of *F. psychrophilum* has proved excellent for typing purposes. [5,6]. Our results suggest a clonal genetic structure with slight differences among the types, which is also found by serotyping and ribotyping. Two different clonal types were seen using an 80% similarity cut off value. It might be suggested that one of the clones contain the virulent strains, whereas the other the non-virulent strains. To get more information about the population structure of *F. psychrophilum* isolated from farm fish and wild fish, the multilocus sequencing approach (MLST) [7] based on seven loci was used for further analysis of the isolated bacteria. The relationship between the different strains shown with the PFGE method was also supported with MLST. Using both methods, the bacteria were divided into the same clonal types, hence separating the suggested virulent and non-virulent strains. The use of both traditional bacteriological tests and DNA-based molecular methods are important approaches to understand the impact of the disease on wild population, transfer of the bacterium between different fish species and the role of the wild fish as reservoir of *F. psychrophilum.*

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