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Publications:

Secondary immune response of rainbow trout following repeated immersion vaccination
Teleosts are able to raise a protective immune response, comprising both innate and adaptive elements, against various pathogens. This is the basis for a widespread use of vaccines, administered as injection or immersion, in the aquaculture industry. It has been described that repeated injection vaccination of fish raises a secondary immune response, consisting of rapid, accelerated and increased antibody reaction. This study reports how rainbow trout responds to repeated immersion vaccination against yersiniosis (ERM) caused by the bacterial pathogen Yersinia ruckeri. It was found that rainbow trout does not raise a classical secondary response following repeated immersion vaccination. Serum antibody titres were merely slightly increased even after three immunizations, using 30-s immersion into a bacterin consisting of formalin-inactivated Y. ruckeri (serotype O1, biotypes 1 and 2), performed over a 3-month period. The densities of IgM-positive lymphocytes in spleen of fish immunized three times were increased compared to control fish, but no general trend for an increase with the number of immunizations was noted. The lack of a classical secondary response following repeated immersion vaccination may partly be explained by limited uptake of antigen by immersion compared to injection.

General information
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Organisations: National Veterinary Institute, Innate Immunology, Bacteriology & Parasitology, University of Copenhagen
Authors: Jaafar, R. M. (Ekstern), Al-Jubury, A. (Ekstern), Chettri, J. K. (Intern), Dalsgaard, I. (Intern), Kania, P. W. (Ekstern), Buchmann, K. (Ekstern)
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Antimicrobial peptide CAP18 and its effect on Yersinia ruckeri infections in rainbow trout Oncorhynchus mykiss (Walbaum): comparing administration by injection and oral routes

The antimicrobial peptide CAP18 has been demonstrated to have a strong in vitro bactericidal effect on Yersinia ruckeri, but its activity in vivo has not been described. In this work, we investigated whether CAP18 protects rainbow trout Oncorhynchus mykiss (Walbaum) against enteric red mouth disease caused by this pathogen either following i.p. injection or by oral administration (in feed). It was found that injection of CAP18 into juvenile rainbow trout before exposure to Y. ruckeri was associated with lowered mortality compared to non-medicated fish although it was less effective than the conventional antibiotic oxolinic acid. Oral administration of CAP18 to trout did not prevent infection. The proteolytic effect of secretions on the peptide CAP18 in the fish gastrointestinal tract is suggested to account for the inferior effect of oral administration.
Detection and quantification of *Aeromonas salmonicida* in fish tissue by real-time PCR

Furunculosis, a septicamia infection caused by the bacterium *Aeromonas salmonicida* subsp. salmonicida, currently causes problems in Danish seawater rainbow trout production. Detection has mainly been achieved by bacterial culture, but more rapid and sensitive methods are needed. A previously developed real-time PCR assay targeting the plasmid encoded aopP gene of *A. salmonicida* was, in parallel with culturing, used for the examination of five organs of 40 fish from Danish freshwater and seawater farms. Real-time PCR showed overall a higher frequency of positives than culturing (65% of positive fish by real-time PCR compared to 30% by a culture approach). Also, no real-time PCR-negative samples were found positive by culturing. *A. salmonicida* was detected by real-time PCR, though not by culturing, in freshwater fish showing no signs of furunculosis, indicating possible presence of carrier fish. In seawater fish examined after an outbreak and antibiotics treatment, real-time PCR showed the presence of the bacterium in all examined organs (1-482 genomic units mg⁻¹). With a limit of detection of 40 target copies (1-2 genomic units) per reaction, a high reproducibility and an excellent efficiency, the present real-time PCR assay provides a sensitive tool for the detection of *A. salmonicida*.

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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Technical University of Denmark, Aarhus University
Authors: Bartkova, S. (Intern), Kokotovic, B. (Ekstern), Skall, H. F. (Ekstern), Lorenzen, N. (Ekstern), Dalsgaard, I. (Intern)
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Scopus rating (2014): CiteScore 1.99
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Scopus rating (2013): CiteScore 1.74
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.7
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.09
ISI indexed (2011): ISI indexed yes
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Web of Science (2009): Indexed yes
Effect of oral booster vaccination of rainbow trout against Yersinia ruckeri depends on type of primary immunization

Vaccination of rainbow trout against Enteric Redmouth Disease (ERM) caused by Yersinia ruckeri can be successfully performed by administering vaccine (a bacterin consisting of formalin killed bacteria) by immersion, bath or injection. Booster immunization is known to increase the protection of fish already primed by one of these vaccination methods. Oral vaccination of trout (administering vaccine in feed) is an even more convenient way of presenting antigen to the fish but the effect of an oral booster has not previously been described in detail. The present work describes to what extent protection may be enhanced by oral boosting following priming with different administration methods. The study confirms that vaccination by 30 s dip into a bacterin (diluted 1:10) may confer a significant protection compared to non-vaccinated fish. The immunity may be optimized by booster immunization either provided as dip (most effective), bath (less effective) or orally (least effective). Oral immunization may be used as booster after dip but applied as a single oral application it induced merely a slight and statistically non-significant response. It is noteworthy that primary oral immunization followed by an oral booster vaccination showed a trend for an even weaker response. It should be investigated if continued exposure to a low antigen concentration - as performed by two oral immunizations - may induce tolerance to the pathogen and thereby leave the fish more vulnerable.

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Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.001 SNIP 1.149 CiteScore 3.11
Epidemiology of Danish Aeromonas salmonicida subsp salmonicida in Fish Farms Using Whole Genome Sequencing

Furunculosis, a serious infection caused by the bacterium *Aeromonas salmonicida* subsp. *salmonicida* is common in sea-reared rainbow trout production in Denmark. Developing an effective control strategy requires knowledge of the epidemiology, as well as the genomic and virulent variability of the Danish *A. salmonicida* subsp. *salmonicida* isolates. To obtain this, the genomes of 101 *A. salmonicida* subsp. *salmonicida*, including 99 Danish isolates, one Scottish strain and the type strain NCIMB 1102, were sequenced using the Illumina HiSeq platform. Isolates were de novo assembled, examined for presence of plasmids, virulence and iron acquisition proteins, genomic islands, and antibiotic resistance genes. Single Nucleotide Polymorphisms were aligned and subjected to Bayesian temporal phylogenetic and maximum likelihood tree reconstruction using the published genome of *A. salmonicida* subsp. *salmonicida* A449 as reference. Bayesian temporal phylogenetic reconstruction suggests that four major introductions of *A. salmonicida* subsp. *salmonicida* into Denmark have occurred. The introductions correlate with the freshwater and subsequent seawater expansion of rainbow trout production. Initial transmission of the bacterium could have been from seawater to freshwater or vice versa, and most minor clades include a mixture of strains from different fresh- and seawater farms. Genomic variation of *A. salmonicida* subsp. *salmonicida* mostly appeared to be associated with their plasmids and plasmid encoded virulence factors. Nine *A. salmonicida* subsp. *salmonicida* isolates harbored worldwide known antibiotic resistance genes against several antibiotics and there is an indication that 33% of the isolates contained the genomic island AsaGEI1b.
These findings not only support the usefulness of whole genome sequencing for genetic studies of homogeneous bacteria in general, but provide novel information about the Danish A. salmonicida subsp. salmonicida population, with implications for vaccine development in efforts to better protect Danish rainbow trout in the future.

**Genomic characterization of Flavobacterium psychrophilum serotypes and development of a multiplex PCR-based serotyping scheme**

*Flavobacterium psychrophilum* is a devastating bacterial pathogen of salmonids reared in freshwater worldwide. So far, serological diversity between isolates has been described but the underlying molecular factors remain unknown. By combining complete genome sequence analysis and the serotyping method proposed by Lorenzen and Olesen (1997) for a set of 34 strains, we identified key molecular determinants of the serotypes. This knowledge allowed us to develop a robust multiplex PCR-based serotyping scheme, which was applied to 244 bacterial isolates. The results revealed a
striking association between PCR-serotype and fish host species and illustrate the use of this approach as a simple and cost-effective method for the determination of *F. psychrophilum* serogroups. PCR-based serotyping could be a useful tool in a range of applications such as disease surveillance, selection of salmonids for bacterial coldwater disease resistance and future vaccine formulation.

**General information**

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Organisations: National Veterinary Institute, Bacteriology & Parasitology, Fish Diseases, Universite Paris Saclay, Kindai University, INRA Institut National de La Recherche Agronomique, Universite d'Evry, Abo Akademi University
Authors: Rochat, T. (Ekstern), Fujiwara-Nagata, E. (Ekstern), Calvez, S. (Ekstern), Dalsgaard, I. (Intern), Madsen, L. (Intern), Calteau, A. (Ekstern), Lunazzi, A. (Ekstern), Nicolas, P. (Ekstern), Wiklund, T. (Ekstern), Bernardet, J. (Ekstern), Duchaud, E. (Ekstern)
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Scopus rating (2016): CiteScore 4.16 SJR 1.731 SNIP 1.172
Web of Science (2016): Indexed yes
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Scopus rating (2015): SJR 1.878 SNIP 1.208 CiteScore 4.15
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.861 SNIP 1.16 CiteScore 3.76
Web of Science (2014): Indexed yes
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ISI indexed (2013): ISI indexed no
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**Infection routes of *Aeromonas salmonicida* in rainbow trout monitored in vivo by real-time bioluminescence imaging**

Recent development of imaging tools has facilitated studies of pathogen infections in vivo in real time. This trend can be exemplified by advances in bioluminescence imaging (BLI), an approach that helps to visualize dissemination of pathogens within the same animal over several time points. Here, we employ bacterial BLI for examining routes of entry and spread of *Aeromonas salmonicida* susbp. salmonicida in rainbow trout. A virulent Danish *A. salmonicida* strain was
tagged with pAKgfplux1, a dual-labelled plasmid vector containing the mutated gfpmut3a gene from Aequorea victoria and
the luxCDABE genes from the bacterium Photorhabdus luminescens. The resulting A. salmonicida transformant exhibited
growth properties and virulence identical to the wild-type A. salmonicida, which made it suitable for an experimental
infection, mimicking natural conditions. Fish were infected with pAKgfplux1 tagged A. salmonicida via immersion bath.
Colonization and subsequent tissue dissemination was followed over a 24-h period using the IVIS spectrum imaging
workstation. Results suggest the pathogen's colonization sites are the dorsal and pectoral fin and the gills, followed by a
progression through the internal organs and an ensuing exit via the anal opening. This study provides a tool for visualizing
colonization of A. salmonicida and other bacterial pathogens in fish.

General information
State: Published
Organisations: Section for Bacteriology, Pathology and Parasitology, National Veterinary Institute
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Scopus rating (2016): CiteScore 2.12
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.71
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.99
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.74
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.7
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.09
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Web of Science (2008): Indexed yes
Web of Science (2007): Indexed yes
Web of Science (2006): Indexed yes
Web of Science (2005): Indexed yes
Web of Science (2004): Indexed yes
In vivo adherence of Flavobacterium psychrophilum to mucosal external surfaces of rainbow trout (Oncorhynchus mykiss) fry

The adherence of Flavobacterium psychrophilum to surfaces of epithelial tissues has been inconclusively suggested as a mechanism, which enables the bacterium to invade the host. Hence, the present study aimed to examine the adherence of the cells of two colony phenotypes, smooth and rough, of F. psychrophilum to mucosal tissues of rainbow trout fry and to test the skin mucus as a nutrient for the growth of F. psychrophilum. Fish were immersed in water containing 10^6 CFU mL^-1 F. psychrophilum for each colony phenotype. Mucosal tissue samples from fins, gills, skin and eyes, and swab samples from spleen and kidney were taken and inoculated onto TYES agar plates. Colony phenotypes of F. psychrophilum were identified and number of colonies counted. The results showed that cells of both phenotypes initially (0 h) adhered to all mucosal surfaces, but only the rough cells were still present on tissues 1 h post-immersion. Both phenotypes showed a tissue tropism with the fin tissue being the most adhered. Furthermore, skin mucus promoted the growth of both colony phenotypes. We suggest that the growth of F. psychrophilum cells in skin mucus apparently facilitates the bacterial adherence to mucosal surfaces, and the subsequent invasion into the host.

General information
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Organisations: National Veterinary Institute, Bacteriology & Parasitology, Novia University of Applied Sciences, Åbo Academy University
Authors: Papadopoulou, A. (Ekstern), Dalsgaard, I. (Intern), Lindén, A. (Ekstern), Wiklund, T. (Ekstern)
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Scopus rating (2015): CiteScore 1.71
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.99
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.74
ISI indexed (2013): ISI indexed yes
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Positive correlation between Aeromonas salmonicida vaccine antigen concentration and protection in vaccinated rainbow trout Oncorhynchus mykiss evaluated by a tail fin infection model

Rainbow trout, Oncorhynchus mykiss (Walbaum), are able to raise a protective immune response against Aeromonas salmonicida subsp. salmonicida (AS) following injection vaccination with commercial vaccines containing formalin-killed bacteria, but the protection is often suboptimal under Danish mariculture conditions. We elucidated whether protection can be improved by increasing the concentration of antigen (formalin-killed bacteria) in the vaccine. Rainbow trout juveniles were vaccinated by intraperitoneal (i.p.) injection with a bacterin of Aeromonas salmonicida subsp. salmonicida strain 090710-1/23 in combination with Vibrio anguillarum serotypes O1 and O2a supplemented with an oil adjuvant. Three concentrations of AS antigens were applied. Fish were subsequently challenged with the homologous bacterial strain administered by perforation of the tail fin epidermis and 60-s contact with live A. salmonicida bacteria. The infection method proved to be efficient and could differentiate efficacies of different vaccines. It was shown that protection and antibody production in exposed fish were positively correlated to the AS antigen concentration in the vaccine.

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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, University of Copenhagen, Vaxxinova Norway AS
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Aeromonas salmonicida subsp. salmonicida is the etiological agent of furunculosis and a major fish health problem in salmonid aquaculture worldwide. Injection vaccination with commercial mineral oil-adjuvanted bacterin vaccines has been partly successful in preventing the disease but in Danish rainbow trout (Oncorhynchus mykiss, Walbaum) aquaculture furunculosis outbreaks still occur. In this study we tested the efficacy of experimental subunit vaccines against A. salmonicida infection in rainbow trout. We utilized in silico screening of the proteome of A. salmonicida subsp. salmonicida strain A449 and identified potential protective protein antigens that were tested by in vivo challenge trial. A total of 14 proteins were recombinantly expressed in Escherichia coli and prepared in 3 different subunit vaccine combinations to immunize 3 groups of rainbow trout by intraperitoneal (i.p.) injection. The fish were exposed to virulent A. salmonicida 7 weeks after immunization. To assess the efficacy of the subunit vaccines we evaluated the immune response in fish after immunization and challenge infection by measuring the antibody levels and monitoring the survival of fish in different groups. The survival of fish at 3 weeks after challenge infection showed that all 3 groups of fish immunized with 3 different protein combinations exhibited significantly lower mortalities (17-30%) compared to the control groups (48% and 56%).
The ELISA results revealed significantly elevated antibody levels in fish against several protein antigens, which in some cases were positively correlated to the survival.

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Scopus rating (2016): CiteScore 3.11 SJR 1.201 SNIP 1.092
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.545 SNIP 1.141 CiteScore 3.54
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 1.74 SNIP 1.147 CiteScore 3.94
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.945 SNIP 1.142 CiteScore 4.15
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BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.369 SNIP 1.23 CiteScore 4.58
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
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BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.473 SNIP 0.985
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Aeromonas salmonicida - Epidemiology, whole genome sequencing, detection and in vivo imaging

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 real-time 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.
A New Furunculosis Challenge Method for Evaluation of Vaccine Efficacy in Rainbow Trout

Experimental infection of fish for vaccine efficacy studies is associated with several limitations. Administration of live bacteria with the purpose of causing disease in fish can be performed by co-habitation, immersion or injection. We have developed a new Aeromonas salmonicida challenge method for rainbow trout and have applied it for evaluation of furunculosis vaccine efficacy. The method reveals development of systemic immunity in fish as live bacteria are introduced in the tail fin epidermis distant from the vaccine injection site (peritoneal cavity). This method seeks to mimic natural infection in fish farms where tail biting and therefore bacterial exposure to tail fin ulcers is widespread. By use of a multi-needle device ten epidermal perforations were introduced in the dorsal part of the tail fin of anaesthetized rainbow trout (vaccinated or naive). Subsequently 100 μL (3.4 × 10^8 colony-forming units (CFU) mL⁻¹) of a 48 hour culture of Aeromonas salmonicida subsp. salmonicida strain 090710-1/23 was placed at the perforation site for 60 sec whereafter fish were allowed to regain consciousness in clean freshwater. Immunohistochemistry and scanning electron microscopy illustrated the spread of bacteria from the injection site. Classical furunculosis symptoms associated with a high morbidity rate were observed in control fish whereas vaccinated fish exhibited a significantly higher survival.

Antigen Uptake during Different Life Stages of Zebrafish (Danio rerio) Using a GFP-Tagged Yersinia ruckeri

Immersion-vaccines (bacterins) are routinely used for aquacultured rainbow trout to protect against Yersinia ruckeri (Yr). During immersion vaccination, rainbow trout take up and process the antigens, which induce protection. The zebrafish was used as a model organism to study uptake mechanisms and subsequent antigen transport in fish. A genetically modified Yr was developed to constitutively express green fluorescent protein (GFP) and was used for bacterin production. Larval, juvenile and adult transparent zebrafish (tra:nac mutant) received a bath in the bacterin for up to 30 minutes. Samples were taken at 1 min, 15 min, 30 min, 2 h, 12 h and 24 h. At each sampling point fish were used for live imaging of the uptake using a fluorescence stereomicroscope and for immunohistochemistry (IHC). In adult fish, the bacterin could be traced within 30 min in scale pockets, skin, oesophagus, intestine and fins. Within two hours post bath (pb) Yr-antigens were visible in the spleen and at 24 h in liver and kidney. Bacteria were associated with the gills, but uptake at this location was limited. Antigens were rarely detected in the blood and never in the nares. In juvenile fish uptake of the bacterin was seen in the intestine 30 min pb and in the nares 2 hpb but never in scale pockets. Antigens were detected in the spleen 12 hpb. Zebrafish larvae exhibited major Yr uptake only in the mid-intestine enterocytes 24 hpb. The different life stages of zebrafish varied with regard to uptake locations, however the gut was consistently a major uptake site. Zebrafish and rainbow trout tend to have similar uptake mechanisms following immersion or bath vaccination, which points towards...
zebrafish as a suitable model organism for this aquacultured species.

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Comparative Genome Analysis Provides Insights into the Pathogenicity of Flavobacterium psychrophilum

Flavobacterium psychrophilum is a fish pathogen in salmonid aquaculture worldwide that causes cold water disease (CWD) and rainbow trout fry syndrome (RTFS). Comparative genome analyses of 11 F. psychrophilum isolates representing temporally and geographically distant populations were used to describe the F. psychrophilum pan-genome and to examine virulence factors, prophages, CRISPR arrays, and genomic islands present in the genomes. Analysis of the genomic DNA sequences were complemented with selected phenotypic characteristics of the strains. The pan genome analysis showed that F. psychrophilum could hold at least 3373 genes, while the core genome contained 1743 genes. On average, 67 new genes were detected for every new genome added to the analysis, indicating that F. psychrophilum possesses an open pan genome. The putative virulence factors were equally distributed among isolates, independent of geographic location, year of isolation and source of isolates. Only one prophage-related sequence was found which corresponded to the previously described prophage 6H, and appeared in 5 out of 11 isolates. CRISPR array analysis revealed two different loci with dissimilar spacer content, which only matched one sequence in the database, the temperate bacteriophage 6H. Genomic Islands (GIs) were identified in F. psychrophilum isolates 950106-1/1 and CSF 259-93, associated with toxins and antibiotic resistance. Finally, phenotypic characterization revealed a high degree of similarity among the strains with respect to biofilm formation and secretion of extracellular enzymes. Global scale dispersion of virulence factors in the genomes and the abilities for biofilm formation, hemolytic activity and secretion of extracellular enzymes among the strains suggested that F. psychrophilum isolates have a similar mode of action on adhesion, colonization and destruction of fish tissues across large spatial and temporal scales of occurrence. Overall, the genomic characterization and phenotypic properties may provide new insights to the mechanisms of pathogenicity in F. psychrophilum.

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Effect of Bacteriophages on the Growth of Flavobacterium psychrophilum and Development of Phage-Resistant Strains

The controlling effect of single and multiple phages on the density of Flavobacterium psychrophilum at different initial multiplicity of infection (MOI) was assessed in batch cultures to explore the potential for phage-based treatment of this important fish pathogen. A high initial phage concentration (MOI = 0.3–4) was crucial for efficient viral lysis, resulting in a 104–105-fold reduction of phage-sensitive cells (both single phages and phage cocktails), which was maintained throughout the incubation (>10 days). Following cell lysis, regrowth of phage-resistant strains was examined and resistant strains were isolated for further characterization. The application of a mathematical model allowed simulation of phage-host interactions and resistance development, confirming indications from strain isolations that phage-sensitive strains dominated the regrowing population (>99.8 %) at low MOI and phage-resistant strains (>87.8 %) dominated at high MOI.

A cross-infectivity test covering 68 isolated strains and 22 phages resulted in 23 different host susceptibility patterns, with 20 of the isolates being resistant to all the applied phages. Eleven isolated strains with different susceptibility patterns had lower growth rates (0.093 to 0.31 h−1) than the host strain (0.33 h−1), while 10 of 14 examined strains had lost the ability to take up specific substrates as shown by BIOLOG profiles. Despite increased selection for phage resistance at high MOI, the results emphasize that high initial MOI is essential for fast and effective control of F. psychrophilum infection and suggest that the small populations of resistant clones had reduced competitive abilities relative to the sensitive ancestral strain.

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Epidemiological cut-off values for Flavobacterium psychrophilum MIC data generated by a standard test protocol

Epidemiological cut-off values were developed for application to antibiotic susceptibility data for Flavobacterium psychrophilum generated by standard CLSI test protocols. The MIC values for ten antibiotic agents against Flavobacterium psychrophilum were determined in two laboratories. For five antibiotics, the data sets were of sufficient quality and quantity to allow the setting of valid epidemiological cut-off values. For these agents, the cut-off values, calculated by the application of the statistically based normalized resistance interpretation method, were ≤16 mg L\(^{-1}\) for erythromycin, ≤2 mg L\(^{-1}\) for florfenicol, ≤0.025 mg L\(^{-1}\) for oxolinic acid (OXO), ≤0.125 mg L\(^{-1}\) for oxytetracycline and ≤20 (1/19) mg L\(^{-1}\) for trimethoprim/sulphamethoxazole. For ampicillin and amoxicillin, the majority of putative wild-type observations were 'off scale', and therefore, statistically valid cut-off values could not be calculated. For ormetoprim/sulphadimethoxine, the data were excessively diverse and a valid cut-off could not be determined. For flumequine, the putative wild-type data were extremely skewed, and for enrofloxacin, there was inadequate separation in the MIC values for putative wild-type and non-wild-type strains. It is argued that the adoption of OXO as a class representative for the quinolone group would be a valid method of determining susceptibilities to these agents.
Oil adjuvant elevates protection of rainbow trout (Oncorhynchus my-kiss) following injection vaccination against Yersinia ruckeri

Enteric redmouth disease (ERM) caused by the fish pathogen Yersinia ruckeri is a major threat to freshwater production of rainbow trout throughout all life stages. Injection vaccination of rainbow trout against Y. ruckeri infection has been shown to confer better protection compared to the traditionally applied immersion vaccination. It may be hypothesized, based on experience from other vaccines, that adjuvants may increase the protective level of ERM injection vaccines even more. Controlled comparative vaccination studies conducted to investigate effects of the oil adjuvant Montanide™ ISA 763 A VG when added to an experimental Y. ruckeri bacterin (containing both biotype 1 and 2 of serotype O1). A total of 1000 fish with mean weight 19 g was divided into five different groups (in duplicated tanks 2 • 100 fish per group) 1) non-vaccinated control fish (NonVac), 2) fish injected with a commercial vaccine (AquaVac® Relera™) (ComVac), 3) fish injected with an experimental vaccine (ExpVac), 4) fish injected with an experimental vaccine + adjuvant (ExpVacAdj) and 5) fish injected with adjuvant alone (Adj). Injection of the experimental vaccine (both adjuvanted and non-adjuvanted) induced a significantly higher antibody (IgM) level, increased occurrence of IgM+ cells in spleen tissue and significant up-regulation of several immune genes. Additional experiments using a higher challenge dosage suggested an immune enhancing effect of the adjuvant as the challenge produced 100% mortality in the NonVac group, 60% mortality in both of ComVac and Adj groups and only 13 and 2.5% mortalities in the ExpVac and the ExpVacAdj groups, respectively.

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Testing experimental subunit furunculosis vaccines for rainbow trout

Aeromonas salmonicida subsp. salmonicida (AS) is the etiological agent of typical furunculosis in salmonid fish. The disease causes bacterial septicemia and is a major fish health problem in salmonid aquaculture worldwide, inducing high morbidity and mortality. In this study we vaccinated rainbow trout with subunit vaccines containing protein antigens that were selected based on an in silico antigen discovery approach. Thus, the proteome of AS strain A449 was analyzed by
an antigen discovery platform and its proteins consequently ranked by their predicted ability to evoke protective immune response against AS. Fourteen proteins were prepared in 3 different experimental subunit vaccine combinations and used to vaccinate rainbow trout by intraperitoneal (i.p.) injection. We tested the proteins for their ability to elicit antibody production and protection. Thus, fish were exposed to virulent AS 7 weeks post-vaccination by applying a novel, multi-puncture challenge method. The immune response in fish was evaluated following vaccination and challenge by measuring antibody levels and recording survival. The control group showed 56 % mortality whereas the groups of fish vaccinated with experimental subunit vaccines exhibited significantly lower mortalities (17-30 %). These results imply that in silico-predicted protective protein antigens of AS have significant protective properties and should be considered for further validation as potential candidates for a subunit vaccine against furunculosis.
Aeromonas salmonicida infection in vaccinated rainbow trout: influence of challenge methods and environmental factors on challenge success

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A novel challenge method with aeromonas salmonicida in rainbow trout for evaluation of furunculosis vaccines

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Bacteriophage Resistance Mechanisms in the Fish Pathogen Flavobacterium psychrophilum: Linking Genomic Mutations to Changes in Bacterial Virulence Factors

Flavobacterium psychrophilum is an important fish pathogen in salmonid aquaculture worldwide. Due to increased antibiotic resistance, pathogen control using bacteriophages has been explored as a possible alternative treatment. However, the effective use of bacteriophages in pathogen control requires overcoming the selection for phage resistance in the bacterial populations. Here, we analyzed resistance mechanisms in F. psychrophilum after phage exposure using whole-genome sequencing of the ancestral phage-sensitive strain 950106-1/1 and six phage-resistant isolates. The phage-resistant strains had all obtained unique insertions and/or deletions and point mutations distributed among intergenic and genic regions. Mutations in genes related to cell surface properties, gliding motility, and biosynthesis of lipopolysaccharides and cell wall were found. The observed links between phage resistance and the genetic modifications were supported by direct measurements of bacteriophage adsorption rates, biofilm formation, and secretion of extracellular enzymes, which were all impaired in the resistant strains, probably due to superficial structural changes. The clustered regularly interspaced short palindromic repeat (CRISPR) region was unaffected in the resistant isolates and thus did not play a role as a resistance mechanism for F. psychrophilum under the current conditions. All together, the results suggest that resistance in F. psychrophilum was driven by spontaneous mutations, which were associated with a number of derived effects on the physiological properties of the pathogen, including reduced virulence under in vitro conditions. Consequently, phage-driven physiological changes associated with resistance may have implications for the impact of the pathogen in aquaculture, and these effects of phage resistance on host properties are therefore important for the ongoing exploration of phage-based control of F. psychrophilum.
Booster immersion vaccination using diluted Yersinia ruckeri bacterin confers protection against ERM in rainbow trout

A single immersion vaccination of rainbow trout fry using a Yersinia ruckeri bacterin confers immunity to reinfection but only for a shorter period. A longer protective period is needed in practical trout farming and we have shown that booster vaccination prolongs immunity. Due to economic considerations and management practices it is not possible to immersion vaccinate large trout (20–30 g) with the recommended high bacterin concentration. We here demonstrate that booster vaccination using dilutions of the bacterin (1:100, 1:1000 and 1:2000) with increased exposure time (1 h, 2 h) confers a higher and longer lasting immunity although a short term (30 s) booster in 1:10 bacterin proved superior. This has practical implications for fish farmers because this diluted booster vaccination over longer time can be applied when farmers handle and transport fish between ponds and farms. Therefore such a practice will not challenge farm management and economy. Increased antibody levels were recorded after challenge of vaccinated fish but not after immersion vaccination alone which suggests that immersion induces priming of memory cells.
Comparative evaluation of infection methods and environmental factors on challenge success: Aeromonas salmonicida infection in vaccinated rainbow trout

When testing vaccine-induced protection an effective and reliable challenge method is a basic requirement and we here present a comparative study on different challenge methods used for infection of rainbow trout Oncorhynchus mykiss with Aeromonas salmonicida, a bacterial pathogen eliciting furunculosis. Fish were vaccinated with three different adjuvanted trivalent vaccines containing formalin killed A. salmonicida, Vibrio anguillarum O1 and O2a. These were 1) the commercial vaccine Alpha Ject 3000, 2) an experimental vaccine with water in paraffin oil adjuvant, 3) an experimental vaccine with water in paraffin oil in water adjuvant. Fish were then exposed to A. salmonicida challenge using i.p. injection, cohabitation in freshwater, cohabitation in saltwater (15 ppt) or combined fresh/saltwater cohabitation. Cohabitation reflects a more natural infection mode and was shown to give better differentiation of vaccine types compared to i.p. injection of live bacteria. The latter infection mode is less successful probably due to the intra-abdominal inflammatory reactions (characterized in this study according to the Speilberg scale) induced by i.p. vaccination whereby injected live bacteria more effectively become inactivated at the site of injection. Compared to cohabitation in freshwater, cohabitation in saltwater was less efficient probably due to reduced survivability of A. salmonicida in saltwater, which was also experimentally verified in vitro.
Danish sea reared rainbow trout suffer from furunculosis despite vaccination - How can applied research help to solve the problem?

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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, Aarhus University, Danish Aquaculture Organisation, University of Copenhagen, Vaxxinova
Authors: Skall, H. F. (Ekstern), Lorenzen, E. (Ekstern), Kjær, T. E. (Ekstern), Henriksen, N. (Ekstern), Dalsgaard, I. (Intern), Madsen, S. B. (Ekstern), Buchmann, K. (Ekstern), Krossøy, B. (Ekstern), Lorenzen, N. (Ekstern)
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Detection of aeromonas salmonicida in fish tissue by real-time PCR

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Effect of hydrogen peroxide and/or Flavobacterium psychrophilum on the gills of rainbow trout, Oncorhynchus mykiss (Walbaum)
The immune response and morphological changes in the gills of rainbow trout fry after immersion in hydrogen peroxide (H2O2), Flavobacterium psychrophilum or combined exposure were examined. The gills were sampled 4, 48, 125 and 192 h after exposure, and the regulation of expression of the following genes was investigated using qPCR: IgT, IgM, CD8, CD4, MHC I, MHC II, IL-4/13A, TcR-β, IL-10, IL-1β, IL-17, SAA and FoxP3. Bacteria were not observed in haematoxylin-and-eosin-stained gill tissue, but the presence of F. psychrophilum 16S rRNA was detected using qPCR. The 16S rRNA levels were correlated with gene expression. Although pretreatment with H2O2 before immersion in F. psychrophilum did not significantly alter the amount of bacteria found in the gill, the immune response was influenced: exposure to F. psychrophilum resulted in a negative correlation with expression of IL-17c1, MHC I and MHC II, while pretreatment with H2O2 resulted in a positive correlation with IL-4/13A and IgM. Exposure to either H2O2 or F. psychrophilum influenced the regulation of gene expression and damaged tissue. Exposure to both combined altered the immune response to infection and postponed healing of gill tissue.

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Effects of adjuvant Montanide™ ISA 763 A VG in rainbow trout injection vaccinated against Yersinia ruckeri

Enteric redmouth disease (ERM) caused by the fish pathogen Yersinia ruckeri is a major threat to freshwater production of rainbow trout (Oncorhynchus mykiss) throughout all life stages. Injection vaccination of rainbow trout against Y. ruckeri infection has been shown to confer better protection compared to the traditionally applied immersion vaccination. It may be hypothesized, based on experience from other vaccines, that adjuvants may increase the protective level of ERM injection vaccines even more. Controlled comparative vaccination studies have been performed to investigate effects of the oil adjuvant Montanide™ ISA 763 A VG (Seppic) when added to an experimental Y. ruckeri bacterin (containing both biotype 1 and 2 of serotype O1). A total of 1000 fish with mean weight 19 g was divided into five different groups (in duplicated tanks 2 × 100 fish per group) 1) non-vaccinated control fish (NonVac), 2) fish injected with a commercial vaccine (AquaVac® Reiela™) (ComVac), 3) fish injected with an experimental vaccine (ExpVac), 4) fish injected with an experimental vaccine + adjuvant (ExpVacAdj) and 5) fish injected with adjuvant alone (Adj). Injection of the experimental vaccine (both adjuvanted and non-adjuvanted) induced a significantly higher antibody (IgM) level, increased occurrence of IgM(+) cells in spleen tissue and significant up-regulation of several immune genes. Additional experiments using a higher challenge dosage suggested an immune enhancing effect of the adjuvant as the challenge produced 100% mortality in the NonVac group, 60% mortality in both of ComVac and Adj groups and only 13 and 2.5% mortalities in the ExpVac and the ExpVacAdj groups, respectively.

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ERM immersion vaccination and adjuvants

Two candidate adjuvants were tested with a commercial ERM dip vaccine (AquaVac™ Relera, MSD Animal Health) for rainbow trout in an experimental design compatible with common vaccination practices at farm level, i.e. immersion of fish in vaccine (±adjuvant) for 30 s. The adjuvants were the commercial product Montanide™ IMS 1312 VG PR (SEPPIC), and a soluble and ≥98% pure β-glucan from yeast (Saccharomyces cerevisiae) (Sigma-Aldrich). Hence, five experimental groups in duplicate were established and exposed to vaccine and adjuvants in the following combinations: AquaVac™ Relera (alone); AquaVac™ Relera + Montanide™; AquaVac™ Relera + β-glucan; Montanide™ (alone); and β-glucan (alone). Approximately 450 degree days post-vaccination, the fish were bath-challenged with live Yersinia ruckeri to produce survival curves. Blood, skin and gills were sampled at selected time points during the course of the experiment to test for plasma Ab levels and lysozyme activity, and the regulation of immune relevant genes and cells in external, mucosal tissues. Preliminary results show 96% to 100% survival of vaccinated fish with and without any of the two adjuvants, whereas unvaccinated controls and fish exposed to β-glucan alone experienced 58% and 60% survival, respectively (calculated at day 24 post-challenge). Montanide™ alone gave rise to an intermediate level of 72% survival. Lysozyme activity levels in plasma were markedly elevated at day 3 and day 24 post-challenge in fish exposed to Montanide™ alone or β-glucan alone compared to fish from any of the three vaccinated groups.

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Evaluation of the immune response in rainbow trout fry, Oncorhynchus mykiss (Walbaum), after waterborne exposure to Flavobacterium psychrophilum and/or hydrogen peroxide

The immune response in rainbow trout fry against Flavobacterium psychrophilum was elucidated using an immersion-based challenge with or without prior exposure to hydrogen peroxide (H2O2). Samples were taken from the head kidney 4, 48, 125 and 192 h after immersion, and the regulation of several genes was examined. Bacterial load was assessed based on the presence of 16S rRNA and correlated with gene expression, and the levels of specific antibodies in the blood were measured 50 days post-infection. Separately, both H2O2 and F. psychrophilum influenced gene expression, and pre-treatment with H2O2 influenced the response to infection with F. psychrophilum. Pre-treatment with H2O2 also affected correlation between gene regulation and pathogen load for several genes. A delay in antibody production in H2O2-treated fish in the early phase of infection was indicated, but H2O2 exposure did not affect antibody levels 50 days post-infection. An increasing amount of F. psychrophilum 16S rRNA was found in the head kidneys of infected fish pre-treated with H2O2 relative to the F. psychrophilum group. The results show that a single pre-treatment with H2O2 impairs the response against F. psychrophilum and may intensify infection.

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Matrix-assisted laser desorption/ionization time of flight, MALDI-TOF, mass spectrometry for identification of fish pathogenic bacteria

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Authors: Jansson, E. (Ekstern), Eriksson, E. (Ekstern), Säker, E. (Ekstern), Dalsgaard, I. (Intern), Nonnemann, B. (Intern), Roozenburg, I. (Ekstern), Haenen, O. (Ekstern)
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Matrix-assisted laser desorption/ionization time of flight, MALDI-TOF, mass spectrometry for identification of fish pathogenic bacteria

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Matrix-assisted laser desorption/ionization time of flight, maldi-tof. mass spectrometry for identification of fish pathogenic bacteria

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Sygdom og sundhed

General information
Variable Number of Tandem Repeats (VNTR) analysis of Flavobacterium psychrophilum from salmonids in Chile and Norway

Background: Flavobacterium psychrophilum causes serious fish diseases such as RTFS and BCWD, affecting the aquaculture industry worldwide. Commercial vaccines are not available and control of the disease depends on the use of antibiotics. Reliable methods for detection and identification of different isolates of this bacterium could play an important role in the development of good management strategies. The aim of this study was to identify genetic markers for discrimination between isolates. A selection of eight VNTRs from 53 F. psychrophilum isolates from Norway, Chile, Denmark and Scotland were analyzed. The results were compared with previous work on the same pathogen using MLST for genetic differentiation.

Results: The VNTR analysis gave a separation between the F. psychrophilum isolates supporting the results of previous MLST work. A higher diversity was found among the Chilean isolates compared to those from Norway, which suggests a more homogenous reservoir in Norway. Transgenerational transmission of F. psychrophilum from other countries, exporting salmon embryos to Chile, may explain the differences in diversity. The same transmission mechanisms could also explain the wide geographical distribution of identical isolates in Norway. But, this could also be a result of movement of smolts and embryos. The selected VNTRs are stable genetic markers and no variation was observed after several passages on agar plates at different temperatures.

Conclusions: These VNTRs are important additions for genotyping of F. psychrophilum isolates. Future studies on VNTRs of F. psychrophilum should include isolates from more host species from a wider geographical area. To get a more robust genotyping the VNTRs should be used in concert with MLST. Future studies of isolates with high and low virulence should focus on identifying virulence markers using VNTRs and MLST.
Detection and Quantification of Flavobacterium psychrophilum-Specific Bacteriophages In Vivo in Rainbow Trout upon Oral Administration: Implications for Disease Control in Aquaculture.

The use of bacteriophages in the treatment and prevention of infections by the fish pathogen Flavobacterium psychrophilum has attracted increased attention in recent years. It has been shown recently that phage delivery via the parenteral route resulted in immediate distribution of phages to the circulatory system and the different organs. However, little is known about phage dispersal and survival in vivo in rainbow trout after delivery via the oral route. Here we examined the dispersal and survival of F. psychrophilum phage FpV-9 in vivo in juvenile rainbow trout after administration by three different methods-bath, oral intubation into the stomach, and phage-coated feed-with special emphasis on the oral route of delivery. Phages could be detected in all the organs investigated (intestine, spleen, brain, and kidney) 0.5 h postadministration, reaching concentrations as high as ~10(5) PFU mg intestine(-1) and ~10(3) PFU mg spleen(-1) within the first 24 h following the bath and ~10(7) PFU mg intestine(-1) and ~10(4) PFU mg spleen(-1) within the first 24 h following oral intubation. Phages were most persistent in the organs for the first 24 h and then decreased exponentially; no phages were detected after 83 h in the organs investigated. Phage administration via feed resulted in the detection of phages in the intestine, spleen, and kidney 1 h after feeding. Average concentrations of ~10(4) PFU mg intestine(-1) and ~10(1) PFU mg spleen(-1) were found throughout the experimental period (200 h) following continuous delivery of phages with feed. These experiments clearly demonstrate the ability of the phages to survive passage through the fish stomach and to penetrate the intestinal barrier and enter the circulatory system after oral delivery, although the quantity of phages found in the spleen was 100- to 1,000-fold lower than that in the intestine. It was also shown that phages could tolerate long periods of desiccation on the feed pellets, with 60% survival after storage at -80°C, and 10% survival after storage at 5°C, for ~8 months. Continuous delivery of phages via coated feed pellets constitutes a promising method of treatment and especially prevention of rainbow trout fry syndrome.
Diet type dictates the gut microbiota and the immune response against Yersinia ruckeri in rainbow trout (Oncorhynchus mykiss)

This study investigated the influence of the rainbow trout (Oncorhynchus mykiss) commensal intestinal microbiota in connection to an experimental Yersina ruckeri infection, the causative agent of enteric redmouth disease. One marine and one plant diet was administered to two different groups of rainbow trout. The plant-based diet gave rise to an intestinal microbiota dominated by the genera Streptococcus, Leuconostoc and Weissella from phylum Firmicutes whereas phylum Proteobacteria/Bacteroidetes/Actinobacteria dominated the community in the marine fed fish. In connection to the Y. ruckeri bath challenge there was no effect of the diet type on the cumulative survival, but the number of Y. ruckeri positive fish as measured by plate count and the number of fish with a 'high' number of reads belonging to genus Yersinia as measured by 16S rRNA next-generation sequencing was higher for marine diet fed fish. Furthermore, the two experimental groups of fish showed a differential immune response, where Y. ruckeri challenged marine fed fish had a higher transcription of IL-1β and MBL-2 relative to challenged plant diet fed fish. The data suggest that the plant diet gave rise to a prebiotic effect favouring the presence of bacterial taxons proving protective in connection to bath challenge by Y. ruckeri.

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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, University of Copenhagen
Authors: Ingerslev, H. (Intern), Strube, M. L. (Intern), Jørgensen, L. V. G. (Forskerdatabase), Dalsgaard, I. (Intern), Boye, M. (Intern), Madsen, L. (Intern)
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Multilocus Sequence Typing Identifies Epidemic Clones of Flavobacterium psychrophilum in Nordic Countries

Flavobacterium psychrophilum is the causative agent of bacterial cold water disease (BCWD), which affects a variety of freshwater-reared salmonid species. A large-scale study was performed to investigate the genetic diversity of F. psychrophilum in the four Nordic countries: Denmark, Finland, Norway, and Sweden. Multilocus sequence typing of 560 geographically and temporally disparate F. psychrophilum isolates collected from various sources between 1983 and 2012 revealed 81 different sequence types (STs) belonging to 12 clonal complexes (CCs) and 30 singleton STs. The largest CC, CC-ST10, which represented almost exclusively isolates from rainbow trout and included the most predominant genotype, ST2, comprised 65% of all isolates examined. In Norway, with a shorter history (<10 years) of BCWD in rainbow trout, ST2 was the only isolated CC-ST10 genotype, suggesting a recent introduction of an epidemic clone. The study identified five additional CCs shared between countries and five country-specific CCs, some with apparent host specificity. Almost 80% of the singleton STs were isolated from non-rainbow trout species or the environment. The present study reveals a simultaneous presence of genetically distinct CCs in the Nordic countries and points out specific F.
psychrophilum STs posing a threat to the salmonid production. The study provides a significant contribution toward mapping the genetic diversity of F. psychrophilum globally and support for the existence of an epidemic population structure where recombination is a significant driver in F. psychrophilum evolution. Evidence indicating dissemination of a putatively virulent clonal complex (CC-ST10) with commercial movement of fish or fish products is strengthened.
Rainbow trout fed diets with varying content of marine and plant origin; how does that influence the outcome of experimental infections of the fry with *Flavobacterium psychrophilum* and *Yersinia ruckeri*?

Feed for rainbow trout aquaculture has traditionally been based on marine resources such as fish meal and fish oil. Because of a shortage of marine resources as well as the growing production of farmed fish, the feed industry has been forced to partially exchange fish meal protein with proteins derived from plants, like soy bean meal. This has been shown to affect the salmonid intestinal mucosa, and in addition, plant-based dietary proteins have been associated with changes in disease susceptibility in salmon and it has been suggested that these special diet types weakens the immune status of the fish. One major cause for losses in Danish freshwater fish farms is the fry disease rainbow trout fry syndrome (RTFS), caused by the bacterium *Flavobacterium psychrophilum*, and experiences of the fish farmers suggest that the diet type is an important factor for disease development. Enteric redmouth disease caused by *Yersinia ruckeri* is also an economically important disease which causes problems in rainbow trout fry as well as larger fish. Rainbow trout were fed from first-feeding with five different diets; diet A with marine fish oil (conventional fry diet), diet B (an organic version of A), diet C with rape seed oil (like B but with rape seed oil exchanging marine fish oil), diet C with pea protein (like B but added pea protein) and diet E with rape seed oil and pea protein. When the fry had reached sizes 1.5 g and 4 g, groups of fish from the five diet groups were infected with *Flavobacterium psychrophilum* and *Yersinia ruckeri*, respectively. An intraperitoneal injection model was used for *F. psychrophilum*, whereas a bath challenge was used for *Y ruckeri*. Before and after infection, samples were taken from internal organs including the intestine for traditional bacteriology and only intestinal samples for next generation sequencing. The cumulative mortalities among the diet groups did not differ between groups in either of the two infection trials, suggesting that the diets did not have different effects on the immune status of the fish, when it comes to survival after infection, meaning that plant content did not seem to weaken the immune status of the rainbow trout fry. Results of the trial as well as the bacteriological examinations and the next generation sequencing results will be presented.

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Organisations: National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology
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The development of the gut microbiota in rainbow trout (Oncorhynchus mykiss) is affected by first feeding and diet type
An influence of the intestinal microbiota in connection to first-feeding of rainbow trout (Oncorhynchus mykiss) fry was demonstrated using Illumina HiSeq sequencing. The period from the end of yolk-sac feeding to seven weeks post first-feeding was examined after administration of either a marine- or plant based diet with or without the probiont Pediococcus acidilactici. Before first feeding the main part of the sequence reads grouped to the genus Sediminibacterium probably originating from the surrounding water. The microbial abundance and diversity increased after first-feeding and the microbiota then changed towards phylum Firmicutes dominance for plant based fed fish and towards dominance of phylum Proteobacteria for the marine fed fish. After first-feeding, there were significantly higher abundances of Streptococcus, Leuconostoc and Weissella in fish fed the plant-based diet. The microbiota clustered separately according to the diet type, but only minor effects were seen from the probiont when using PCA-analysis. The constitutive transcription level of most examined immune genes increased during the ontogenic shift, but the results could not explain the differences in the composition of the microbiota dependent on diet treatment after first-feeding. The results suggest that the intestine of rainbow trout is colonised at an early state, but is guided in new and different directions dependent on the diet type.

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Rapid and accurate identification of microbial pathogens is a cornerstone for timely and correct treatment of diseases of livestock and fish. The utility of the MALDI-TOF technique in the diagnostic laboratory is directly related to the quality of mass spectra and quantity of different microbial species in the database. Since commercial MALDI-TOF spectral database providers mainly focus on human pathogens there is a need for improving the datasets in order to extend the applicability of the technique to the veterinary field. Here we report upgrading of a commercial MALDI-TOF database with the mass spectra of fish and mastitis pathogens as well as pathogens relevant for surveillance of diseases in farm animals and wildlife.

**General information**

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Authors: Nonnemann, B. (Intern), Dalsgaard, I. (Intern), Pedersen, K. (Intern), Andresen, L. O. (Intern), Kokotovic, B. (Intern)
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Vibriosis in aquaculture

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Authors: Haenen, O. (Ekstern), Fouz, B. (Ekstern), Amaro, C. (Ekstern), Isern, M. (Ekstern), Zrncic, S. (Ekstern), Travers, M. (Ekstern), Wardle, R. (Ekstern), Hellström, A. (Ekstern), Dalsgaard, I. (Intern)
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Scopus rating (2012): SJR 0.257 SNIP 0.49 CiteScore 0.47
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Scopus rating (2010): SJR 0.34 SNIP 0.469
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BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.325 SNIP 0.373
Scopus rating (2007): SJR 0.31 SNIP 0.386
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A simple and low-toxic method of preparing small specimens of bacteria, flagellates and their likes for Scanning Electron Microscopy

The preparation of samples of bacteria and other very small organisms (<50 μm) for Scanning Electron Microscopy is often complex and intricate, which typically involve the use of specialized filter systems, complex handling and toxic chemicals. Based on the methods described in the literature and our own tests, we have distilled a simpler (although slightly crude) method to prepare bacterial samples in a fast way. We only employ readily available chemicals requiring no more than a fume hood, and low-cost, standard lab equipment like single use filters. The method is excellent for achieving relatively quick results for illustration purposes and does not require handling of highly toxic substances like Osmium-tetroxide, which typically necessitates skilled/trained lab personnel. Thus, this method is well-suited for testing different bacterial concentrations, biotypes, and other variables relatively quickly. So far, this method has yielded good results on several pathogenic bacteria and parasites; Aeromonas salmonicida, Yersinia ruckeri, Ichthyobodo necator and theronts of Ichthyophthirius multifiliis.

Comparative evaluation of administration methods for a vaccine protecting rainbow trout against Yersinia ruckeri O1 biotype 2 infections

Numerous outbreaks of enteric red mouth disease (ERM) caused by Yersinia ruckeri O1 biotype 2 in rainbow trout farms are currently being recorded despite established vaccination procedures against this disease. This could indicate that the currently used application of single immersion vaccination (using a commercial vaccine AquaVac® RELERA™) does not provide full protection. We elucidated by a controlled duplicated experiment if different vaccine administration methods can improve level and extent of protection. Rainbow trout, Oncorhynchus mykiss were vaccinated by: (1) a single immersion in bacterin diluted 1:10 for 30s (only primary vaccination); (2) two times 30s immersion (primary immersion vaccination followed by booster immersion vaccination 1 month later); (3) a single i.p. injection (only primary vaccination); (4) immersion vaccination followed by injection booster 1 month later; (5) a single 1h bath in bacterin diluted 1:2000; and (6) immersion (30s, 1:10) plus booster (1h in diluted 1:2000 vaccine) 5 months later. Injection challenge experiments were performed 3, 5 and 7 months post primary vaccination with 8.5×10⁶CFU/fish, 10.6×10⁶CFU/fish and 1×10⁸CFU/fish, respectively. In the first challenge trial, control fish exhibited a mortality of 76%, one time immersion group had a 37% mortality, two times immersion vaccinated fish had a 4% mortality, the one-time injection vaccinated group showed a mortality of 2% and the immersion plus injection boostered fish showed no mortality at all. When rainbow trout were challenged 5 months post primary vaccination, 26% mortality occurred in control fish, 21% in one time immersion group, 12% in two times immersion group, 5% in the one-time injection vaccinated group whereas immersion plus injection boostered fish again showed no mortality at all. When challenged 7 months post vaccination, one-time immersion vaccinated were not protected at all compared to the control group whereas injection vaccinated fish showed lower
mortality (17%) compared to booster immersed fish (32% mortality) which was still better than un-vaccinated controls (44% mortality). It was noteworthy that a diluted bacterin (1:2000 for 1h after 5 months post primary vaccination) booster showed the same effect as a booster with 1:10 bacterin dilution for 30s applied 1 month after primary vaccination. Antibody levels showing significant elevations 28 days post challenge in vaccinated fish point to this immune parameter as a protective element. The superior and extended protection offered by booster vaccination or simply injection is noteworthy and may be applied in future vaccination strategies at farm level.

**General information**

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**Organisations:** National Veterinary Institute, Section for Bacteriology, Pathology and Parasitology, University of Copenhagen  
**Authors:** Chettri, J. K. (Forskerdatabase), Deshmukh, S. (Forskerdatabase), Holten-Andersen, L. (Intern), Jafaar, R. M. (Ekstern), Dalsgaard, I. (Intern), Buchmann, K. (Intern)  
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Scopus rating (2014): SJR 0.768 SNIP 0.719 CiteScore 1.6  
Web of Science (2014): Indexed yes  
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Scopus rating (2013): SJR 0.808 SNIP 0.805 CiteScore 1.89  
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Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 2  
Scopus rating (2012): SJR 0.837 SNIP 0.922 CiteScore 2.15  
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Web of Science (2012): Indexed yes  
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Scopus rating (2011): SJR 0.849 SNIP 0.996 CiteScore 2.16  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 2  
Scopus rating (2010): SJR 0.77 SNIP 0.945  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 2  
Scopus rating (2009): SJR 0.768 SNIP 0.852  
Web of Science (2009): Indexed yes  
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Detection and quantification of Flavobacterium psychrophilum specific bacteriophages in rainbow trout upon different administration methods: Implications for disease control in aquaculture

Flavobacterium psychrophilum is the pathogen causing the disease rainbow trout fry syndrome (RTFS), which has important implications for aquaculture production and trade worldwide. RTFS can be treated by antibiotic administration, but with the increasing problem of antibiotic resistant bacteria, the use of lytic bacteriophages is a promising alternative approach to disease control in aquaculture. Bacteriophage control of bacterial infections depends on efficient delivery of the phages to the infected organs, and in this study we therefore examined the occurrence and persistence of phages in the internal organs in rainbow trout, following different administration methods. Three phage administration methods using phage FpV-9 were used: phage bath, oral administration of phagesuspension directly into the stomach and feeding with phage-coated feed pellets. Phages were detected in all the four examined organs (intestine, brain, spleen and liver) with all three administration methods, demonstrating that the phages are capable of passing the intestinal wall and entering the bloodstream. The highest phage concentration was found in the intestine where a maximum of 3x10^{10} phages g^{-1} was obtained after oral administration of phage-suspension, but also phage addition via phage-coated feed pellets resulted in high phage titers (5x10^{6} phages g^{-1} intestine). The concentration of phages in the spleen was 100 fold lower than in the intestine, suggesting a large phage decay during transport to the inner organs. These results provide the basis for future phage treatment of RTFS.

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Authors: Christiansen, R. H. (Intern), Madsen, L. (Intern), Dalsgaard, I. (Intern), Middelboe, A. (Ekstern)
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Diet type and challenge by *Yersinia Ruckeri* influence the intestinal microbiota in rainbow trout (*Oncorhynchus Mykiss*)

In warm-blooded animals such as humans and pigs the intestinal microbial composition is dependent on the type of ingested diet. It is known that it also influences the immune system and prevent colonization of pathogenic bacteria. The question is if the gut microbiota has the same impact in lower vertebrates such as fish?

To examine this rainbow trout fry were fed two different diets of either a marine or vegetable origin. At a size of about four gram a subset of the fish was bath challenged by *Yersinia ruckeri* serotype O1 and intestines were then sampled 5 days post challenge from challenged fish (n = 40) and non-challenged control fish (n = 40). Subsequent metagenomic examination based on the 16S rDNA gene was then performed using the Illumina HiSeq 2000 platform. Obtained sequences were paired, sorted, filtered, checked for chimeras and finally mapped against the Greengene database using the open-source package Bion-meta. Analysis of the microbial communities when then made for the non-infected control fish and *Yersinia ruckeri* challenged fish.

For the non-infected control fish, the results showed two distinctly different microbial patterns in the intestines dependent on the administered diet type. Fish fed a marine based diet overall had a significantly higher amount of the class β-proteobacteria, while phylum Firmicutes was significantly higher abundant in the intestines of vegetable fed fish. The genera within phylum Firmicutes present in significantly higher amounts in vegetable fed fish were Weissella, Leuconostoc and Streptococcus. Genus Aeromonas from the γ-proteobacteria class was also present in significantly higher amounts in the vegetable fed fish.

The microbial community was different in the fish that were challenged by *Yersinia ruckeri*. Challenged fish clustered into two groups according to the load of *Yersinia ruckeri* specific reads in their intestine; the main part of challenged fish (n = 34) had a low amount of *Yersinia* specific reads (≤ 1.2 % of total amount of reads), while a minor group (n = 6) had a high load ranging between 13.9 and 23.2 % of all reads. These ‘high *Yersinia* level’ fish had a significantly lower amount of reads from the order Burkholderiales relative to the ‘low *Yersinia* level’ fish and non-infected control fish. Further, the ‘high *Yersinia* level’ fish further clustered separately when analyzing the bacterial community on a PCA plot.

The immunological examinations were performed by RT-qPCR in order to measure the expression of selected immune genes. The results showed a similar expression pattern between the two diet groups of non-infected fish, but the response differed between the two diet groups in challenged fish. Overall, the results indicate that the gut microbiota in rainbow trout, like in warm-blooded animals, is highly plastic according to the type of diet and does also seem to be involved in the immunological response in connection to pathogenic challenge.

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**Dispersal and Survival of Flavobacterium psychrophilum Phages In Vivo in Rainbow Trout and In Vitro under Laboratory Conditions: Implications for Their Use in Phage Therapy**

Attention has been drawn to phage therapy as an alternative approach for controlling pathogenic bacteria such as Flavobacterium psychrophilum in salmonid aquaculture, which can give rise to high mortalities, especially in rainbow trout fry. Recently, phages have been isolated with a broad host range and a strong lytic potential against pathogenic F. psychrophilum under experimental conditions. However, little is known about the fate of phages at environmental conditions. Here, we quantified the dispersal and fate of F. psychrophilum phages and hosts in rainbow trout fry after intraperitoneal injection. Both phages and bacteria were isolated from the fish organs for up to 10 days after injection, and coinfection with both bacteria and phages resulted in a longer persistence of the phage in the fish organs, than when the fish had been injected with the phages only. The occurrence of both phage and bacterium was most prevalent in the kidney and spleen, with only minor occurrence in the brain. The experiment showed that injected phages were rapidly spread in the internal organs of the fish, also in the absence of bacteria. Parallel examination of the regulation of bacteriophage infectivity in controlled laboratory experiments at various environmental conditions showed that pH had only minor effects on long-term (3 months) phage infectivity within a pH range of 4.5 to 7.5, whereas phage infectivity was immediately lost at pH 3. In the absence of host cells, phage infectivity decreased by a factor of 10,000 over 55 days in untreated pond water, while the sterilization and removal of particles caused a 100-fold increase in phage survival relative to the control. In addition, F. psychrophilum-specific phages maintained their infectivity for ~2 months in glycerol at ~80°C, whereas infectivity decreased by a factor 10 when kept in a buffer at 20°C. Only a very small degradation in infectivity was
seen when bacteriophages were added and dried on fish feed pellets. Together, these results indicate that application of bacteriophages represents a promising approach for the control of F. psychrophilum infections in trout and suggest fish feed as a potential delivery method.
Effect of Hydrogen Peroxide on Immersion Challenge of Rainbow Trout Fry with Flavobacterium psychrophilum

An experimental model for immersion challenge of rainbow trout fry (Oncorhynchus mykiss) with Flavobacterium psychrophilum, the causative agent of rainbow trout fry syndrome and bacterial cold water disease was established in the present study. Although injection-based infection models are reliable and produce high levels of mortality attempts to establish a reproducible immersion model have been less successful. Various concentrations of hydrogen peroxide (H2O2) were evaluated before being used as a pre-treatment stressor prior to immersion exposure to F. psychrophilum. H2O2 accelerated the onset of mortality and increased mortality approximately two-fold; from 9.1% to 19.2% and from 14.7% to 30.3% in two separate experiments. Clinical signs observed in the infected fish corresponded to symptoms characteristically seen during natural outbreaks. These findings indicate that pre-treatment with H2O2 can increase the level of mortality in rainbow trout fry after exposure to F. psychrophilum.

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Yersinia ruckeri infection of rainbow trout: entrance portals and spread in the host

The portal of entrance of either formalin inactivated or live Yersinia ruckeri organism in rainbow trout fish was studied by applying immunohistochemistry and in-situ hybridization. The sequential study involved a specific monoclonal antibody and a specific oligonucleotide probe binding to Yersinia ruckeri. It demonstrated the differential and regional uptake of both formalin inactivated and live bacterial organism in rainbow trout. The uptake dynamics in various organs/tissues demonstrated a site specific propensity between formalin inactivated and live bacteria. The possibility that lateral lines, dorsal fins and the gastro-intestinal tract could act as an active avenue to bacterial entrance was shown by both immunohistochemistry and in situ hybridization. The translocations of systemically absorbed formalin inactivated and live bacteria within different host body compartments were elucidated.
Environmental factors affecting challenge success in vaccination studies
We have tested the efficacies of two different vaccines (a commercial versus an experimental vaccine – both being oil
adjuvanted) for rainbow trout against furunculosis caused by Aeromonas salmonicida infections. However, when
challenging fish with live bacteria in order to assess protection following vaccination, the administration of the pathogen is
important for the outcome of the experiments. We have therefore also compared injection challenge with cohabitation
challenge. In addition, when doing so we also investigated the influence of environmental conditions such as salinity and
temperature on the protection recorded. Thus, challenge studies were conducted at two temperatures (12 and 19°C) and
at two salinities (0 and 15 ppt). Mortalities following challenge were recorded and RPS calculated for each group. Side
effects of the vaccines were evaluated by using the Speilberg scale. Results from the challenge studies will be presented.

Flavobacterium psychrophilum - Experimental challenge and immune response
The disease rainbow trout fry syndrome (RTFS) is caused by the bacterial fish pathogen Flavobacterium psychrophilum. It
has been the cause of great losses of rainbow trout in aquacultures both in Denmark and around the world. It was
estimated that RTFS resulted in the death of 88 million fry in 1998, which amounted to a loss of 18 million DKK. Disease
outbreaks are typically seen at water temperatures below 15 °C and typically with fry mortality rates from 50-60 %.

Disease outbreaks are usually first observed shortly after the onset of feeding after the yolk sack is depleted. At this point
the immune system of the fry is not fully developed. Theoretically, the infection pressure could be subdued by vaccinating
larger fish, but no commercial vaccine is yet available. Diagnostic methods are well described and the disease is treated
with antibiotics. To prevent disease outbreaks and subsequent use of antibiotics, further knowledge of the disease is
needed.

Previous studies focusing on various types of aquacultures demonstrated the presence of F. psychrophilum in all
examined farms. The bacterium was demonstrated in gills, skin, internal organs and wounds both during RTFS outbreaks
and periods without disease.

The main purpose and focus of the present thesis was to increase knowledge of the immune response following infection
with F. psychrophilum, which may contribute to the future development of vaccines and other preventive measures. The
project consisted of three main tasks: 1) Establish an experimental infection model imitating natural infection, 2) examine
the immune response in blood and selected organs, and 3) examine potential portals of entry for the bacterium.

Previous experimental immersion-challenges involving F. psychrophilum have resulted in none or low mortality in rainbow
tROUT fry, unless the fish are stressed or have their surface compromised through e.g. injuries to the skin. The effect of a
range of hydrogen peroxide (H2O2) concentrations was tested on fry in order to assess mortality. An appropriate dose
was subsequently combined with immersion in a diluted bacterial broth. The method was shown to increase the mortality
of RTFS significantly and as a result, this model for infection was used for the planned studies. All subsequent
investigations thus include two infected groups, one of which was pretreated with hydrogen peroxide, while the other was not.

Real-time PCR (RT-PCR) was used to examine the immune response in the head kidney during the first eight days after
infection, and enzyme-linked immunosorbent assay (ELISA) was used to evaluate the production of antibodies 50 days
post-exposure. A pro-inflammatory response was observed in both groups infected with F. psychrophilum. However, only
a weak pattern was observed in the regulation of the adaptive response, while numerous correlations between the regulation of genes and the amount of F. psychrophilum 16S rRNA were demonstrated. Exposure to H2O2 before immersion in F. psychrophilum also influenced correlation between regulation of genes and pathogen load in several cases. Pre-treatment with H2O2 e.g. delayed the positive correlation between IgM and pathogen load in the head kidney. Antibodies against F. psychrophilum were present in the blood of both infected groups 50 days after infection, but no significant difference could be observed at this time point.

Morphological changes in the gill tissue after exposure to F. psychrophilum and H2O2 was examined on tissue sections stained with hematoxylin and eosin (H&E). Exposure to F. psychrophilum or H2O2 resulted in epithelial lifting and formation of edemas, but in both cases the tissue was regenerating after 192 hours. However, when the fish had been exposed to both H2O2 and F. psychrophilum, the damage was still evident at this time point. The relative pathogen load measured as 16S rRNA was highest at the first sampling and decreased steadily with no significant effect from pre-treatment with H2O2. No bacteria were observed on the H&E-stained tissue or visualized using a F. psychrophilum-specific probe with fluorescence in situ hybridization. Changes in gene expression in the gills after exposure to F. psychrophilum and H2O2 was also examined using RT-PCR. The observed immune response was limited, and neither a typical Th1 or Th2 response was observed.

The results indicate that immersion in F. psychrophilum may suppress the immune response in its host and induce lesions in the gills. A single exposure to H2O2 influenced the immune response to subsequent exposure to F. psychrophilum in both head kidney and gills, and leads to increased mortality. The results show a need for further investigation regarding the potential connection between routine non-medical treatments like H2O2 in aquaculture and disease outbreaks.
Insight from Molecular, Pathological, and Immunohistochemical Studies on Cellular and Humoral Mechanisms Responsible for Vaccine-Induced Protection of Rainbow Trout against *Yersinia ruckeri*

The immunological mechanisms associated with protection of vaccinated rainbow trout, *Oncorhynchus mykiss*, against enteric redmouth disease (ERM), caused by *Yersinia ruckeri*, were previously elucidated by the use of gene expression methodology and immunohistochemical methods. That approach pointed indirectly to both humoral and cellular elements being involved in protection. The present study correlates the level of protection in rainbow trout to cellular reactions in spleen and head kidney and visualizes the processes by applying histopathological, immunohistochemical, and in situ hybridization techniques. It was shown that these cellular reactions, which were more prominent in spleen than in head kidney, were associated with the expression of immune-related genes, suggesting a Th2-like response. *Y. ruckeri*, as shown by in situ hybridization (ISH), was eliminated within a few days in vaccinated fish, whereas nonprotected fish still harbored bacteria for a week after infection. Vaccinated fish reestablished normal organ structure within a few days, whereas nonprotected fish showed abnormalities up to 1 month postinfection. Protection in the early phase of infection was mainly associated with the expression of genes encoding innate factors (complement factors, lysozyme, and acute phase proteins), but in the later phase of infection, increased expression of adaptive immune genes dominated. The histological approach used has shown that the cellular changes correlated with protection of vaccinated fish. They comprised transformation of resident cells into macrophage-like cells and increased occurrence of CD8α and IgM cells, suggesting these cells as main players in protection. Future studies should investigate the causality between these factors and protection.
Is the intestinal microbiota in rainbow trout (*Oncorhynchus Mykiss*) influenced by diet type and challenge by *Yersinia Ruckeri*?

In recent years it has become more and more evident that the bacterial flora in the gut of warm-blooded animals modulates physiological processes and the immunological status of the host. Besides effects on growth parameters, commensal intestinal bacteria balance the immune system and prevent colonization of pathogenic bacteria. The question is if the gut microbiota is also important in lower vertebrates such as fish? And does it play a role in connection to pathogenic challenge? To examine these questions rainbow trout fry were fed two different diets of either a marine or vegetable origin directly after first feeding. At a size of about four gram the fish were bath challenged by *Yersinia ruckeri* and intestines were then sampled 5 and 18 days post challenge for subsequent metagenomic and immunological examinations. Next-generation sequencing was applied for the metagenomic studies using the Illumina HiSeq platform. The results clearly showed two different microbial patterns in the intestines dependent on the diet type. Control fish fed a marine based diet overall had a higher amount of proteobacteria, while high amount of reads belonging to phylum Firmicutes dominated in the intestines of vegetable fed fish. Several genera within the order Lactobacillales belonged to the many reads from Firmicutes. In challenged fish with a high load of reads from genus *Yersinia* there was a significantly lower amount of reads from the order Burkholderiales. Further, these fish further clustered separately when analyzing the bacterial community on a PCA plot. The immunological examinations using RT-qPCR showed no different expression patterns between the diet groups in control fish, but the response was very different in connection to challenge. Here, the general pattern was a pro-inflammatory response in the intestine of marine fed fish challenged with *Yersinia ruckeri*, while several immune genes were down-regulated in vegetable fed fish. Overall, the results indicate that the gut microbiota in rainbow trout is highly plastic according to the type of diet and does further seem to be involved in the immunological response in connection to pathogenic challenge.

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**Preventing Disease in Rainbow Trout Caused by Aeromonas Salmonicida**

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**The immune response of rainbow trout to Flavobacterium psychrophilum following immersion-challenge model with and without hydrogen peroxide pre-treatment**

The bacterial fish pathogen Flavobacterium psychrophilum is a major cause of mortality in farmed rainbow trout (Oncorhynchus mykiss) and other salmonid fish. The disease following infection is called bacterial coldwater disease (BCWD) or rainbow trout fry syndrome (RTFS). To our knowledge, no commercial vaccine is currently available and the disease is treated with antibiotics. Injection-based challenges with F. psychrophilum are standardized but the route of infection does not reflect a natural situation. Therefore, we established an immersion-based model investigating if hydrogen peroxide (H2O2) pre-treatment could elevate infection and mortality.

The model consisted of four groups: 1) Un-exposed control, 2) H2O2 exposure, 3) F. psychrophilum immersion and 4) H2O2 + F. psychrophilum. Pre-treatment with H2O2 increased mortality two-fold if fish also were exposed to F. psychrophilum after pretreatment. Tissue samples were taken from the involved groups 4 h, 48 h, 125 h and 192 h post-exposure and investigated for regulation of immune genes. Following genes were examined in the head kidney and gills by qPCR: IgT, IgM, CD8, CD4, MHC I, MHC II, IL-4/13A, TcR-β, IL-10, IL-6, IL-1β, IL-17, SAA and FoxP3.

A pro-inflammatory response was indicated, but only a weak indication of an adaptive response was recorded (most evident in the F. psychrophilum group). Further, pre-treatment with H2O2 affected the correlation gene expression and pathogen load in several cases. Morphological changes in the gill tissue were evaluated using hematoxylin and eosin stained tissue sections. Exposure to both H2O2 and F. psychrophilum intensified tissue damage and postponed healing. The results indicate that F. psychrophilum may have an immunosuppressive action and that environmental stress may be one of several factors playing a role in RTFS outbreaks.

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The intestinal microbiota in rainbow trout (Oncorhynchus Mykiss) is influenced by diet type and Yersinia Ruckeri challenge

In recent years it has become more and more evident that the bacterial flora in the gut of warm-blooded animals modulates physiological processes and the immunological status of the host. Besides effects on growth parameters, commensal intestinal bacteria balance the immune system and prevent colonization of pathogenic bacteria. The question is if the gut microbiota is also important in lower vertebrates such as fish? Is the microbiota related to the diet type and does it play a protective role in connection to pathogenic challenge? To examine these questions rainbow trout fry were fed two different diets of either a marine or vegetable origin from first feeding and onwards. At a size of about four gram the fish were bath challenged by Yersinia ruckeri serotype O1 and intestines were then sampled 5 days post challenge for subsequent metagenomic examination. Next-generation sequencing was applied for the metagenomic studies using the Illumina HiSeq 2000 platform. The results showed two distinctly different microbial patterns in the intestines dependent on the diet type. Fish fed a marine based diet overall had a significantly higher amount of the class β-proteobacteria, while the amount of reads belonging to phylum Firmicutes were significantly higher in the intestines of vegetable fed fish. The genera within phylum Firmicutes present in significantly higher amounts in vegetable fed fish were Weissella, Leuconostoc and Streptococcus. Genus Aeromonas from the γ-proteobacteria class was also present in significantly higher amounts in the vegetable fed fish. When challenged with Yersinia ruckeri, fish with a high amount of sequence reads belonging to genus Yersinia had a significantly lower amount of reads from the order Burkholderiales relative to non-infected control fish and fish with a low amount of Yersinia specific sequences. Further, these infected fish further clustered separately when analyzing the bacterial community on a PCA plot. The immunological examinations using RT-qPCR showed similar constitutive expression between the two diet groups, but the response differed between the two diet groups in challenged fish. Here, the general pattern was a pro-inflammatory response in the intestine of marine fed fish challenged with Yersinia ruckeri relative to non-infected control fish, while several immune genes were down-regulated in vegetable fed fish relative to non-infected control fish. Overall, the results indicate that the gut microbiota in rainbow trout is highly plastic according to the type of diet and does further seem to be involved in the immunological response in connection to pathogenic challenge.

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Tissue specific uptake of inactivated and live Yersinia ruckeri in rainbow trout (Oncorhynchus mykiss): Visualization by immunohistochemistry and in situ hybridization

Understanding of uptake and invasion routes of Yersinia ruckeri, causing Enteric Red Mouth Disease (ERM) in rainbow trout (Oncorhynchus mykiss), is essential for improved understanding of the pathogenicity and immune response mechanisms associated this disease. The present work shed light on areas of invasion in rainbow trout by the use of immunohistochemistry and in situ hybridization techniques. Fish were exposed to live or formalin inactivated bacteria and samples were subsequently taken for histology from various outer and inner surfaces. We applied a specific monoclonal antibody and specific oligonucleotide probes binding to Y. ruckeri (serotype O1, biotype 2) in tissue sections and were able to demonstrate a tissue specific uptake of this bacterium (both formalin inactivated and live form). Uptake and subsequent translocation dynamics at various surfaces demonstrated different site specific propensities between the formalin inactivated and live bacterial organisms. Lateral lines, dorsal fin, epidermis and gastro-intestinal tract mucosal tissue were the primary areas where bacterial uptake was demonstrated readily after exposure. The fate of internalized bacterial organisms within the host suggested that central immune organs are involved in the final antigen processing.

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Baltic Salmon, Salmo salar, from Swedish River Lule Älv Is More Resistant to Furunculosis Compared to Rainbow Trout.

Background: Furunculosis, caused by Aeromonas salmonicida, continues to be a major health problem for the growing salmonid aquaculture. Despite effective vaccination programs regular outbreaks occur at the fish farms calling for repeated antibiotic treatment. We hypothesized that a difference in natural susceptibility to this disease might exist between Baltic salmon and the widely used rainbow trout. Study Design: A cohabitation challenge model was applied to investigate the relative susceptibility to infection with A. salmonicida in rainbow trout and Baltic salmon. The course of infection was monitored daily over a 30-day period post challenge and the results were summarized in mortality curves. Results: A. salmonicida was recovered from mortalities during the entire test period. At day 30 the survival was 6.2% and 34.0% for rainbow trout and Baltic salmon, respectively. Significant differences in susceptibility to A. salmonicida were demonstrated between the two salmonids and hazard ratio estimation between rainbow trout and Baltic salmon showed a 3.36 higher risk of dying from the infection in the former. Conclusion: The finding that Baltic salmon carries a high level of natural resistance to furunculosis might raise new possibilities for salmonid aquaculture in terms of minimizing disease outbreaks and the use of antibiotics.
For many years it has been known that the bacterial microflora in the gut of warm-blooded animals exists in harmony with the host and exert various beneficial effects on the health by their metabolic activities. Hence, the gut microbiota has a high importance for the animal. In many studies from e.g. humans and the pig mapping of the bacterial flora from the gut have shown dominance by some specific bacterial groups, and this bacterial profile is termed as a ‘core microbiota’. For lower vertebrates like fish mapping of the bacterial flora in the gastrointestinal system is to date a relatively new research field and previous studies have mainly been done on bacterial species that can be cultured or by classical molecular techniques like T-RFLP and DGGE. In the last recent years deep-sequencing techniques have enabled sequencing and mapping of entire microbial communities from for instance an environmental sample or a tissue / fecal sample from an animal. These metagenomic studies have provided new and deeper insight into the microbial ecology and the influence of the microbiota in warm-blooded animals.

In this study next-generation sequencing of the 16S rDNA gene on the Illumina HiSeq platform was used to examine the composition of the microbial flora in the gut of rainbow trout (Oncorhynchus mykiss) fry. The fish were examined before and after first-feeding and after administration by commercial probiotic lactobacilli to the feed.
Comparative protection of two different commercial vaccines against Yersinia ruckeri serotype O1 and biotype 2 in rainbow trout (Oncorhynchus mykiss)

Differentially extended specific protection by two commercial vaccines against Yersinia ruckeri serotype O1 biotype 2 was studied following 30s immersion exposure. Rainbow trout were challenged intra-peritoneally (i.p.) with Y. ruckeri serotype O1, biotype 2 (≈10^6 to 10^7 CFU/fish) at 4, 6 and 8 months after vaccination with vaccines containing either biotype 1 (AquaVac® ERM) or both biotypes 1 and 2 (AquaVac® RELERA™). The specific pattern of vaccine-mediated protection was evaluated by relative percentage survival (RPS) analysis at 4 and 6 months post-vaccination and by obtaining gross pathological observations at 4 and 8 months respectively. We determined specific significant and superior protection in terms of increased survivability in AquaVac® RELERA™ vaccinated fish and observed correspondingly fewer pathological changes. The challenge trials indicated a longer protection for at least 6 months without any booster vaccination. A specific and adaptive response induced by AquaVac® RELERA™ vaccine against Y. ruckeri biotype 2 was clearly indicated. In addition, some degree of cross protection rendered by AquaVac® ERM containing biotype 1 during infection with Y. ruckeri biotype 2 was also noted.

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Determining Vaccination Frequency In Farmed Rainbow Trout Using Vibrio anguillarum O1 Specific Serum Antibody Measurements

Background
Despite vaccination with a commercial vaccine with a documented protective effect against Vibrio anguillarum O1 disease outbreaks caused by this bacterium have been registered among rainbow trout at Danish fish farms. The present study examined specific serum antibody levels as a valid marker for assessing vaccination status in a fish population. For this purpose a highly sensitive enzyme-linked immunosorbent assay (ELISA) was developed and used to evaluate sera from farmed rainbow trout vaccinated against V. anguillarum O1.

Study Design
Immune sera from rainbow trout immunised with an experimental vaccine based on inactivated V. anguillarum O1 bacterin in Freund’s incomplete adjuvant were used for ELISA optimisation. Subsequently, sera from farmed rainbow trout...
vaccinated with a commercial vaccine against V. anguillarum were analysed with the ELISA. The measured serum antibody levels were compared with the vaccine status of the fish (vaccinated/unvaccinated) as evaluated through visual examination.

Results
Repeated immunisation with the experimental vaccine lead to increasing levels of specific serum antibodies in the vaccinated rainbow trout. The farmed rainbow trout responded with high antibody levels to a single injection with the commercial vaccine. However, the diversity in responses was more pronounced in the farmed fish. Primary visual examinations for vaccine status in rainbow trout from the commercial farm revealed a large pool of unvaccinated specimens (vaccination failure rate = 20%) among the otherwise vaccinated fish. Through serum analyses using the ELISA in a blinded set-up it was possible to separate samples collected from the farmed rainbow trout into vaccinated and unvaccinated fish.

Conclusions
Much attention has been devoted to development of new and more effective vaccines. Here we present a case from a Danish rainbow trout farm indicating that attention should also be directed to the vaccination procedure in order to secure high vaccination frequencies necessary for optimal protection with a reported effective vaccine.

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Diversity of Flavobacterium psychrophilum and the potential use of its phages for protection against bacterial cold water disease in salmonids

Flavobacterium psychrophilum causes rainbow trout fry syndrome (RTFS) and cold water disease (CWD) in salmonid aquaculture. We report characterization of F. psychrophilum strains and their bacteriophages isolated in Chilean salmonid aquaculture. Results suggest that under laboratory conditions phages can decrease mortality of salmonids from infection by their F. psychrophilum host strain. Twelve F. psychrophilum isolates were characterized, with DNA restriction patterns showing low diversity between strains despite their being obtained from different salmonid production sites and from different tissues. We isolated 15 bacteriophages able to infect some of the F. psychrophilum isolates and characterized six of them in detail. DNA genome sizes were close to 50 Kbp and corresponded to the Siphoviridae and Podoviridae families. One isolate, 6H, probably contains lipids as an essential virion component, based on its chloroform sensitivity and low buoyant density in CsCl. Each phage isolate rarely infected F. psychrophilum strains other than the strain used for its enrichment and isolation. Some bacteriophages could decrease mortality from intraperitoneal injection of its host strain when added together with the bacteria in a ratio of 10 plaque-forming units per colony-forming unit. While we recognize the artificial laboratory conditions used for these protection assays, this work is the first to demonstrate that phages might be able protect salmonids from RTFS or CWD.
Hydrogen peroxide as pre-treatment stressor in experimental immersion challenge of rainbow trout fry with Flavobacterium psychrophilum

Several models for experimental infection with Flavobacterium psychrophilum in rainbow trout (Oncorhynchus mykiss) have been carried out with varying success, including challenge through injection, immersion and cohabitation. Intraperitoneal challenge is highly reproducible, while immersion challenge results in low mortality rates unless combined with various forms of stress or scarification. Although reproducibility has generally been an issue regarding immersion challenges, the method is more appropriate for investigations concerning the immune system, since natural transmission is imitated. Non-medical therapeutic substances are routinely used against pathogens in aquacultures, including copper sulphate, chloramine-T, sodium carbonates, sodium chloride, formalin and hydrogen peroxide (H2O2). One of the more successful immersion models used formalin as a stressor, but a less harmful substance was desirable, since formalin is to be phased out in Danish farms by 2014 due to health considerations. The aim of this study was to establish a reproducible method for immersion challenge of rainbow trout fry to be used in investigations concerning immune response and vaccine testing. Various concentrations of H2O2 were tested before being combined with immersion exposure to the virulent F. psychrophilum isolate950106-1/1, which was used for infections. Pre-treatment with H2O2 elevated the cumulative mortality following immersion challenge with F. psychrophilum, although variation between replicates was very high.
Immersion challenge with Flavobacterium psychrophilum in rainbow trout fry (Oncorhynchus mykiss)
The fish pathogen Flavobacterium psychrophilum is one of the main causes of mortality in farmed rainbow trout (Oncorhynchus mykiss) and other salmonid fish. Presently no commercial vaccine exists, although several are under development.

Various models for experimental infection have been carried out with varying success, including challenge through injection, bath and cohabitation. Intraperitoneal challenge and bath challenges combined with various forms of stress have shown to be reproducible. Bath challenge is more appropriate for vaccine testing, since natural transmission of infection is imitated and is also more suitable due to the small size of the fry. Various chemicals are used against pathogens in aquacultures, including copper sulphate, chloramine-T, sodium carbonates, sodium chloride, formalin and hydrogen peroxide (H2O2). One of the more successful immersion models made use of formalin as a stressor, but a less harmful chemical was desirable, since the use of formalin is to be phased out in the Danish farms by 2014 due to health considerations.

The aim of this study was to establish a reproducible method for immersion challenge of rainbow trout fry to be used in investigations concerning the immune response and vaccine testing. Various concentrations of H2O2 were tested, before being combined with immersion exposure to the well-known, virulent strain 950106-1/1, which was used for all infections. The mortality of F. psychrophilum infection in fry was increased by pre-treating with H2O2, although variation was very high.

Improved ERM vaccination efficacy using combined vaccine administration methods
We have previously shown that immersion vaccination (30 sec) using the Aquavac Relera vaccine (containing formalin killed Yersinia ruckeri serotype O1 of both biotypes 1 and 2) provides the best protection (when compared to other commercial ERM vaccines on the Danish market) against infection following i. p. challenge using Y. ruckeri O1, biotype 2, which at present is the main bacterial pathogen in fingerling trout production in Denmark. Despite a significant protection conferred by this vaccine (immersion) some mortality could be observed following challenge.

We have therefore performed a study in order to elucidate if different vaccine administration methods (using Aquavac Relera) can improve protection and reduce mortality of exposed trout following challenge with this particular pathogen. Rainbow trout (mean weight 7.8 g) reared at the Bornholm Salmon Hatchery under pathogen free conditions were vaccinated by 1) a single immersion (30 sec) and no booster, 2) a single immersion (30 sec) and a second immersion booster (30 sec) 410 degree-days later, 3) a single injection i.p. (0.1 ml) and no booster and finally 4) a single immersion (30 sec) and a booster injection i.p. (0.1 ml) 410 degree-days later. Fish were compared to naïve and non-vaccinated control fish. All experiments were performed in duplicate. Fish were brought to our university facilities, acclimated for 14 days and then challenged by i.p. injection of Y. ruckeri O1, biotype 2 (8.5 x 106 cfu/fish).

Control fish exhibited a mortality of 65 %, fish immersed only once showed a mortality of 36 %, fish immersed twice had 4 % mortality, fish injected once reached 2 % mortality. Rainbow trout immersed once and subsequently boosted by
injection showed no mortality at all. This superior protection offered by a combination of primary immersion vaccination and a booster vaccination by injection may lead to improved health and reduced drug application in Danish trout farms.

**Improved Protection of Rainbow Trout Against Furunculosis by an Autologous Vaccine Under Experimental Conditions**

Despite vaccination with oil-adjuvanted vaccines against vibriosis and furunculosis, sea reared rainbow trout in Denmark often encounter outbreaks of furunculosis during warm summer periods. To address this issue under experimental conditions, two groups of rainbow trout were vaccinated by i.p. injection with two different oil-adjuvanted vaccines: (1) a commercial vaccine comprising Vibrio anguillarum serotype O1 and O2, and Aeromonas salmonicida subspecies salmonicida bacteria, with all bacteria originating from Atlantic salmon, and (2) an experimental vaccine based on cultures of the same bacterial species originating from rainbow trout reared in Danish sea farms. The experiment also included a third group of non-vaccinated controls. All fish were individually chip-tagged to allow mixing of all groups in three replicate aquaria. After 770 dg (day degrees) or 77 days at 10°C, half of the fish in each group were challenged by i.p. injection of 1x10⁵ cells of the A. salmonicida isolate used in the experimental vaccine. The other half was tagged by cutting off the adipose fin (non-injected cohabitants). While the non-vaccinated, i.p.-injected fish all died within 2 weeks, a certain level of protection was evident among the vaccinated groups although high mortality also occurred here. No mortality/clinical disease was evident among the non-injected cohabitants. However, when the water temperature was gradually risen to 15-17-20°C, the cohabitants started to die. Some variability was evident between replicate tanks, but the experimental vaccine tended to provide better protection than the commercial counterpart. The results indicate that tailor-making of a vaccine against furunculosis for sea reared rainbow trout in DK is an important approach for optimal protection.
Danish rainbow trout fry died due to infections with this bacterium [1]. A further challenge for production of organic fry is that diets with high plant contents cause enteritis and injury to the intestine, which in the end will affect the overall health status of the fish and result in a higher risk of disease following exposure to pathogenic microorganisms.

The aim of the Danish project OPTIFISH is to optimize growth and survival for organic cultured rainbow trout. OPTIFISH investigates 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 following exposure to pathogens. The project consists of several work packages, among them the backbone of the project, namely the feed experiments with fry as well as sampling from the different diet groups. The samples will be used for studies of the bacterial microbiota in the fish by e.g. traditional bacteriology and molecular methods (16S rRNA PCR combined with next generation sequencing) as well as for the immunological investigation of the intestine by gene expression studies by quantitative RT-PCR. Fish health will be investigated by experimental exposure of fry to pathogens, e.g. F. psychrophilum.

In the current experiment four diet types were tested on fry, a conventional type (Inicio®, BioMar A/S) with and without probiotics, as well as an organic type with and without probiotic. Bactocell® (Lallemand) was used as the probiotic. Studies were done in triplicates, whereas the sampling and experimental infections were done in duplicates. The rainbow trout were fed the different diet types from first feeding and onwards. Just before first feeding yolk sac fry were sampled for reference. Thereafter sampling was done when the fry had been fed for at least 10 days, just before experimental infection with F. psychrophilum as well as 5 and 26 days after infection. Until the fish reached average weight 5 g two more samplings were done.

An experimental bath infection model with F. psychrophilum was used [2]. A 48 hour old bacterial culture of the strain 950106-1/1 was diluted to 107 CFU/ml in bore-hole water and the fish were exposed to this dilution for 0.5 h. Thereafter the fish were moved to 100 l aquaria. During the whole feed experiment the water temperature was 12 °C.

The bath exposure challenge experiment was terminated after 35 days. The cumulative mortalities were less than 10 % in the groups exposed to the bacterium, whereas no mortalities were seen in the controls. A traditional bacteriological examination of the microflora in the fry was done by taking out samples from inner organs for detecting potential pathogens as well as samples from the intestine to examine the intestinal microflora. Tryptone yeast extract salts (TYES) agar [3] was used for isolating F. psychrophilum. The random sampling done 5 days post bath exposure infection showed that F. psychrophilum could be isolated in 1 of 10 fry, 3 of 10 fry, 3 of 10 fry and 4 of 10 fry, respectively, in the four different diet groups, in total 27.5 % of the sampled fry. In all cases apart from one the bacterium was only isolated from the spleen. When the random sampling was repeated 26 days post infection, a total of 3 of 40 fish hosted the bacterium (each fish sampled from a different group), corresponding to 7.5 % of the sampled fish. The isolation site this time was spleen and kidney. F. psychrophilum was not isolated from the control groups.

The conclusions based on this study are that bath exposure under the given conditions resulted in low mortalities in diet groups no matter if the fish had been fed diets that were organic or conventional nor if the feed had been added probiotic. No significance in mortalities was seen between the diet groups. The bacteriological examinations showed that the bacterium had entered the fish, as it was found in inner organs in one fourth of the sampled fish 5 days post infection, but it seems that the fish were able to clear the infection, because the cumulative mortalities after 35 days were less than 10 percent in each group. 26 days post infection only 7.5 % of the sampled fish still harboured the bacterium.

Further analyses include next generation sequencing of samples from the diet groups and comparison of all data. The OPTIFISH project is aiming at a production of a more robust and healthy rainbow trout fry/fingerling, so the production of organic fry can become a reality and the result can be a higher production in organic aquaculture.

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**Local and global scale host range patterns and distribution of bacteriophages infecting the fish pathogen Flavobacterium psychrophilum**

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Økologisk regnbueørred – hvordan påvirker fodersammensætningen fiskenes sundhed?

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Optimering af driften på klassiske dambrug

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Potential Role of Specific Antibodies as Important Vaccine Induced Protective Mechanism against Aeromonas salmonicida in Rainbow Trout.

Furunculosis caused by infection with Aeromonas salmonicida subsp. salmonicida has been a known threat to aquaculture for more than a century. Efficient prophylactic approaches against this disease are essential for continued growth of salmonid aquaculture. Since the introduction of successful oil-adjuvanted vaccines in the early 1990’s, a number of studies have been published on the protective as well as adverse effects of these vaccines. Most studies focus on vaccination of salmon (Salmo salar). However, rainbow trout (Oncorhynchus mykiss) are also very susceptible to infection and are vaccinated accordingly. In this study we have examined the protection against infection with a Danish strain of A. salmonicida in both vaccinated and non-vaccinated rainbow trout. A commercial and an experimental auto-vaccine were tested. The protective effects of the vaccines were evaluated through an A. salmonicida challenge 18 weeks post vaccination. Both vaccines resulted in a significantly increased survival in the vaccinated fish during a 28 day challenge period relative to non-vaccinated fish (P = 0.01 and P = 0.001 for the commercial and experimental vaccine, respectively).

Throughout the entire experiment, the presence of specific antibodies in plasma was monitored using ELISA. A significant increase in specific antibody levels was seen in fish vaccinated with both vaccines during the 18 weeks between vaccination and challenge. Within 3 days post challenge, a significant decrease in specific antibodies occurred in vaccinated fish. A positive correlation was found between mean levels of specific antibodies pre challenge and overall survival. This correlation, along with the observed depletion of antibodies during the initial phase of infection, suggests that specific antibodies play an essential role in vaccine mediated protection against A. salmonicida in rainbow trout.
Furunculosis caused by infection with Aeromonas salmonicida subsp. salmonicida has now been a known threat to aquaculture for more than a century. Efficient prophylactic precautions against this disease are essential for continued growth of salmonid fish aquaculture. Ever since the introduction of successful oil-adjuvanted vaccines in the 1990’s, a number of studies have been published on the protective effects of these vaccines. While most of these studies mainly focus on vaccination of salmon (Salmo salar), rainbow trout (Oncorhynchus mykiss) are also highly susceptible to infection and are therefore vaccinated as well.

In this study we have examined the levels of protection against infection with a Danish strain of A. salmonicida subsp. salmonicida in both non-vaccinated, as well as vaccinated rainbow trout. Both a commercial vaccine (AlphaJect 3000, PHARMAQ AS) as well as an experimental auto-vaccine was tested. For comparison, the isolated adjuvant used in the commercial vaccine, as well as the one used in the experimental vaccine was included in the experimental setup. The protective effects of the vaccines were tested by bacterial challenge 18 weeks post vaccination, and during the 18 weeks, the development of specific antibodies was monitored using ELISA assays.

Both vaccines resulted in significantly increased survival during a 28 day challenge period. None of the two adjuvant systems provided increased protection. A significant increase in specific antibody levels was seen in both vaccinated groups during the 18 weeks between vaccination and challenge.

Additionally, further analysis showed a significant correlation between the mean level of specific antibodies measured for each group and the final survival percentages of these groups. The positive correlation between specific antibodies and survival seems to indicate a prominent role of antibodies as a vaccine mediated protective mechanism.
Quality control ranges for testing broth microdilution susceptibility of Flavobacterium columnare and F. psychrophilum to nine antimicrobials

A multi-laboratory broth microdilution method trial was performed to standardize the specialized test conditions required for the fish pathogens Flavobacterium columnare and F. psychrophilum. Nine laboratories tested the quality control (QC) strains Escherichia coli ATCC 25922 and Aeromonas salmonicida subsp. salmonicida ATCC 33658 against 10 antimicrobials (ampicillin, enrofloxacin, erythromycin, florfenicol, flumequine, gentamicin, ormetoprim/sulfadimethoxine, oxolinic acid, oxytetracycline, and trimethoprim/sulfamethoxazole) in diluted (4 g l−1) cation-adjusted Mueller-Hinton broth incubated at 28 and 18°C for 44–48 and 92–96 h, respectively. QC ranges were set for 9 of the 10 antimicrobials. Most of the minimal inhibitory concentration (MIC) distributions (16 of 18, 9 drugs at both temperatures) for A. salmonicida ATCC 33658 were centered on a single median MIC ± 1 two-fold drug dilution resulting in a QC range that spanned 3 dilutions. More of the E. coli ATCC 25922 MIC distributions (7 of 16) were centered between 2 MIC dilutions requiring a QC range that spanned 4 dilutions. A QC range could not be determined for E. coli ATCC 25922 against 2 antimicrobials at the low temperature. These data and their associated QC ranges have been approved by the Clinical and Laboratory Standards Institute (CLSI), and will be included in the next edition of the CLSI M49-A Guideline. This method represents the first standardized reference method for testing fish pathogenic Flavobacterium spp.
The gut microbiota in rainbow trout changes after first feeding and is dependent on the type of diet

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The role of the gills as potential portal of entry in rainbow trout fry syndrome

The Gram negative fish pathogen Flavobacterium psychrophilum is the causative agent of rainbow trout fry syndrome (RTFS), also known as BCWD (bacterial cold water disease). While injection-based experimental challenges with F. psychrophilum have been standardized and result in high mortality rates, the same has not been the case for immersion based models. However, injection is not a proper approach for investigations of the immune response since the first line of defense is bypassed. This study aims at understanding the immune response in the gills following infection as well as their possible role as portal of entry. A bath model, using hydrogen peroxide (H2O2) as a pre-treatment stressor to increase the number of infected fish, was used for sampling. The experimental setup consisted of four treatment groups; 1) untreated
controls, 2) H2O2, 3) F. psychrophilum and 4) H2O2 and F. psychrophilum. The use of this model also provides information regarding the impact of H2O2 alone, though this was not the main objective. Samples were taken 4 hours, 2 days, 5 days and 8 days after infection and preserved for later processing. Two methods are employed in this study. Firstly, quantitative real-time PCR will be used to investigate the immune response in the gills after immersion exposure to F. psychrophilum. Secondly, fluorescent in situ hybridization will be used to visualize pathogen entry and spread in gills and head region. Samples are still undergoing analysis and results will be presented at the workshop.

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**Anvendelse af bakteriofager til kontrol af Flavobacterium psychrophilum i ørreddambrug**

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**Challenge models for RTFS in rainbow trout fry (Oncorhynchus mykiss)**

The fish pathogen Flavobacterium psychrophilum is one of the main causes of mortality in fry of farmed rainbow trout (Oncorhynchus mykiss) and other salmonid fish. The disease following infection is often called bacterial coldwater disease (BCWD) in USA and rainbow trout fry syndrome (RTFS) in Europe. Presently no commercial vaccine exists, although several are under development. Various models for experimental infection have been carried out with varying success, including challenge through injection, bath and cohabitation. Intraperitoneal challenge and bath challenges combined with various forms of stress have shown to be reproducible. Bath challenge is more appropriate for vaccine testing, since natural transmission of infection is imitated and is also more suitable due to the small size of the fry. A bath-model using H2O2 as a stressor is currently being tested on 1.4g rainbow trout fry in four experimental groups: 1) no H2O2/no bath infection, 2) H2O2/no bath infection, 3) no H2O2/ bath infection and 4) H2O2/ bath infection. Mortality will be evaluated over approximately 25 days. The project is currently in its preliminary phase and presently focused on development of a model of infection. The overall goal is also to examine gene expression and location of transcription products in rainbow trout fry, in order to optimize vaccination or immune-stimulation. The presentation will focus on previous experimental models and the experimental design of the current model as well as the future plans for the project.

**General information**
Flavobacterium psychrophilum, prevention and immune response

The fish pathogen Flavobacterium psychrophilum is one of the main causes of mortality in farmed rainbow trout and other salmonid fish. The disease following infection is often called bacterial coldwater disease (BCWD) in USA or rainbow trout fry syndrome (RTFS) in Europe. An infected farm can expect mortality rates around 50-60% in fry and 2-10% in juvenile fish within few weeks, which causes significant economical losses worldwide. Presently no commercial vaccine exists, and fish farmers control the disease with antibiotics. The project is currently in its preliminary phase but the overall goal is to examine gene expression and location of transcription products in rainbow trout fry, in order to optimize vaccination or immune-stimulation. The presentation will focus on the future plans for the project, since no data have yet been obtained.

Immune response in rainbow trout against infection with Flavobacterium psychrophilum

The fish pathogen Flavobacterium psychrophilum is one of the main causes of mortality in farmed rainbow trout and other salmonid fish. An infected farm can expect mortality rates around 50-60% in fry and 2-10% in juvenile fish within few weeks. Presently no commercial vaccine exists, and fish farmers control the disease with antibiotics. The pathogen has a limited ability to cause disease in an experimental setting without applying a stressor. A bath-model using 150mg/L H2O2 for 60 minutes as a stressor was used on 1.4g rainbow trout fry in four experimental groups: 1) no H2O2/no bath infection, 2) H2O2/no bath infection, 3) no H2O2/bath infection and 4) H2O2/bath infection. Bath infections were carried out in 107 CFU/ml F. psychrophilum bath solution and control groups were bathed in sterile medium. Samples from all internal organs, head and skin were taken before pathogen exposure and 4 hours, 2 days, 5 days, 8 days and 17 days after exposure. The samples will be used for examining the gene expression in rainbow trout related to infection with F. psychrophilum and results will be presented. Furthermore, antibody production will be assessed using ELISA on serum samples. The results will be used in order to optimize vaccination or immune-stimulation.
Independent Emergence of Yersinia ruckeri Biotype 2 in the United States and Europe

Biotype 2 (BT2) variants of the bacterium Yersinia ruckeri are an increasing disease problem in U.S. and European aquaculture and have been characterized as serovar 1 isolates that lack both peritrichous flagella and secreted phospholipase activity. The emergence of this biotype has been associated with an increased frequency of enteric redmouth disease (ERM) outbreaks in previously vaccinated salmonid fish. In this study, four independent specific natural mutations that cause the loss of both motility and secreted lipase activity were identified in BT2 strains from the United States, United Kingdom, and mainland Europe. Each of these was a unique mutation in either flIR, flhA, or flhB, all of which are genes predicted to encode essential components of the flagellar secretion apparatus. Our results demonstrate the existence of independent mutations leading to the BT2 phenotype; thus, this phenotype has emerged separately at least four times. In addition, BT2 strains from the United Kingdom were shown to have the same mutant allele found in U.S. BT2 strains, suggesting a common origin of this BT2 lineage. This differentiation of distinct BT2 lineages is of critical importance for the development and validation of alternative vaccines or other treatment strategies intended for the control of BT2 strains.

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Authors: Welch, T. J. (Ekstern), Verner-Jeffreys, D. W. (Ekstern), Dalsgaard, I. (Intern), Wiklund, T. (Ekstern), Evenhuis, J. P. (Ekstern), Garcia Cabrera, J. A. (Ekstern), Hinshaw, J. M. (Ekstern), Drennan, J. D. (Ekstern), LaPatra, S. E. (Ekstern)
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Phage-host interactions in Flavobacterium psychrophilum and the potential for phage therapy in aquaculture

**General information**
State: Published
Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, University of Copenhagen
Authors: Christiansen, R. H. (Intern), Madsen, L. (Intern), Dalsgaard, I. (Intern), Middelboe, M. (Ekstern)
Number of pages: 19
Pages: P6
Publication date: 2011

**Host publication information**
Title of host publication: Abstracts
Main Research Area: Technical/natural sciences
Conference: Aquatic Virus Workshop, Texel, Netherlands, 01/01/2011
Electronic versions: AVW6 Abstract.pdf
Links:
http://www.avw6.org/avw6

**Bibliographical note**
Poster presentation.
Source: orbit
Source-ID: 316672
Publication: Research - peer-review › Conference abstract in proceedings – Annual report year: 2011

Phage-host interactions in Flavobacterium psychrophilum and the potential for phage therapy in aquaculture

**General information**
State: Published
Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Authors: Christiansen, R. H. (Intern), Madsen, L. (Intern), Dalsgaard, I. (Intern), Middelboe, M. (Ekstern)
Number of pages: 20
Pages: P2
Publication date: 2011

**Host publication information**
Resistance of Salmons Against Aeromonas Salmonicida: Host Genetics as a Main Player

Furunculosis, caused by Aeromonas salmonicida, continues to be a health problem for the growing salmonid aquaculture. Despite effective vaccination programs regular outbreaks occur at Danish trout farms calling for repeated antibiotic treatment. We hypothesized that a difference in natural susceptibility to this disease might exist between Baltic salmon and the widely used rainbow trout. Hence, a cohabitation challenge model was applied to investigate the relative susceptibility to infection with Aeromonas salmonicida in rainbow trout and Baltic salmon. The course of infection was monitored daily over a 30-day period post challenge and the results were summarized in mortality curves. A. salmonicida was recovered from mortalities during the entire test period. At day 30 the survival was 6.2 % and 34.0 % for rainbow trout and Baltic salmon, respectively. Significant differences in susceptibility to A. salmonicida were demonstrated between the two salmonids and hazard ratio estimation between rainbow trout and Baltic salmon showed a 3.36 higher risk of dying from the infection in the former. The finding that Baltic salmon carries a high level of natural resistance to furunculosis might raise new possibilities for salmonid aquaculture in terms of minimizing disease outbreaks and the use of antibiotics.

Vaccine Induced Specific Protection Against Enteric Red Mouth Disease (ERM) Caused by Yersinia Ruckeri Serotype 1 Biotype 2

In European fish farms there is evidence of enteric red mouth disease (ERM) outbreaks in previously vaccinated farmed fish. It has been suggested that the occurrence of a Yersinia ruckeri variant (biotype 2) may explain this situation. Recent development of commercial vaccines has included both biotype 1 and 2. In this study, the specificity of immune protection extended by three commercial vaccines viz; AQUAVAC ERM® Intervet Schering Plough (based on biotype 1 only), ERMGEN VET® Novartis (based on biotype 1 only) and AQUAVAC RELERA® Intervet Schering Plough (based on both biotype 1and 2) developed against ERM was investigated following intraperitoneal (IP) challenge with Yersinia ruckeri serotype1 biotype 2. Fish were immersion vaccinated for 30 s and challenged 2, 4 and 6 months post vaccination. The onset and severity of various pathological lesions along with their disappearance during the course of disease was also carried out to evaluate the protective index conferred by three different vaccines. After IP challenge, the overall best relative percentage survival was observed in AQUAVAC RELERA® followed by ERMGEN VET® with least survival rates in AQUAVAC ERM® among all vaccinated groups. Interestingly a marginal better immune protection was observed between AQUAVAC RELERA® and ERMGEN VET® vaccinated group during the last two challenge trial. The onset and severity of pathological lesions observed during challenge 2 (i.e. 4 month post vaccination) suggested a beneficial efficacy shown by AQUAVAC RELERA® in terms of milder and lesser degree of certain pathological lesions like haemorrhages in or around the buccal cavity, base of fins and intestines, when compared to ERMGEN VET®, AQUAVAC ERM®
Chemical surface disinfection of eggs of Baltic cod, Gadus morhua L.

The effect of two disinfectants on eggs and larvae of Baltic cod, Gadus morhua, was investigated. The eggs were disinfected for 10 min using various concentrations of either glutaraldehyde (100, 200, 400, 600 and 800 mg L⁻¹) or iodophor (10, 50, 100 and 150 mg L⁻¹), 1–4-days post-fertilization. Bactericidal effect of disinfection, survival to hatching, hatching success and larval abnormalities were assessed. Larval survival was recorded at 5-, 10- and 15-days post-hatch (dph). Although Baltic cod eggs have an unusually thin chorion, they could tolerate surface disinfection. A reduction in bacterial growth was observed with increased concentrations of disinfectant (3.0 × 10⁷–1.6 × 10⁸ CFU mL⁻¹). Abnormalities in newly hatched larvae were not related to disinfection. Survival of the yolk sac larvae was significantly better for eggs treated with 400 mg L⁻¹ glutaraldehyde for 10 min at 10 and 15 dph. Effective disinfection was also recorded using 100 mg L⁻¹ Actomar K30. Egg batch effect rather than initial bacterial concentration, disinfectant type or incubation method determined the survival of the eggs to hatching and survival of larvae. Because of the carcinogenic effect of glutaraldehyde, iodophor is recommended for routine disinfection of cod eggs.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, AquaPri Innovation
Authors: Overton, J. L. (Ekstern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern)
Pages: 707-716
Publication date: 2010
Main Research Area: Technical/natural sciences
Publication information
Journal: Journal of Fish Diseases
Volume: 33
Issue number: 9
ISSN (Print): 0140-7775
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.12
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Experimental vaccination of small turbot against bacterial and viral pathogens

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, Fishlab, Intervet/Schering-Plough Animal Health, University of Copenhagen
Publication date: 2010
Event: Abstract from Dafinet-Scofda workshop, Copenhagen, Denmark, .
Main Research Area: Technical/natural sciences
Electronic versions:
E Lorenzen Dafinet november uændringer 2010v3[1].pdf
Source: orbit
Source-ID: 273817
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2010
Flavobacterium psychrophilum infections in rainbow trout: possible control methods

General information
State: Published
Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
Publication date: 2010
Event: Abstract from DAFlinet workshop: Vaccination of early life cycle stages of fish, Copenhagen, Denmark.
Main Research Area: Technical/natural sciences
Electronic versions:
F86EDC9Fd01.pdf
Source: orbit
Source-ID: 274048
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2010

Risikovurdering - stamfiskovervåking og vertikal smitteoverføring: Uttalelse fra Fagruppe for dyrehelse og dyrevelferd i Vitenskapskomiteen for mattrygghet

General information
State: Published
Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Authors: Rimstad, E. (Ekstern), Dalsgaard, I. (Intern), Hjeltnes, B. (Ekstern), Håstein, T. (Ekstern)
Number of pages: 44
Publication date: 2010

Publication information
Publisher: Vitenskapskomiteen for mattrygghet (VKM)
ISBN (Print): 978-82-8082-384-7
Original language: Norwegian
Main Research Area: Technical/natural sciences
Electronic versions:
551A1F7Bd01.pdf
Source: orbit
Source-ID: 272662
Publication: Research › Report – Annual report year: 2010

Yersinia ruckeri challenge with rainbow trout fed different diet

General information
State: Published
Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
Publication date: 2010
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 273809
Publication: Research - peer-review › Poster – Annual report year: 2010

Antibiotic residues in fish from recirculated aquaculture

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, National Veterinary Institute
Authors: Bruun, M. S. (Intern), Henriksen, N. H. (Intern), Dalsgaard, I. (Intern)
Publication date: 2009
Event: Poster session presented at 14th EAFP International Conference on Diseases of Fish and Shellfish, Prague, Czech Republic.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 252788
Publication: Research › Poster – Annual report year: 2009
Bakterielle infektioner hos torsk i opdræt

**General information**

State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Madsen, L. (Intern)
Publication date: 2009
Main Research Area: Technical/natural sciences

**Bibliographical note**

Published in: Program and abstracts, s. 139
Source: orbit
Publication: Research › Poster – Annual report year: 2009

Bath vaccination with Flavobacterium psychrophilum outer membrane vesicles: Up regulation of toll-like receptor in liver tissue

**General information**

State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Bruun, M. S. (Intern), Raida, M. (Intern), Dalsgaard, I. (Intern)
Publication date: 2009
Event: Poster session presented at 14th EAFP International Conference on Diseases of Fish and Shellfish, Prague, Czech Republic.
Main Research Area: Technical/natural sciences

**Bibliographical note**

Published in: Book of abstract, p. 200
Source: orbit
Publication: Research › Poster – Annual report year: 2009

Characterization of Flavobacterium psychrophilum isolates originating from rainbow trout farms with a high degree of water recirculation

**General information**

State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern)
Publication date: 2009
Main Research Area: Technical/natural sciences
Source: orbit
Publication: Research › Conference abstract for conference – Annual report year: 2009

**Characterization of Flavobacterium psychrophilum isolates originating from rainbow trout farms with a high degree of water recirculation**

**General information**

State: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern)
Publication date: 2009

**Host publication Information**

Title of host publication: Flavobacterium 2009 Scientific Program + Proceedings
Main Research Area: Technical/natural sciences
Fate of Flavobacterium psychrophilum bacteria and phages in rainbow trout

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Bertelsen, S. (Ekstern), Middelboe, M. (Ekstern), Dalsgaard, I. (Intern)
Publication date: 2009
Event: Abstract from 14th International Conference, European Association of Fish Pathologists, Tjekkiet.
Main Research Area: Technical/natural sciences

Bibliographical note
Published in: Abstracts Book, P-158, p. 347
Source: orbit
Source-ID: 252678
Publication: Research › Conference abstract for conference – Annual report year: 2009

Identification of flagellar motility genes in Yersinia ruckeri by transposon mutagenesis
Here we demonstrate that flagellar secretion is required for production of secreted lipase activity in the fish pathogen Yersinia ruckeri and that neither of these activities is necessary for virulence in rainbow trout. Our results suggest a possible mechanism for the emergence of nonmotile biotype 2 Y. ruckeri through the mutational loss of flagellar secretion.

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Evenhuis, J. P. (Ekstern), LaPatra, S. E. (Ekstern), Verner-Jeffreys, D. W. (Ekstern), Dalsgaard, I. (Intern), Welch, T. J. (Ekstern)
Pages: 6630-6633
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 75
Isolering af Vibrio-arter fra østersyngel

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern)
Publication date: 2009
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 252677
Publication: Research › Conference abstract in proceedings – Annual report year: 2009

Produktion af torskelarver til udsætning i den østlige Østersø – RESTOCK

General information
State: Published
Organisations: Section for Coastal Ecology, National Institute of Aquatic Resources, Section for Population- and Ecosystem Dynamics, Section for Aquaculture, Section for Fish Diseases
Number of pages: 143
Publication date: 2009
Host publication information
Title of host publication: Program og abstracts
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 253917
Publication: Research › Conference abstract in proceedings – Annual report year: 2009

Proteinafgrøder til økologiske regnbueørreder (Oncorhynchus mykiss)

General information
State: Published
Organisations: Section for Aquaculture, National Institute of Aquatic Resources, Section for Fish Diseases, Section for Aquatic Protein Biochemistry
Authors: Jokumsen, A. (Intern), Lund, I. (Intern), Dalsgaard, A. J. T. (Intern), Dalsgaard, I. (Intern), Nielsen, H. H. (Intern), Rasmussen, H. T. (Ekstern), Larsen, V. J. (Ekstern), Jessen, P. B. (Ekstern), Holm, J. (Ekstern)
Publication date: 2009
Event: Poster session presented at Økologi-kongres, Odense, Danmark.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 252715
Publication: Research › Poster – Annual report year: 2009

Recent knowledge of Flavobacterium psychrophilum in Denmark

General information
State: Published
Organisations: Section for Veterinary Diagnostics, Division of Veterinary Diagnostics and Research, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Andersen, J. H. (Intern), Madsen, L. (Intern)
Yersinia ruckeri biotype 2 isolates from mainland Europe and the UK likely represent different clonal groups

There have been increased reports of outbreaks of enteric redmouth disease (ERM) caused by Yersinia ruckeri in previously vaccinated salmonids in Europe, with some of these outbreaks being attributed to emergent non-motile, Tween 80-negative, biotype 2 isolates. To gain information about their likely origins and relationships, a geographically and temporally diverse collection of isolates were characterised by serotyping, biotyping, pulsed-field gel electrophoresis (PFGE) and outer membrane protein (OMP) profiling. A total of 44 pulsotypes were identified from 160 isolates by PFGE, using the restriction enzyme NotI. Serotype O1 isolates responsible for ERM in rainbow trout in both the US and Europe, and including biotype 2 isolates, represented a distinct subgroup of similar pulsotypes. Biotype 2 isolates, responsible for outbreaks of the disease in rainbow trout in the UK, Denmark and Spain, had different pulsotypes, suggesting that they represented different clones that may have emerged separately. Danish biotype 2 isolates recovered since 1995 were indistinguishable by PFGE from the dominant biotype 1 clone responsible for the majority of outbreaks in Denmark and the rest of mainland Europe. In contrast, US biotype 2 isolate YRNC10 had an identical pulsotype and OMP profile to UK biotype 2 isolates, suggesting that there had been exchange of these isolates between the UK and the US in the past. UK Atlantic salmon isolates were genetically and serologically diverse, with 12 distinct pulsotypes identified among 32 isolates.
**Bakterier og antibiotika: Fra: Drift og fiskesygdomme i modeldambrug: Master management system**

**General information**
State: Published
Organisations: National Institute of Aquatic Resources, Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute
Authors: Bruun, M. S. (Intern), Madsen, L. (Intern), Dalsgaard, I. (Intern)
Number of pages: 18
Publication date: 2008

**Publication information**
Publisher: Dansk Akvakultur
Original language: English
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 277732
Publication: Research › Report – Annual report year: 2008

**Isolation and characterization of bacteriophages infecting the fish pathogen Flavobacterium psychrophilum**

**General information**
State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Stenholm, A. R. (Intern), Dalsgaard, I. (Intern), Middelboe, M. (Ekstern)
Pages: 4070-4078
Publication date: 2008
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Applied and Environmental Microbiology
Volume: 74
Issue number: 13
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12

General information
State: Published
Organisations: Bacteriology & Pathology, Division of Veterinary Diagnostics and Research, National Veterinary Institute, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Overton, J. L. (Intern)
Publication date: 2008

Publication information
Place of publication: Charlottenlund
Publisher: DTU Aqua. Institut for Akvatiske Ressourcer
Original language: English
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 227922
Publication: Research - peer-review › Journal article – Annual report year: 2008

Permanent network to strengthen expertise on infectious diseases of aquaculture species and scientific advice to EU policy. PANDA. WP4: Report on the current best methods for rapid and accurate detection on the main disease hazards in aquaculture, requirements for improvement, their eventual standardisation and validation, and how to achieve harmonised implementation throughout Europe of the best diagnostic methods
Rainbow trout farms with a high degree of recirculation: Are pathogenic bacteria a problem?

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Haenen, O. (Ekstern), Dalsgaard, I. (Intern), Bonami, J. (Ekstern), Joly, J. (Ekstern), Olesen, N. (Ekstern), Jensen, B. (Ekstern), Ariel, E. (Ekstern), Miossec, L. (Ekstern), Arzul, I. (Ekstern)
Publication date: 2008

Publication information
Publisher: [s.n.]
Original language: English
Main Research Area: Technical/natural sciences

Bibliographical note
Project no. SSPE-CT-2003-502329
Source: orbit
Source-ID: 231503
Publication: Research › Report – Annual report year: 2008

Water recirculation and good management: potential methods to avoid disease outbreaks with Flavobacterium psychrophilum

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Madsen, L. (Intern)
Publication date: 2008

Host publication information
Title of host publication: Programme and abstract book : SCOFDA workshop: Diagnosis and Control of Fish Diseases
Publisher: Københavns Universitet
Main Research Area: Technical/natural sciences
Conference: SCOFDA workshop: Diagnosis and Control of Fish Diseases, 4-5/11, 2008, KU-LIFE, 01/01/2008
Source: orbit
Source-ID: 231501
Publication: Research › Article in proceedings – Annual report year: 2008

Publication information
Journal: Journal of Fish Diseases
Volume: 31
Issue number: 11
ISSN (Print): 0140-7775
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.12
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.71
Web of Science (2015): Indexed yes
Bacterial infections in cod from "pre-production trials"

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Madsen, L. (Intern)
Publication date: 2007

Host publication information
Title of host publication: EAFP, 13th International Conference "Diseases of Fish and Shellfish", Grado, Italy
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225223
Publication: Research › Conference abstract in proceedings – Annual report year: 2007

Characterisation of Yersinia ruckeri by pulsed-field gel electrophoresis (PFGE) and multiple locus sequence typing (MLST)

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Wheeler, R. (Ekstern), Davies, R. (Ekstern), Dalsgaard, I. (Intern), Wagley, S. (Ekstern), Verner-Jeffreys, D. (Ekstern)
Isolation of Vibrio species from flat oyster (Ostrea edulis) larvae

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern)
Publication date: 2007

Host publication information
Title of host publication: Abstract Book - EAFP, 13th International Conference "Diseases of Fish and Shellfish", Grado, Italy
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 226560
Publication: Research › Conference abstract in proceedings – Annual report year: 2007

Profiling acylated homoserine lactones in Yersinia ruckeri and influence of exogenous acyl homoserine lactones and known quorum-sensing inhibitors on protease production

To profile the quorum-sensing (QS) signals in Yersinia ruckeri and to examine the possible regulatory link between QS signals and a typical QS-regulated virulence phenotype, a protease. Methods and Results: Liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) showed that Y. ruckeri produced at least eight different acylated homoserine lactones (AHLs) with N-(3-oxooctanoyl)-l-homoserine lactone (3-oxo-C8-HSL) being the dominant molecule. Also, some uncommon AHL, N-(3-oxoheptanoyl)-l-homoserine lactone (3-oxo-C7-HSL) and N-(3-oxononanoyl)-l-homoserine lactone (3-oxo-C9-HSL), were produced. 3-oxo-C8-HSL was detected in organs from fish infected with Y. ruckeri. Protease production was significantly lower at temperatures above 23 degrees C than below although growth was faster at the higher temperatures. Neither addition of sterile filtered high-density Y. ruckeri culture supernatant nor the addition of pure exogenous AHLs induced protease production. Furthermore, three QS inhibitors (QSI), sulfur-containing AHL analogues, did not inhibit protease production in Y. ruckeri. Conclusions: Exogenous AHL or sulfur-containing AHL analogues did not influence the protease production indicating that protease production may not be QS regulated in Y. ruckeri. Significance and Impact of the Study: The array of different AHLs produced indicates that the QS system of Y. ruckeri is complex and could involve several regulatory systems. In this case, neither AHLs nor QSI would be likely to directly affect a QS-regulated phenotype.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Center for Microbial Biotechnology, Department of Systems Biology, National Veterinary Institute, Center for Biomedical Microbiology
Authors: Kastbjerg, V. G. (Intern), Nielsen, K. F. (Intern), Dalsgaard, I. (Intern), Rasch, M. (Intern), Bruhn, J. B. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)
Pages: 363-374
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 102
Issue number: 2
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
Quorum sensing signals are produced by Aeromonas salmonicida and quorum sensing inhibitors can reduce production of a potential virulence factor.

Many pathogens control production of virulence factors by self-produced signals in a process called quorum sensing (QS). We demonstrate that acyl homoserine lactone (AHL) signals, which enable bacteria to express certain phenotypes in relation to cell density, are produced by a wide spectrum of Aeromonas salmonicida strains. All 31 typical strains were AHL producers as were 21 of 26 atypical strains, but on a strain population basis, production of virulence factors such as protease, lipase, A-layer or pigment did not correlate with the production and accumulation of AHLs in the growth medium. Pigment production was only observed in broth under highly aerated conditions. Quorum sensing inhibitors (QSIs) are compounds that specifically block QS systems without affecting bacterial growth and 2 such compounds, sulphur-containing AHL-analogues, reduced production of protease in a typical strain of Aeromonas salmonicida. The most efficient compound N-(heptylsulfanylacetyl)-L-homoserine lactone (HepS-AHL), reduced protease production by a factor of 10. Five extracellular proteases were detected on gelatin-containing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gels and 3 of these were completely down regulated by HepS-AHL. Hence, QSIs can curb...
virulence in some strains and could potentially be pursued as bacterial disease control measures in aquaculture.

**General information**

State: Published
Organisations: National Institute of Aquatic Resources, Department of Systems Biology, Section for Fish Diseases
Authors: Rasch, M. (Intern), Kastbjerg, V. G. (Intern), Bruhn, J. B. (Intern), Dalsgaard, I. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)
Pages: 105-113
Publication date: 2007
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Diseases of Aquatic Organisms
Volume: 78
Issue number: 2
ISSN (Print): 0177-5103
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.95 SJR 0.858 SNIP 0.929
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.949 SNIP 0.935 CiteScore 1.96
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.889 SNIP 0.881 CiteScore 1.86
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.812 SNIP 0.918 CiteScore 1.77
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.912 SNIP 1.092 CiteScore 2.04
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.11 SNIP 1.165 CiteScore 2.29
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.91 SNIP 0.951
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.889 SNIP 0.99
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.859 SNIP 0.998
Scopus rating (2007): SJR 0.949 SNIP 1.054
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.868 SNIP 0.964
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.898 SNIP 1.046
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.972 SNIP 1.105
Rainbow trout farms with a high degree of recirculation: Pathogenic bacteria and antimicrobial resistance

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Bruun, M. S. (Intern), Madsen, L. (Intern), Dalsgaard, I. (Intern)
Publication date: 2007

Host publication information
Title of host publication: Abstract Book - EAFP, 13th International Conference "Diseases of Fish and Shellfish", Grado, Italy and: The Research School SCOFDA, October 30-31
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225020
Publication: Research › Conference abstract in proceedings – Annual report year: 2007

The fate of chemical additives and antimicrobial agents applied in Danish freshwater fish farms

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, Section for Aquaculture
Authors: Bruun, M. S. (Intern), Pedersen, L. (Intern), Dalsgaard, I. (Intern), Pedersen, P. B. (Intern), Sortkjaer, O. (Ekstern)
Pages: 57-61
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: World Aquaculture
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ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
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Original language: English
Source: orbit
Source-ID: 225021
The impact of CWD/RTFS on rainbow trout farming in Denmark

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Madsen, L. (Intern)
Number of pages: 9
Publication date: 2007

Host publication information
Title of host publication: Flavobacterium 2007 Workshop, Shepherdstown, West Virginia, USA, 2-4 September
Main Research Area: Technical/natural sciences
Conference: Flavobacterium 2007 Workshop, Shepherdstown, West Virginia, USA, 2-4 September, 01/01/2007
Source: orbit
Source-ID: 225232

The tetracycline resistance gene tet(E) is frequently occurring and present on large horizontally transferable plasmids in Aeromonas spp. from fish farms

General information
State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute, Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Agersø, Y. (Intern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern), Larsen, J. L. (Ekstern)
Pages: 47-52
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Aquaculture
Volume: 266
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Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Common errors in surveillance and monitoring programs on fish populations

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dopazo, C. (Ekstern), De Blas, I. (Ekstern), Miossec, L. (Ekstern), Cameron, A. (Ekstern), Vallejo, A. (Ekstern), Dalsgaard, I. (Intern)
Publication date: 2006
Event: Poster session presented at 11th International Society for Veterinary Epidemiology and Economics, Cairns, Australia.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225339
Publication: Research › Poster – Annual report year: 2006

Isolation and characterization of lytic Flavobacterium psychrophilum bacteriophages

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Økologisk fiskeopdræt: Rapport fra en vidensyntese om udviklingsmuligheder inden for økologisk fiskeopdræt i Danmark

General information
State: Published
Organisations: Section for Aquaculture, National Institute of Aquatic Resources, Section for Fish Diseases, Section for Aquatic Protein Biochemistry
Authors: Jokumsen, A. (Intern), Larsen, V. (Ekstern), Dalsgaard, I. (Intern), Nielsen, H. H. (Intern), Jessen, P. (Ekstern), Kold, J. (Ekstern), Jokumsen, A. (ed.) (Intern)
Number of pages: 110
Publication date: 2006

Publication Information
Publisher: FØJO
Original language: Danish
Series: FØJO rapport
Number: 21
Main Research Area: Technical/natural sciences
Links:
Source: orbit
Source-ID: 226076
Publication: Research › Report – Annual report year: 2006

Rainbow trout farms with a high degree of recirculation: are pathogenic bacteria a problem?

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern)
Number of pages: 173
Publication date: 2006

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Title of host publication: Abstract Book - Fifth International Symposium on Aquatic Animal Health, San Francisco, USA, 2.-6. september
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 226563
Publication: Research › Conference abstract in proceedings – Annual report year: 2006

Some aspects in epidemiology studies in shrimp farms and their impact in wild populations

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: De Blas, I. (Ekstern), Vallejo, A. (Ekstern), Cameron, A. (Ekstern), Miossec, L. (Ekstern), Dopazo, C. (Ekstern), Dalsgaard, I. (Intern)
Publication date: 2006
Event: Poster session presented at Joint International Conference of European Aquaculture Society & World aquaculture Society, Firenze, Italy.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225253
Transfer of pathogens between farmed and wild aquatic animals - epidemiological basis for demonstrating causality

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Cameron, A. (Ekstern), De Blas, I. (Ekstern), Miossec, L. (Ekstern), Dopazo, C. (Ekstern), Vallejo, A. (Ekstern), Dalsgaard, I. (Intern)
Publication date: 2006
Event: Poster session presented at 11th International Society for Veterinary Epidemiology and Economics, Cairns, Australia.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225077
Publication: Research › Poster – Annual report year: 2006

Winter ulcer disease of post-smolt Atlantic salmon: an unsuitable case for treatment?

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Coyne, R. (Ekstern), Smith, P. (Ekstern), Dalsgaard, I. (Intern), Nielsen, H. (Ekstern), Kongshaug, H. (Ekstern), Bergh, Ø. (Ekstern), Samuelsen, O. (Ekstern)
Pages: 171-178
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Main Research Area: Technical/natural sciences

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BFI (2018): BFI-level 2
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Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.294 SNIP 1.542 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Characterisation of surface blebbing and membrane vesicles produced by Flavobacterium psychrophilum

The surface of Flavobacterium psychrophilum was examined by electron microscopy to determine if previous findings of haemagglutination positive (HA+) and haemagglutination negative (HA-) abilities could be correlated with expression of pili or of a capsular layer. A thin capsular layer was observed in both HA+ and HA- strains but typical pili were absent. However, long, tubular blebs that released membrane vesicles (MVs) into the supernatant were observed on up to 94% of cells within 1 sample. The surface blebbing was increased for 1 strain following growth on media with restricted iron availability. The MVs had an intact membrane bilayer and were released from blebbing cells of both strains. The protein profiles of MVs, while containing some banding similarity with the profile of outer membrane preparations (OMPs) and of lysed whole cells (WCs), showed several bands that reacted strongly with rabbit anti-whole-cell antisera. Two distinct bands of approximately 62 and 58 kDa were highly expressed in the MVs and not seen in the OMP. MVs contained proteolytic activity towards gelatine but not towards casein and elastin, which were only degraded by live cells. Low molecular weight lipopolysaccharides (LPS) or lipooligosaccharides (LOS) were associated with the MVs. Only the MVs of the HA+ strain possessed haemagglutinin activity. These findings suggest that the F. psychrophilum may, through surface blebbing, release antigenic MVs that contain some proteolytic activity and may aid the bacterium in releasing nutrients from its surrounding environment as well as playing a role in impeding the immune response of its host.

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State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Møller, J. D. (Intern), Barnes, A. (Ekstern), Dalsgaard, I. (Intern), Ellis, A. (Ekstern)
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Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Diseases of Aquatic Organisms
Volume: 64
Flavobacterium psychrophilum in rainbow trout, Oncorhynchus mykiss (Walbaum), hatcheries: studies on broodstock, eggs, fry and environment

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Møller, J. D. (Intern), Dalsgaard, I. (Intern)
Pages: 39-47
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Fish Diseases
Volume: 28
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ISSN (Print): 0140-7775
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.12
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.71
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.99
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.74
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.7
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.09
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Web of Science (2008): Indexed yes
Web of Science (2007): Indexed yes
Web of Science (2006): Indexed yes
Web of Science (2005): Indexed yes
Iron acquisition mechanisms of Flavobacterium psychrophilum

Forty strains of Flavobacterium psychrophilum were tested for the production of siderophores using the universal Chrome Azurol S (CAS) assay. The majority of the strains (85%) were CAS positive (CAS+) and some (15%) were CAS negative (CAS-). The cryptic plasmid pCP1 was carried by all positive strains and was lacking from negative strains. While a weak catechol reaction was detectable in CAS+ culture supernatants, the CAS reaction was, to some extent, heat sensitive, questioning whether the positive reaction was caused only by siderophores. The ability to grow in vitro under iron-restricted conditions did not correlate with the CAS reactivity, as growth of both CAS+ and CAS- strains was similarly impaired under iron restriction induced by 2,2 dipyridyl. Suppressed growth under these conditions was restored by addition of FeCl3, haemoglobin and transferrin for both CAS+ and CAS- strains.

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State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Møller, J. D. (Intern), Ellis, A. (Ekstern), Barnes, A. (Ekstern), Dalsgaard, I. (Intern)
Pages: 391-398
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Main Research Area: Technical/natural sciences

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Journal: Journal of Fish Diseases
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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.12
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.71
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.99
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 1.74
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.7
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.09
Novel strategies for production of RTFS vaccines

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Møller, J. D. (Intern), Dalsgaard, I. (Intern)
Pages: 18-19
Publication date: 2005

Host publication information
Title of host publication: SCOFDA Workshop "Diagnosis and Control of Fish Diseases", The Royal Veterinary and Agricultural University, November 2 and 3
Main Research Area: Technical/natural sciences
Conference: SCOFDA Workshop "Diagnosis and Control of Fish Diseases", The Royal Veterinary and Agricultural University, November 2 and 3, 2005, 01/01/2005
Source: orbit
Source-ID: 238776
Publication: Research › Article in proceedings – Annual report year: 2005

Quorum sensing signal molecules (acylated homoserine lactones) in Gram-negative fish pathogenic bacteria
The aim of the present study was to investigate the production of quorum sensing signals (specifically acylated homoserine lactones, AHLs) among a selection of strains of Gram-negative fish bacterial pathogens. These signals are involved in the regulation of virulence factors in some human and plant-pathogenic bacteria. A total of 59 strains, representing 9 different fish pathogenic species, were tested against 2 AHL monitor bacteria (Agrobacterium tumefaciens NT1 [pZLR4] and Chromobacterium violaceum CV026) in a well diffusion assay and by thin-layer chromatography (TLC). Representative samples were further characterized by high performance liquid chromatography-high resolution mass spectrometry (HPLC-HR-MS). AHLs were produced by all strains of Aeromonas salmonicida, Aeromonas hydrophila, Yersinia ruckeri, Vibrio salmonicida, and Vibrio vulnificus. Some strains of atypical Aeromonas salmonicida and Vibrio splendidus were also positive. Aeromonas species produced N-butanoyl homoserine lactone (BHL) and N-hexanoyl homoserine lactone (HHL) and 1 additional product, whereas N-3-oxo-hexanoyl homoserine lactone (OHL) and HHL were detected in V. salmonicida. N-3-oxo-octanoyl homoserine lactone (OOHL) and N-3-octanoyl homoserine lactone (OHL) were detected in V. ruckeii. AHLs were not detected from strains of Photobacterium damselae, Flavobacterium psychrophilum or Moritella viscosa. AHLs were extracted from fish infected with Y. ruckeri but not from fish infected with A. salmonicida. In conclusion, the production of quorum sensing signals, AHLs, is common among the strains that we examined. If the AHL molecules regulate the expression of the virulence phenotype in these bacteria, as shown to occur in some bacterial pathogens, novel disease control measures may be developed by blocking AHL-mediated communication and suppressing virulence.

General information
Rainbow trout fry syndrome: Diagnosis and control

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Bruun, M. S. (Intern), Madsen, L. (Intern)
Pages: 17-18
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Main Research Area: Technical/natural sciences
Conference: SCOFDA Workshop "Diagnosis and Control of Fish Diseases", The Royal Veterinary and Agricultural University, November 2 and 3, 01/01/2005
Source: orbit
Source-ID: 229011
Publication: Research › Article in proceedings – Annual report year: 2005

Standardization of a broth microdilution susceptibility testing method to determine minimum inhibitory concentrations of aquatic bacteria

A multiple laboratory study was conducted in accordance with the standards established by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS), for the development of quality control (QC) ranges using dilution antimicrobial susceptibility testing methods for bacterial isolates from aquatic animal species. QC ranges were established for Escherichia coli ATCC 25922 and Aeromonas salmonicida subsp. salmonicida ATCC 33658 when testing at 22, 28 and 35 degrees C (E. coli only) for 10 different antimicrobial agents (ampicillin, enrofloxacin, erythromycin, florfenicol, flumequine, gentamicin, ormetoprim/sulfadimethoxine, oxolinic acid, oxytetraacycline and trimethoprim/sulfamethoxazole). Minimum inhibitory concentration (MIC) QC ranges were determined using dry- and frozen-form 96-well plates and cation-adjusted Mueller-Hinton broth. These QC ranges were accepted by the CLSI/NCCLS Subcommittee on Veterinary Antimicrobial Susceptibility Testing in January 2004. This broth microdilution testing method represents the first standardized method for determining MICs of bacterial isolates whose preferred growth temperatures are below 35 degrees C. Methods and QC ranges defined in this study will enable aquatic animal disease researchers to reliably compare quantitative susceptibility testing data between laboratories, and will be used to ensure both precision and inter-laboratory harmonization
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.95 SJR 0.858 SNIP 0.929
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.949 SNIP 0.935 CiteScore 1.96
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.889 SNIP 0.881 CiteScore 1.86
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.812 SNIP 0.918 CiteScore 1.77
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.912 SNIP 1.092 CiteScore 2.04
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.11 SNIP 1.165 CiteScore 2.29
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.91 SNIP 0.951
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.889 SNIP 0.99
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.859 SNIP 0.998
Scopus rating (2007): SJR 0.949 SNIP 1.054
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.868 SNIP 0.964
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.898 SNIP 1.046
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.972 SNIP 1.105
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.931 SNIP 1.187
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.083 SNIP 1.187
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.347 SNIP 1.197
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.091 SNIP 1.221
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.192 SNIP 1.136
Original language: English
DOIs:
10.3354/dao064211
A comparison of oxolinic acid concentrations in farmed and laboratory held rainbow trout (Oncorhynchus mykiss) following oral therapy

Plasma oxolinic acid (OXA) concentrations were measured in fish from a cage of farmed rainbow trout (Oncorhynchus mykiss) 1 day after the termination of medication. The fish were experiencing significant mortalities and following a diagnosis of vibriosis, OXA had been orally administered at 50 mg/kg for 6 days over a 9-day period. Samples from healthy fish (n=20), moribund (n=26) and dead fish (n=10) were analysed by HPLC. There was a dramatic difference in the OXA concentrations between healthy and moribund fish. In the moribund group, none of which showed signs of recent feeding, 85% of the fish had OXA levels below the LOQ (0.005 mg/l). In contrast, 95% of the healthy fish had OXA concentrations >0.015 mg/l and the mean OXA concentration (±standard deviation) was 0.156±0.152 mg/l. The mean OXA concentrations detected in the healthy fish in the farm were similar to those achieved in 30 laboratory held rainbow trout (O. mykiss) following the administration of OXA under similar conditions of salinity, temperature and dosing regimen. In these laboratory held fish, the mean plasma OXA concentration was 0.133±0.068 mg/l. The major difference between the distributions of OXA concentrations in the farm and laboratory populations was in the extent of fish to fish variation observed. In the healthy farmed fish, the percentage coefficient of variation (%CV) was 97% compared to a %CV of 51% in the laboratory held fish. The patterns of the daily mortality in the farmed population were analysed from 20 days before the initiation of therapy to 20 days after its completion but these data failed to provide unambiguous evidence as to the success or other wise of the therapeutic intervention. The moribund fish examined at the end of the therapy showed signs of systemic disease but from the majority, no bacteria were isolated. Strains of Vibrio anguillarum were isolated from some dead and moribund fish and these had a MIC of 0.0625 mg/l. It was, therefore, not possible to use the data generated in this work on the OXA plasma concentrations, the efficacy of the therapy and the MIC values of the infecting bacteria, to investigate the validity of any formula for estimating breakpoint MIC values.
Concentrations of oxolinic acid (OXA) were measured in the plasma, muscle, liver, and kidney of 48 Atlantic salmons (Salmo salar) 1 day after the end of an oral administration. OXA was administered over a period of 13 days to control an outbreak of winter ulcer disease in a commercial marine farm. On the basis of their behaviour, the fish were classified as healthy (n=18), moribund (n=20), or dead (n=10). There was a dramatic difference in the OXA concentrations in the healthy fish and those classified as moribund or dead. There was no evidence of bacterial infection in the 18 healthy fish, all of which were shown to be feeding. In these fish, the mean concentrations of OXA (±standard deviation) in the plasma, muscle, liver, and kidney were 0.40±0.36 mg/l, 1.0±0.71 mg/kg, 0.93±0.67 mg/kg, and 1.13±0.87 mg/kg, respectively. Within the healthy group, there were considerable individual fish-to-fish variations in OXA concentrations and the mean coefficient of variation (CV) for the concentrations in the four tissues was 77%. In contrast, all 20 moribund fish showed external lesions and 19 showed signs of systemic infection. Only 2 showed signs of feeding, and the concentrations of OXA were below the limit of quantitation (LOQ) in 68%, 85%, 70%, and 80% of the plasma, muscle, liver, and kidney, respectively. These data suggest that the major function of the therapy was to assist healthy fish to resist de novo infection and that moribund fish had gained little or no benefit from the oral administration of OXA. A numerical description of the concentration of the antimicrobial agent achieved in therapy is necessary to determine the resistance or sensitivity of the bacteria involved in the infection. The degree of fish-to-fish variation in the concentrations of OXA, both within the healthy fish and between healthy and moribund fish, presents difficulties in generating a clinically meaningful description relevant to the whole population. This issue is discussed, and it is suggested that for this application, the minimum concentration achieved by at least 80% of the treated population might represent a useful parameter for describing the concentrations of agents achieved during therapy. The plasma data from this investigation were used to estimate clinically relevant breakpoint minimum inhibitory concentration (MIC) values. The validity of these breakpoint values was discussed with reference to the outcome of the therapy and the susceptibility of the bacteria isolated from infected fish.
On the validity of setting breakpoint minimum inhibition concentrations at one quarter of the plasma concentration achieved following oral administration of oxytetracycline

Plasma concentrations of oxytetracycline (OTC) were established in two Atlantic salmon (Salmo salar) pre-smolts populations after they had received OTC medicated feed at a rate of 75 mg OTC/kg over 10 days. One population was experiencing an epizootic of furunculosis in a commercial freshwater farm and the other was held in a laboratory. Both populations were maintained at approximately 13 °C. The mean plasma concentration in 26 health farm fish was 0.25±0.06 and the 80th percentile was 0.21 mg/l. The mean concentration for 26 laboratory fish was 0.21±0.06 mg/l with an 80th percentile of 0.15 mg/l. The validity of setting a breakpoint minimum inhibitory concentration (MIC) at a quarter of these plasma concentrations was investigated. The MIC of the Aeromonas salmonicida isolated from the farmed fish (n=7) was 0.5 mg/l and the breakpoints generated by application of the 4:1 ratio were in the range 0.03125–0.0625 mg/l. These breakpoint values would, therefore, predict that the therapy should have had no beneficial effect and that any strain of A. salmonicida with MIC>0.0625 mg/l must be considered as resistant. A consideration of the pattern of the mortalities before and during the period of therapy suggests that the therapy was probably beneficial. Thus, the data obtained in this work suggest that the application of the 4:1 ratio is not a valid method of generating meaningful breakpoint MIC values. Published values for the MIC of OTC against A. salmonicida and the plasma concentrations achieved after oral administration of OTC medicated feed were applied to investigate the validity of the application of the 4:1 ratio.
Breakpoints generated by the application of this ratio to these data would suggest that OTC could never have had any value in combating A. salmonicida infections. As this conclusion is contrary to experience, it is argued that examination of the published data reinforces the conclusion that the 4:1 ratio has little value in the oral therapy of fish disease.

**General information**

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources  
Authors: Coyne, R. (Ekstern), Samuelsen, O. (Ekstern), Bergh, Ø. (Ekstern), Andersen, K. (Ekstern), Pursell, L. (Ekstern), Dalsgaard, I. (Intern), Smith, P. (Ekstern)  
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Main Research Area: Technical/natural sciences

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Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 2  
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12  
Web of Science (2015): Indexed yes  
BFI (2014): BFI-level 2  
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 1  
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 1  
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 1  
Scopus rating (2011): SJR 1.294 SNIP 1.542 CiteScore 2.39  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 1  
Scopus rating (2010): SJR 1.151 SNIP 1.394  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 1  
Scopus rating (2009): SJR 0.941 SNIP 1.263  
Web of Science (2009): Indexed yes  
BFI (2008): BFI-level 2  
Scopus rating (2008): SJR 0.909 SNIP 1.173  
Web of Science (2008): Indexed yes  
Scopus rating (2007): SJR 1.019 SNIP 1.318  
Web of Science (2007): Indexed yes  
Scopus rating (2006): SJR 1.008 SNIP 1.689  
Web of Science (2006): Indexed yes
The precision and robustness of published protocols for disc diffusion assays of antimicrobial agent susceptibility: an inter-laboratory study

The precision of the disc diffusion protocols previously published by Alderman and Smith (Aquaculture 196 (2002) 211) was analysed in a seven-laboratory trial using Escherichia coli ATCC 25922 as the test strain. Discs containing seven antimicrobial agents were employed and 2899 zone size measurements were generated. The total data generated in the trial was used to quantify the intra- and inter-laboratory precisions. The study design also facilitated the investigation of the influence of the source of media and the source of discs on zone sizes. A smaller two-laboratory trial was employed to investigate the influence of incubation time of zone size. The intra-laboratory precision was relatively high with the mean of the coefficients of variation calculated for each laboratory and each agent being 4.7%. In contrast, the inter-laboratory precision was very much lower with the mean of the values for each agent being 11.1%. Significant influences on zone size were detected for all three parameters of the protocol. Media source effects were particularly notable with respect to oxytetracycline and oxolinic acid discs, disc source effects with respect to ampicillin and sulphamethoxazole/trimethoprim discs and incubation times with the ampicillin and amoxycillin discs. ANOVA analysis of the total data set confirmed that inter-laboratory variation was the major factor influencing the low precision of the protocol. The overall precision of the protocols used here was found to be significantly lower than that implied by the control limits associated with the same bacterium in other validated disc diffusion protocols. The implications of these results, for the further development of the protocols under investigation, are discussed.
Association of Flavobacterium psychrophilum with rainbow trout (Oncorhynchus mykiss) kidney phagocytes in vitro

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Wiklund, T. (Ekstern), Dalsgaard, I. (Intern)
Pages: 387-395
Publication date: 2003
Main Research Area: Technical/natural sciences

Publication information
Journal: Fish & Shellfish Immunology
Volume: 15
Issue number: 5
ISSN (Print): 1050-4648
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.36 SJR 1.114 SNIP 1.16
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.268 SNIP 1.171 CiteScore 3.19
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.138 SNIP 1.089 CiteScore 2.92
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.001 SNIP 1.149 CiteScore 3.11
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.151 SNIP 1.174 CiteScore 3.02
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.196 SNIP 1.265 CiteScore 3.52
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.131 SNIP 1.056
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.96 SNIP 1.101
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 0.952 SNIP 1.062
Scopus rating (2007): SJR 0.842 SNIP 1.378
Web of Science (2007): Indexed yes
Concomitant exposure of rainbow trout fry to Gyrodactylus derjavini and Flavobacterium psychrophilum: effects on infection and mortality of host

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Busch, S. (Ekstern), Dalsgaard, I. (Intern), Buchmann, K. (Ekstern)
Pages: 117-122
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Journal: Veterinary Parasitology
Volume: 117
ISSN (Print): 0304-4017
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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.49 SJR 1.173 SNIP 1.228
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.21 SNIP 1.339 CiteScore 2.46
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.316 SNIP 1.421 CiteScore 2.53
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.251 SNIP 1.45 CiteScore 2.63
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.165 SNIP 1.454 CiteScore 2.6
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Conjugal transfer of large plasmids conferring oxytetracycline (OTC) resistance: Transfer between environmental aeromonads, fish-pathogenic bacteria, and Escherichia coli

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Bruun, M. S. (Intern), Schmidt, A. (Ekstern), Dalsgaard, I. (Intern), Larsen, J. (Ekstern)
Pages: 69-79
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Journal: Journal of Aquatic Animal Health
Volume: 15
Issue number: 1
ISSN (Print): 0899-7659
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.472 SNIP 0.583 CiteScore 1.09
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.549 SNIP 0.698 CiteScore 1.06
BFI (2014): BFI-level 1
Efficiency of oxytetracycline treatment in rainbow trout experimentally infected with Flavobacterium psychrophilum strains having different in vitro antibiotic susceptibilities

The medication effect of oxytetracycline on groups of rainbow trout fry experimentally infected with three strains of Flavobacterium psychrophilum was investigated. The infection model was based on intraperitoneal injection of the pathogen and treatment was done using medicated feed resulting in 100 mg oxytetracycline/kg fish for 10 days. The three F. psychrophilum strains had different antimicrobial susceptibilities and successful treatment was only obtained in the trial using a strain with a MICOTC of 0.25 μg/ml. No effect of treatment was seen in the group infected with a strain having MICOTC of 8.0 μg/ml and only little effect was seen when the strain MICOTC was 4.0 μg/ml. This shows that it is valid to predict the treatment efficiency of OTC from in vitro data facing an outbreak of rainbow trout fry syndrome. The importance of doing susceptibility testing is emphasized, and as shown the selection of media for antimicrobial susceptibility testing of F. psychrophilum is important. (C) 2003 Elsevier Science B.V. All rights reserved

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Bruun, M. S. (Intern), Madsen, L. (Intern), Dalsgaard, I. (Intern)
Pages: 11-20
Publication date: 2003
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Journal: Aquaculture
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BFI (2018): BFI-level 2
Forebyggelse af YDS (yngeledødelighedssyndrom) og begrænsning af medicinføring i æg- og yngelopdræt i danske dambrug

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State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Jensen, P. (Ekstern), Henriksen, N. (Ekstern), Michelsen, K. (Ekstern), Madsen, L. (Intern), Dalsgaard, I. (Intern)
Number of pages: 129
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Links:
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Involvement of a sialic acid-binding lectin with hemagglutination and hydrophobicity of Flavobacterium psychrophilum

General information
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Organisations: National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Møller, J. D. (Intern), Larsen, J. (Ekstern), Madsen, L. (Intern), Dalsgaard, I. (Intern)
Pages: 5275-5280
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Journal: Applied and Environmental Microbiology
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Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Forebyggelse af YDS (yngeldødelighedssyndrom) og begrænsning af medicinforbrug i æg- og yngelopdræt i danske dambrug. Projektfase III : Slutrapport, oktober 2002

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Jensen, P. (Ekstern), Henriksen, N. (Ekstern), Michelsen, K. (Ekstern), Madsen, L. (Intern), Dalsgaard, I. (Intern)
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Publication date: 2002

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Original language: Danish
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 226003
Misidentification of Vibrio cholerae O155 isolated from imported shrimp as O serogroup O139 due to cross-agglutination with commercial O139 antiserum

Fish and shellfish products imported into Denmark are routinely analyzed for pathogenic Vibrio spp., particularly Vibrio cholerae, if products originate from subtropical or tropical areas. A V. cholerae strain that agglutinated commercial O139 antiserum but not the O1, Inaba, or Ogawa antiserum was isolated from imported raw frozen shrimp. The toxigenicity of the strain was analyzed, and the results of a polymerase chain reaction showed that the V. cholerae strain did not contain the virulence genes ctx, tcp9, and zot, which are normally found in V. cholerae O1 and O139. The strain was resistant to colistin and spectinomycin. The high susceptibility of the strain to antimicrobial agents was confirmed by the lack of an SXT element, a self-transmissible, chromosomal genetic element that is normally present in O139 strains and encodes resistance to sulfonamides, trimethoprim, and streptomycin. The strain contained two plasmids, in contrast to other O139 strains, which normally do not contain plasmids. The characteristics of the strain led to further agglutination testing with other antiserum that are not commercially available, and the strain was found to agglutinate O155 antiserum in repeated testing. Manufacturers of O139 antiserum should be aware of the closely related O antigens of the O139, O22, and O155 serogroups and should be aware that their commercial diagnostic O139 antiserum must be absorbed to remove cross-reacting agglutinins of O22 and O155 strains.
Occurrence of Flavobacterium psychrophilum in fish-farming environments

Occurrence of Flavobacterium psychrophilum in fish farms and fish-farming environments was studied using agar plate cultivation, the immunofluorescence antibody technique (IFAT) and nested PCR. Characteristics of 64 F. psychrophilum isolates from rainbow trout Oncorhynchus mykiss, fish farm rearing water, ovarian fluid and wild fish were serotyped, ribotyped and compared biochemically. Virulence of F. psychrophilum isolates from different sources was compared by injection into rainbow trout. Additionally, the number of F. psychrophilum cells shed by naturally infected rainbow trout was determined. F. psychrophilum was detected and isolated from skin mucus, skin lesions and internal organs of diseased rainbow trout and from fish without clinical disease. The pathogen was also present in wild perch Perca fluviatilis, roach Rutilus rutilus, and ovarian fluids of farmed rainbow trout brood fish. Isolates were biochemically homogenous, excluding the capability to degrade elastin. Five different agglutination patterns with different antisera against F. psychrophilum were found among the isolates studied. Although several different ribopatterns were found (ClaI: 12 ribopatterns and HaeIII: 9 ribopatterns), ribotype A was the most dominant. Farmed rainbow trout brood fish carried a broad-spectrum of serologically and genetically different F. psychrophilum in ovarian fluids. Virulence of the tested isolates in rainbow trout varied and naturally infected rainbow trout shed 104 to 108 cells fish⁻¹ h⁻¹ of F. psychrophilum into the surrounding water.

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madetoja, J. (Ekstern), Dalsgaard, I. (Intern), Wiklund, T. (Ekstern)
Pages: 109-118
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Diseases of Aquatic Organisms
Volume: 52
Issue number: 2
ISSN (Print): 0177-5103
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
Survival of Flavobacterium psychrophilum in rainbow trout (Oncorhynchus mykiss) serum in vitro

Virulent and non-virulent strains of Flavobacterium psychrophilum of different serotypes were examined for survival and growth in non-immune and immune rainbow trout serum, in vitro. A majority of the examined strains consumed complement of non-immune serum, but the complement cascade was not able to cause an immediate (after 3 h incubation) notable reduction in viability of the inoculated cells. After 24 h incubation a more pronounced reduction in the number of viable bacteria was observed in untreated serum as well as in serum heated at 45 degrees C. In serum heated at 56 degrees C this reduction in cell number was not observed, but an increase in cell number did not occur either. The serum survival of one of the examined strains was different from the others in showing cell multiplication after 24 h incubation in normal as well as heat-treated (45 and 56 degrees C) serum. In immune serum no immediate reduction in viability of inoculated cells, of all tested strains, was observed. The number of viable cells showed a slow decrease or remained almost unchanged for up to 72 h post-inoculation in untreated serum, at 5 degrees C as well as at 15 degrees C. In heat-treated serum (45 degrees C) the number of viable cells decreased slowly at 5 degrees C and 15 degrees C for up to 72 h. The results suggest that the examined strains were unaffected by the alternative complement reaction present in fish serum as well as by antibodies against F. psychrophilum. However, some unknown component(s) in the fish sera, or lack of nutrients or essential growth factors, inhibited the growth of most of the examined strains in the tested fish sera. (C) 2002 Academic Press.
Characterization of class 1 integrons associated with R-plasmids in clinical Aeromonas salmonicida isolates from various geographical areas

Class 1 integrons were found in 26 of 40 antibiotic-resistant isolates of the fish pathogen Aeromonas salmonicida from Northern Europe and North America. Three different dhfr genes, conferring trimethoprim resistance, and one ant(3")1a aminoglycoside resistance gene were identified as gene inserts. The gene cassettes tended to be conserved among isolates from a particular geographical area. Nineteen isolates transferred R-plasmids carrying different tet determinants to Escherichia coli in filter mating assays, and in 15 cases, the class 1 integrons were co-transferred. Transferable sulphadiazine, trimethoprim and streptomycin resistances were invariably encoded by integrons. It thus appears that integron-encoded antibiotic resistance genes contribute substantially to the horizontal spread of antimicrobial resistance within this species, being associated with conjugative plasmids.

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Schmidt, A. (Ekstern), Bruun, M. S. (Intern), Larsen, J. (Ekstern), Dalsgaard, I. (Intern)
Pages: 735-743
Publication date: 2001
Main Research Area: Technical/natural sciences
Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment

A collection of 313 motile aeromonads isolated at Danish rainbow trout farms was analyzed to identify some of the genes involved in high levels of antimicrobial resistance found in a previous field trial (A. S. Schmidt, M. S. Bruun, I. Dalsgaard, K. Pedersen, and J. L. Larsen, Appl. Environ. Microbiol. 66:4908-4915, 2000), the predominant resistance phenotype (37%) being a combined oxytetracycline (OTC) and sulphadiazine/trimethoprim resistance. Combined sulphonamide/trimethoprim resistance (135 isolates) appeared closely related to the presence of a class 1 integron (141 strains). Among the isolates containing integrons, four different combinations of integrated resistance gene cassettes occurred, in all cases including a dihydrofolate reductase gene and a downstream aminoglycoside resistance insert (87 isolates) and occasionally an additional chlor-amphenicol resistance gene cassette (31 isolates). In addition, 23 isolates had "empty" integrons without inserted gene cassettes. As far as OTC resistance was concerned, only 66 (30%) out of 216 resistant aeromonads could be assigned to resistance determinant class A (19 isolates), D (n = 6), or E (n = 39); three isolates contained two tetracycline resistance determinants (AD, AE, and DE). Forty OTC-resistant isolates containing large plasmids were selected as donors in a conjugation assay, 27 of which also contained a class I integron. Out of 17 successful R-plasmid transfers to Escherichia coli recipients, the respective integrons were cotransferred along with the tetracycline resistance determinants in 15 matings. Transconjugants were predominantly tetA positive (10 of 17) and contained class I integrons with two or more inserted antibiotic resistance genes. While there appeared to be a positive correlation between conjugative R-plasmids and tetA among the OTC-resistant aeromonads, tetE and the unclassified OTC resistance genes as well as class I integrons were equally distributed among isolates with and without plasmids. These findings indicate the implication of other mechanisms of gene transfer besides plasmid transfer in the dissemination of antibiotic resistance among environmental motile aeromonads.
Phenotypic and genotypic characterization of Flavobacterium psychrophilum from Finnish fish farms

Finnish isolates (n = 37) of Flavobacterium psychrophilum isolated from farmed salmonids were studied using phenotypic and genotypic characteristics. The characteristics of isolates were compared with the characteristics of Swedish and Estonian F. psychrophilum isolates and the type strain, F. psychrophilum NCIMB 1947(T). Multiple isolates from eight disease outbreaks were examined to determine differences between isolates from a single outbreak. The F. psychrophilum isolates represented a biochemically homogeneous group. However, some minor differences in
biochemical and physiological characteristics were observed. Seven different antigenic patterns among Finnish isolates were detected and the results suggest a new serotype of F. psychrophilum. Using CIA HaeIII and PvuII restriction enzymes in ribotyping analyses 13 different genotypes were demonstrated and a possible relationship between serotype Fd and genotype F1 was determined. There were no significant differences between the isolates from Finland, Estonia, Sweden and the type strain of F. psychrophilum.

**General information**

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark  
Authors: Madetoja, J. (Ekstern), Hanninen, M. (Ekstern), Hirvela-Koski, V. (Ekstern), Dalsgaard, I. (Intern), Wiklund, T. (Ekstern)  
Pages: 469-479  
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BFI (2018): BFI-level 1  
Web of Science (2018): Indexed yes  
BFI (2017): BFI-level 1  
Web of Science (2017): Indexed yes  
BFI (2016): BFI-level 1  
Scopus rating (2016): CiteScore 2.12  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 1  
Scopus rating (2015): CiteScore 1.71  
Web of Science (2015): Indexed yes  
BFI (2014): BFI-level 1  
Scopus rating (2014): CiteScore 1.99  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 1  
Scopus rating (2013): CiteScore 1.74  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 1  
Scopus rating (2012): CiteScore 1.7  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 1  
Scopus rating (2011): CiteScore 2.09  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 1  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 1  
Web of Science (2009): Indexed yes  
BFI (2008): BFI-level 2  
Web of Science (2008): Indexed yes  
Web of Science (2007): Indexed yes  
Web of Science (2006): Indexed yes  
Web of Science (2005): Indexed yes  
Web of Science (2004): Indexed yes  
Web of Science (2003): Indexed yes
Radiological examination of the spinal column in farmed rainbow trout Oncorhynchus mykiss (Walbaum): experiments with Flavobacterium psychrophilum and oxytetracycline

Flavobacterium psychrophilum and oxytetracycline have both been associated with spinal deformities in salmonids. Experiments were carried out to investigate whether infection with F. psychrophilum or medication with oxytetracycline (OTC) at the fry stage would result in an increased occurrence of vertebral column deformities in rainbow trout, Oncorhynchus mykiss (Walbaum). Fish were on-grown for 9 months and examined by radiology at the end of the experiments. There was a relationship between infection by F. psychrophilum and deformities of the spinel column, if fish with more than 10 affected vertebrae were classified as deformed. The deformities found among infected fish were often visible externally and were more severe than those seen among control fish (most deformities found among controls were only seen on X-ray photographs). Deformities were evenly spread along the vertebral column of infected fish. OTC treatments of up to 200 mg of OTC(kgfish)(-1) day(-1) for 10 days and repeated three times did not result in increased spinal deformities relative to untreated control groups; therefore, medication of rainbow trout with oxytetracycline did not cause deformities of the spinal column under our treatment conditions.
Selection of media for antimicrobial susceptibility testing of fish pathogenic bacteria

The available data concerning antimicrobial susceptibility testing of fish pathogens showed that there is no consensus to the basal medium currently being employed. Different media recommended for susceptibility testing of human pathogens (Mueller-Hinton Agar, Tryptone Soya Agar, Antibiotic Medium 3, Diagnostic Sensitivity Test Agar) have been used in addition to media (Brain Heart Infusion Agar, Heart Infusion Agar, Columbia Blood Agar) normally utilized for cultivating fastidious bacteria. When testing marine pathogens, sodium chloride or seawater has been included in the media. Media normally used for cultivation of pathogens with specific growth requirements like Flavobacterium species and Renibacterium salmoninarum have been used for susceptibility testing. The Mueller-Hinton Agar and different modifications of this medium was used most frequently in published studies on resistant pattern in fish pathogenic bacteria. The American guideline from The National Committee for Clinical Laboratory Standards (NCCLS) recommends Mueller-Hinton Agar for susceptibility testing of human pathogens and this validated medium appears to be adequate for the rapidly growing fish pathogens. Following the NCCLS guideline with susceptibility testing of fastidious organisms, the use of modified Mueller-Hinton Agar for testing of pathogens with specific growth requirements, like, e.g. Flavobacterium species and R. salmoninarum, has been initiated. For the testing of susceptibility of fish pathogenic bacteria, it is suggested that, if possible, a chemically defined well-known medium such as Mueller-Hinton Agar or modification of Mueller-Hinton Agar be used for further investigations. (C) 2001 Elsevier Science B.V. AH rights reserved.
Antimicrobial resistance patterns in Danish isolates of Flavobacterium psychrophilum

The resistance pattern of Flavobacterium psychrophilum to the antimicrobial agents used in fish farming in Denmark was assessed in vitro using an agar dilution method. After identification of 387 isolates from clinical outbreaks of rainbow trout fry syndrome (RTFS) and the environment, the isolates were tested and the resulting antibiograms were used to predict the theoretical therapeutic efficacy and to evaluate if resistance had changed as a course of time. Antimicrobial agents included in this investigation were oxolinic acid (OXA), amoxicillin (AMX), potentiated sulfadiazine, oxytetracycline (OTC) and florfenicol (FLO). We found that F. psychrophilum isolates divided in susceptible and resistant clusters reflecting the reduced efficacy in practice when using OTC and AMX. The most recent isolates were less susceptible to AMX and OXA, whereas resistance to OTC seemed stable over the last 5 years. Apparently, F. psychrophilum carries intrinsic resistance towards the potentiated sulfonamides, and in correlation with this, we found very few susceptible isolates. All isolates were susceptible to FLO. The method used for determining minimum inhibitory concentration (MIC) follows the American guideline from The National Committee for Clinical Laboratory Standards (NCCLS) with some modifications to comply with the growth demand of this fastidious bacterium. We suggest future investigations use these guidelines as a benchmark.

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General information
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Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.294 SNIP 1.542 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.151 SNIP 1.394
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.941 SNIP 1.263
Bacterial pathogens in rainbow trout, Oncorhynchus mykiss (Walbaum), reared at Danish freshwater farms

During a 2-year period, bacterial fish pathogens were monitored on five rainbow trout, Oncorhynchus mykiss (Walbaum), freshwater farms in Denmark. A total of 1206 fish were examined and 361 bacterial isolates were identified phenotypically. Enteric redmouth disease, furunculosis and rainbow trout fry syndrome/coldwater disease were recorded. Infections caused by Flavobacterium psychrophilum occurred most frequently, but only one outbreak of enteric redmouth disease caused by Yersinia ruckeri serotype O1 and one of furunculosis caused by Aeromonas salmonicida were recorded during the monitoring period. Flavobacterium psychrophilum was isolated on all farms, both during disease outbreaks and from fish without any signs of disease. Serological investigations of F. psychrophilum showed that serotype Th was the dominant serotype found. The serotypes Th and Fd were involved in disease outbreaks of fry and larger fish. All isolates of F. psychrophilum showed proteolytic activities; however, a few isolates, belonging to serotype Fp(T) did not degrade elastin and were not associated with mortality. Increasing resistance problems to oxytetracycline were demonstrated. More than half of the F. psychrophilum isolates showed resistance to oxolinic acid and oxytetracycline. No antibiotic resistant isolates were found among Y. ruckeri and A. salmonicida.

General information
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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Madsen, L. (Intern)
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BFI (2018): BFI-level 1
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BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Comparative studies of Danish Flavobacterium psychrophilum isolates: ribotypes, plasmid profiles, serotypes and virulence

Ribotyping and plasmid profiling were carried out on 299 Danish Flavobacterium psychrophilum isolates from farmed rainbow trout, Oncorhynchus mykiss (Walbaum). The isolates had been characterized biochemically and serologically in another study. The isolates were very homogeneous, 254 isolates had the same ribotype A (restriction enzyme EcoRI) and 284 isolates harboured one 3.3 kb plasmid. Seventy-five per cent of the F. psychrophilum isolates had ribotype A, one 3.3 kb plasmid and belonged to either serotype Th or Fd. Virulence studies with representatives of the dominant groups classified such isolates as virulent, and an extra small or large plasmid did not change the virulence level. A relationship between the serotypes Fd and Th, certain ribotypes, and virulence was found. The isolates belonging to serotype Fp(T) and ribotype B were less virulent. Only a few isolates with other ribotypes were found and these were also less virulent. Isolates that did not have the 3.3 kb plasmid were also less virulent. The virulent isolates all harboured the 3.3 kb plasmid; however, there was no clear correlation between virulence and plasmid content as a 3.3 kb plasmid was also found in less virulent isolates.
Detection of Flavobacterium psychrophilum from fish tissue and water samples by PCR amplification

Rainbow trout fry syndrome and cold-water disease, caused by Flavobacterium psychrophilum, are important diseases in farmed salmonids. Some of the presently available techniques for the detection of Fl. psychrophilum are either time consuming or lack sufficient sensitivity. In the present investigation, the possible detection of Fl. psychrophilum from fish tissue and water samples was examined using nested PCR with DNA probes against a sequence of the 16S rRNA genes.
The DNA was extracted using Chelex(R) 100 chelating resin. The primers, which were tested against strains isolated from diseased fish, healthy fish, fish farm environments and reference strains, proved to be specific for Fl. psychrophilum. The obtained detection limit of Fl. psychrophilum seeded into rainbow trout brain tissue was 0.4 cfu in the PCR tube, corresponding to 17 cfu mg(-1) brain tissue. The PCR-assay proved to be more sensitive than agar cultivation of tissue samples from the brain of rainbow trout injected with Fl. psychrophilum. In non-sterile fresh water seeded with Fl. psychrophilum the detection limit of the PCR-assay was 1.7 cfu in the PCR tube, corresponding to 110 cfu ml(-1) water. The PCR-assay detected Fl. psychrophilum in water samples taken from a rainbow trout farm, but Fl. psychrophilum could not be isolated using inoculation on selective agar. The method presented here has the potential to detect low levels of Fl. psychrophilum in fish tissue and in water samples, and the technique can be a useful tool for understanding the epidemiology of Fl. psychrophilum.
Occurrence of Antimicrobial Resistance in Fish-Pathogenic and Environmental Bacteria Associated with Four Danish Rainbow Trout Farms

Surveillance of bacterial susceptibility to five antimicrobial agents was performed during a 1-year period in and around four freshwater fish farms situated along a stream in western Denmark. Besides assessing the levels of antibiotic resistance among the culturable fraction of microorganisms in fish, water, and sediment samples, two major fish pathogens (88 Flavobacterium psychrophilum isolates and 134 Yersinia ruckeri isolates) and 313 motile Aeromonas isolates, representing a group of ubiquitous aquatic bacteria, were isolated from the same samples. MICs were obtained applying a standardized agar dilution method. A markedly decreased susceptibility of F. psychrophilum isolates to most antimicrobial agents presently available for use in Danish aquaculture was detected, while the collected Y. ruckeri isolates remained largely sensitive to all therapeutic substances. Comparing the inlet and outlet samples, the increase of the antibiotic-resistant proportions observed among the culturable microflora was more pronounced and statistically significant among the motile aeromonads. High levels of individual and multiple antimicrobial resistances were demonstrated within the collected flavobacteria and aeromonads, thus indicating a substantial impact of fish farming on several groups of bacteria associated with aquacultural environments.

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, National Veterinary Institute, Royal Veterinary and Agricultural University
Authors: Schmidt, A. S. (Ekstern), Bruun, M. S. (Intern), Dalsgaard, I. (Intern), Pedersen, K. (Intern), Larsen, J. (Ekstern)
Pages: 4908-4915
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Main Research Area: Technical/natural sciences

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BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Pulsed-field gel electrophoresis analysis of *Aeromonas salmonicida* ssp *salmonicida*

A total of 133 strains of *Aeromonas salmonicida* ssp. *salmonicida*, isolated from a wide variety of sources, were characterized by pulsed-field gel electrophoresis patterns. Sixteen profiles were demonstrated, with one profile being predominant in samples from all the countries and species of fish. Our results suggest a clonal distribution of this subspecies, with a predominant clone being responsible for most of the outbreaks worldwide. (C) 2000 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.
Spinal deformities in triploid all-female rainbow trout (Oncorhynchus mykiss)

A batch of experimental rainbow trout was found to have a high level of spinal deformities. An equal deformity level was found in fish from the same batch, but reared at the fish farm from where the fry originated, suggesting that the all-female triploid status of the rainbow trout might account for the high level of deformity.

General information
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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Ambjerg, J. (Ekstern), Dalsgaard, I. (Intern)
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BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.49 SJR 0.234 SNIP 0.421
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.27 SNIP 0.496 CiteScore 0.64
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.32 SNIP 0.414 CiteScore 0.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.365 SNIP 0.431 CiteScore 0.62
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.257 SNIP 0.49 CiteScore 0.47
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.236 SNIP 0.364 CiteScore 0.41
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.34 SNIP 0.469
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.292 SNIP 0.459
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.325 SNIP 0.373
Scopus rating (2007): SJR 0.31 SNIP 0.386
Scopus rating (2006): SJR 0.436 SNIP 0.592
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.623 SNIP 0.672
Undersøgelse af eventuelle miljøpåvirkninger af hjælpestoffer og medicin i ferskvandsdambrug samt metoder til at reducere/eliminere sådanne påvirkninger

General information
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Organisations: Section for Aquaculture, National Institute of Aquatic Resources, Section for Fish Diseases
Authors: Sortkjær, O. (Ekstern), Pedersen, P. B. (Intern), Dalsgaard, I. (Intern), Nielsen, P. (Ekstern), Aarup, P. (Ekstern)
Number of pages: 206
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Publication: Research › Report – Annual report year: 2000

Indole-positive Vibrio vulnificus isolated from disease outbreaks on a Danish eel farm
Vibrio vulnificus was isolated in 1996 from 2 disease outbreaks on a Danish eel farm which used brackish water. A characteristic clinical sign was extensive, deep muscle necrosis in the head region. V. vulnificus was isolated from kidney, mucus, spleen, gill and intestine of diseased eels. Thirty-two isolates were examined phenotypically and serologically for pathogenicity to eels and for correlation to ribotype and plasmid profile. Biochemically, the isolates showed properties similar to those described previously for eel-pathogenic strains of V. vulnificus, with the exception of indole production. Virulence was evaluated by LD50 (the 50 % lethal dose), which ranged from

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Publication date: 1999
Main Research Area: Technical/natural sciences

Publication information
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Volume: 35
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ISSN (Print): 0177-5103
Ratings:
Polymerase chain reaction (PCR)-based typing analysis of atypical isolates of the fish pathogen Aeromonas salmonicida

Two hundred and five isolates of atypical Aeromonas salmonicida, recovered from a wide range of hosts and countries were characterized by polymerase chain reaction (PCR) targeting four genes. The chosen genes were those encoding the extracellular A-layer protein (AP), the serine protease (Sprot), the glycerophospholipid:cholestrol acetyltransferase protein (GCAT), and the 16S rRNA (16S rDNA). All the atypical A. salmonicida isolates could be assigned to 4 PCR groups. Group 1 comprised 45 strains which rested positive for PCR amplification, using the 16S rDNA, GCAT2, Sprot2, and AP primer-sets. Group 2 comprised 88 strains with produced PCR products using the 16S rDNA, GCAT2 and AP primer-sets. Group 3 comprised 21 strains which produced PCR products using 16S rDNA, GCAT2 and Sprot2 primer-sets, and group 4 comprised 51 strains which produced PCR products using the 16S rDNA and GCAT2 primer-sets only. A. salmonicida subsp. salmonicida isolates tested, belonged to group 1. The PCR primer-sets separated A. salmonicida from other reference strains of Aeromonas species and related bacteria with the exception of Aeromonas hydrophila. The results indicated that PCR typing is a useful framework for characterization of the increasing number of isolations of atypical A. salmonicida.

General information
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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Høie, S. (Ekstern), Dalsgaard, I. (Intern), Aase, I. (Ekstern), Heum, M. (Ekstern), Thornton, J. (Ekstern), Powell, R. (Ekstern)
Pages: 403-411
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Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.337 SNIP 1.368 CiteScore 3.35
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.285 SNIP 1.344 CiteScore 3.42
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.494 SNIP 1.166 CiteScore 3.37
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.413 SNIP 1.326 CiteScore 3.64
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.818 SNIP 1.213 CiteScore 3.42
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.9 SNIP 1.457 CiteScore 3.75
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.555 SNIP 1.232
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.459 SNIP 1.272
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.499 SNIP 1.288
Reproducible methods for experimental infection with Flavobacterium psychrophilum in rainbow trout Oncorhynchus mykiss

Experiments were done in order to achieve a reproducible method that can be used to infect rainbow trout Oncorhynchus mykiss with Flavobacterium psychrophilum, the causal agent of coldwater disease and rainbow trout fry syndrome. The main method investigated was intraperitoneal injection, and this method was tested using isolates with different elastin-degrading profiles and representing different serotypes. Injecting trout, average weight 1 g, with $10^4$ CFU (colony-forming units) per fish caused cumulative mortalities around 60 to 70%. The virulent strains belonged to certain serotypes and degraded elastin. The intraperitoneal injection challenge method could be used on larger fish, but the infection dose was $10^7$ CFU per fish before mortalities occurred. Bath infection and bath infection in combination with formalin treatment (stress) seemed to be reproducible methods that could be used as alternatives to the intraperitoneal method, although the mortalities among infected trout were lower. The results of investigated methods were influenced by parameters such as the challenge isolate, number of fish in the tank affecting the infection pressure, origin of fish and weight of fish.

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Web of Science (2017): Indexed Yes
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Scopus rating (2016): CiteScore 1.95 SJR 0.858 SNIP 0.929
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.949 SNIP 0.935 CiteScore 1.96
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Vertebral column deformities in farmed rainbow trout (Oncorhynchus mykiss)

Farmed rainbow trout (Oncorhynchus mykiss) were fed diets with either different levels of vitamin C, or diets enriched with glucan or chitin, from feeding start and 6 months onwards. At an average weight of 100 g, the trout were X-rayed to determine the deformity level. The investigations showed deformity levels from 4.8% to 12.5% among the different diet groups. Fish fed the chitin-enriched diet, the low vitamin C diet, the high vitamin C diet, and the control diet had the highest deformity levels, ranging from 8.9% to 12.5%, while the group fed the glucan-enriched diet had the lowest level of deformities (4.8%). In all groups examined, the deformities were spread over the whole vertebral column. The deformities in the group fed the low vitamin C diet were more severe than those found in the other groups. An outbreak of the disease rainbow trout fry syndrome (RTFS) caused by the bacterium Flavobacterium psychrophilum was observed in all groups during the investigation. The findings of deformities in all examined groups indicate a possible role of F. psychrophilum in the aetiology of the observed deformities. The lower level of deformities in the group fed the glucan-enriched diet might be caused by the ability of glucan to stimulate the immune response and increase the disease resistance of fish. (C) 1999 Elsevier Science B.V. All rights reserved.
Characterization of atypical Aeromonas salmonicida by different methods

Fifty two isolates of atypical Aeromonas salmonicida, recovered from a wide range of hosts and geographical locations, were heterogeneous in terms of molecular and phenotypic characteristics, and represented taxa which could not be accommodated by the current classification of four subspecies. Generally, there was incongruence between the molecular (PCR, RAPD and ribotyping) and phenotypic methods in terms of cluster membership. By PCR, 6 groups were described of which Group 1 encompassed 12 isolates including the type strain of A. salmonicida subsp. smithia. Group 2 accommodated 23 isolates including the reference cultures of subspecies achromogenes and masoucida. The named culture of Haemophilus piscium was recovered in Group 6. By ribotyping and RAPD, the reference cultures were recovered in separate groups. All methods pointed to the uniqueness of subspecies smithia. Most isolates contained 2-6 plasmids, of 2.3 to 150 kb in length. Nevertheless, all isolates possessed certain key characteristics, including Gram-negativity, and the absence of motility.

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Organizations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Austin, B. (Ekstern), Austin, D. (Ekstern), Dalsgaard, I. (Intern), Gudmundsdottir, B. (Ekstern), Høie, S. (Ekstern), Thornton, J. (Ekstern), Larsen, J. (Ekstern), O’Hici, B. (Ekstern), Powell, R. (Ekstern)
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Characterization of Danish Flavobacterium psychrophilum strains isolated from rainbow trout (Oncorhynchus mykiss) by ribotyping and plasmid profiles

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
Number of pages: 95
Publication date: 1998
Characterization of Flavobacterium psychrophilum; comparison of proteolytic activity and virulence of strains isolated from rainbow trout (Oncorhynchus mykiss)

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
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Publication date: 1998

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Place of publication: Aberdeen
Publisher: Fisheries Research Services
Editors: Barnes, A., Davidson, G., Hiney, M., McIntosh, D.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 226553
Publication: Research - peer-review › Book chapter – Annual report year: 1998

Forekomst af bakterielle infektioner i regnbueørreder i 5 ferskvandsdambrug i årene 1994 og 1995: Med vægt på bakterien Flavobacterium psychrophilum

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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Madsen, L. (Intern)
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Publisher: Ministeriet for Fødevarer, Landbrug og Fiskeri, Danmarks Jordbrugsforskning
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Series: Intern rapport
Number: 105
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225226
Publication: Research › Report – Annual report year: 1998

Heterogeneity among isolates of Vibrio vulnificus recovered from eels (Anguilla anguilla) in Denmark
The findings of this study demonstrate that Vibrio vulnificus isolates recovered from diseased eels in Denmark are heterogeneous as shown by O serovars, capsule types, ribotyping, phage typing, and plasmid profiling. The study includes 85 V. vulnificus isolates isolated from the gills, intestinal contents, mucus, spleen, and kidneys of eels during five disease outbreaks on two Danish eel farms from 1995 to 1997, along with a collection of 12 V. vulnificus reference strains, The results showed that more than one serovar may be capable of causing disease in eels and that these isolates are genetically heterogenous as shown by ribotyping. Ribotyping also showed that the same isolates map persist in an eel farm and cause recurrent outbreaks, Phage typing did not correlate with ribotyping or serotyping. However, we observed that 26 of 28 isolates, which were not susceptible to any of the phages, showed the same ribotype, O serovar, and capsule type, This suggests that these isolates may possess features that make them resistant to lysis by the phages used in this study. Ninety-three of 97 isolates harbored between one and three high-molecular-weight plasmids which previously had been suggested to be associated with eel virulence, The subdivision of V. vulnificus into two biotypes based on the indole reaction can no longer be supported, since 82 of 97 isolates in this study were indole positive, and a subdivision into serovars appears to be more correct.
General information
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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Høi, L. (Ekstern), Dalsgaard, I. (Intern), DePaola, A. (Ekstern), Siebeling, R. (Ekstern), Dalsgaard, A. (Ekstern)
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BFI (2017): BFI-level 2
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BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.054 SNIP 1.602
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.074 SNIP 1.653
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.108 SNIP 1.648
Web of Science (2004): Indexed yes
Identification of atypical Aeromonas salmonicida: Inter-laboratory evaluation and harmonization of methods

The atypical isolates of Aeromonas salmonicida are becoming increasingly important as the frequency of isolation of bacteria belonging to this group continues to rise. The primary object of this study was to compare and evaluate the results obtained in various laboratories concerning the biochemical identification of atypical Aer. salmonicida before and after standardization of media and methods. Five laboratories examined 25 isolates of Aer. salmonicida from diverse fish species and geographical locations including the reference strains of Aer. salmonicida subsp. salmonicida (NCMB 1102) and Aer. salmonicida subsp. achromogenes (NCMB 1110). Without standardization of the methods, 100% agreement was obtained only for two tests: motility and ornithine decarboxylase. The main reason for the discrepancies found was the variation of the incubation time prior to reading the biochemical reactions. After standardization, improvement was obtained with the identification; however, disagreement was still observed between the different laboratories. These findings demonstrate the difficulties involved in a proper identification of atypical Aer. salmonicida and also that data presented in the literature on various strains of Aer. salmonicida are not readily comparable. This paper seems to be the first on standardization of microbiological tests for identification of fish pathogens and the results obtained show the need for standardization of methods both within and between laboratories.

General information
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Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Gudmundsdottir, B. (Ekstern), Helgason, S. (Ekstern), Høie, S. (Ekstern), Thoresen, O. (Ekstern), Wichardt, U. (Ekstern), Wiklund, T. (Ekstern)
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Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.56
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.69
ISI indexed (2013): ISI indexed yes
Improved isolation of Vibrio vulnificus from seawater and sediment with cellobiose-colistin agar

An improved selective medium, cellobiose-colistin (CC) agar, gave a significantly higher (P <0.05) isolation rate of Vibrio vulnificus from water and sediment samples than did modified cellobiose-polymyxin B-colistin (mCPC) agar. In a total of 446 alkaline peptone water preenrichments amended with polymyxin B, V. vulnificus was isolated from 154 preenrichments (35%) with mCPC agar and from 179 preenrichments (40%) with CC agar. CC agar gave a higher plating efficiency of V. vulnificus cells than did cellobiose-polymyxin B-colistin (CPC) agar, mCPC agar, or thiosulfate-citrate-bile salts-sucrose (TCBS) agar; the only significant difference was observed with TCBS agar, which gave much lower plating efficiencies than the other selective media. Determination of MICs demonstrated that the concentrations of colistin and polymyxin B in CPC agar inhibit growth of a proportion of V. vulnificus strains.

General information
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Journal: Applied and Environmental Microbiology
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Issue number: 5
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Occurrence of Vibrio vulnificus biotypes in Danish marine environments

General information
State: Published
Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: A review

Bacterial strains of *Aeromonas salmonicida* included in the recognized subsp. *achromogenes*, subsp. *masoucida*, and subsp. *smithia* in addition to the large number of strains not included in any of the described subspecies are referred to as atypical *A. salmonicida*. The atypical strains form a very heterogeneous group with respect to biochemical characteristics, growth conditions, and production of extracellular proteases. Consequently, the present taxonomy of the species *A. salmonicida* is rather ambiguous. Atypical *A. salmonicida* has been isolated from a wide range of cultivated and wild fish species, non-salmonids as well as salmonids, inhabiting fresh water, brackish water and marine environments in northern and central Europe, South Africa, North America, Japan and Australia. In non-salmonid fish species, infections with atypical strains often manifest themselves as superficial skin ulcerations. The best known diseases associated with atypical *A. salmonicida* are carp *Cyprinus carpio* erythrodermatitis, goldfish *Carassius auratus* ulcer disease, and ulcer disease of flounder *Platichthys flesus*, but atypical strains are apparently involved in more disease outbreaks than previously suspected. Macroscopical and microscopical studies of ulcerated fish indicate internal organs are infrequently invaded by atypical *A. salmonicida*. This view is supported by the fact that atypical strains are irregularly isolated from visceral organs of ulcerated fish. High mortality caused by atypical *A. salmonicida* has been observed in populations of wild non-salmonids and farmed salmonids, although the association between the mortality in the wild fish stocks and atypical *A. salmonicida* has not always been properly assessed. In injection experiments the pathogenicity of the atypical strains examined showed large variation. An extracellular A-layer has been detected in different atypical strains, but virulence mechanisms different from those described for (typical) *A. salmonicida* subsp. *salmonicida*, for example an extracellular metallo-protease and a different iron utilization mechanism, have been described. Limited information is available about the ecology, spread and survival of atypical strains in water. The commonly used therapeutic methods for the control of diseases in farmed fish caused by atypical *A. salmonicida* are generally effective against the atypical strains. Resistance to different antibiotics and transferable plasmids encoding multiple drug resistance have been observed in atypical *A. salmonicida*. Studies aimed at producing a vaccine against atypical strains are in progress.

**General information**

State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Wiklund, T. (Ekstern), Dalsgaard, I. (Intern)
Pages: 49-69
Publication date: 1998
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Diseases of Aquatic Organisms
Volume: 32
Issue number: 1
ISSN (Print): 0177-5103
Ratings:
- BFI (2018): BFI-level 1
- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 1
- Web of Science (2017): Indexed Yes
- BFI (2016): BFI-level 1
- Scopus rating (2016): CiteScore 1.95 SJR 0.856 SNIP 0.929
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 1
- Scopus rating (2015): SJR 0.949 SNIP 0.935 CiteScore 1.96
- Web of Science (2015): Indexed yes
An experimental model for infection of rainbow trout (Oncorhynchus mykiss) with Flavobacterium psychrophilum.
Characterization of isolates of Flavobacterium psychrophilum associated with coldwater disease or rainbow trout fry syndrome I: phenotypic and genomic studies

Isolates of Flavobacterium psychrophilum (formerly Cytophaga psychrophila and Flexibacter psychrophilus) mainly originating from clinical outbreaks of either coldwater disease (CWD) or rainbow trout fry syndrome (RTFS) were studied for selected biochemical, physiological, morphological and genomic characteristics, and compared with previously characterized French and American strains. DNA hybridization studies showed that the Danish isolates were highly related to the type strain, F. psychrophilum NCIMB 1947(T). Plasmid profiling of Danish isolates and those from other European countries revealed differences, which might be related to differences in pathogenicity. European isolates originating from clinical outbreaks of either RTFS or CWD usually harboured one plasmid of 3.2 kb, whereas isolates originating from fish with different or no disease signs had other profiles. Phenotypically, the Danish isolates appeared very homogeneous and shared most characteristics with the type strain, and with French and American strains studied by other authors. Further studies on the importance of the plasmids and the proteolytic activities of the bacterium might help in elucidating possible virulence factors.
Er der sammenhæng mellem "skæve" fisk, antibiotikabehandling og/eller yngeldødelighedssyndromet (YDS)?

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
Pages: 190-191
Publication date: 1997
Main Research Area: Technical/natural sciences

Publication information
Journal: Ferskvandsfiskeribladet
Volume: 95
ISSN (Print): 0015-0223
Ratings:
ISI indexed (2013): ISI indexed no
**Karakterisering af Flavobacterium psychrophilum; sammenligning af proteolytisk aktivitet og virulens hos isolater fra regnbueørred**

**General information**
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
Pages: 21-22
Publication date: 1997
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Meddelande / Laxforskningsinstitut
Volume: 2
ISSN (Print): 0346-7066
Original language: Danish
Source: orbit
Source-ID: 226561
Publication: Research › Journal article – Annual report year: 1997

**Occurrence of Vibrio vulnificus in the Danish marine environment**

**General information**
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Høi, L. (Ekstern), Dalsgaard, I. (Intern), Larsen, J. (Ekstern), Dalsgaard, A. (Ekstern)
Pages: 486-486
Publication date: 1997

**Host publication information**
Title of host publication: Abstracts of the General Meeting of the American Society for Microbiology: 97th General Meeting of the American Society for Microbiology
Volume: 97, Issue 0
Main Research Area: Technical/natural sciences
Conference: 97th General Meeting of the American Society for Microbiology, Florida, USA, 01/01/1997
Source: orbit
Source-ID: 225867
Publication: Research › Conference abstract in proceedings – Annual report year: 1997

**SJVF forskningsprogrammet "Sygdomsforebyggelse, genetik og emøring ved produktion af regnbueørred": Forskningsaktiviteter på Fiskepatologisk laboratorium, Danmarks Fiskeriundersøgelser**

**General information**
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, I. (Intern), Madsen, L. (Intern)
Pages: 153-156
Publication date: 1997
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Ferskvandsfiskeribladet
Volume: 95
ISSN (Print): 0015-0223
Ratings:
ISI indexed (2013): ISI indexed no
Vaccination improves survival of Baltic salmon (Salmo salar) smolts in delayed release sea ranching (net-pen period)

Baltic salmon (Salmo salar) of the Finnish Iijoki stock were hatched and reared in freshwater in a salmon hatchery on the Danish island of Bornholm in the Baltic sea. Salmon parr were divided in three groups each comprising 22,000 fish. One group was vaccinated by intraperitoneal injection with a non-mineral oil-adjuvanted vaccine consisting of formalin killed Aeromonas salmonicida, Yersinia ruckeri and Vibrio anguillarum (serotype O1 and O2). A second group was vaccinated by 1 h bathing in a corresponding vaccine without adjuvant. A third group was left as untreated control. Subsequently, presmolts were transferred to three identical net-pens located next to each other in the Baltic Sea (salinity 8 ppt), 500 m from the north-eastern coast of Bornholm, where they were studied for four months until tagging and release for restocking purposes. Mortality during this period in the ip vaccinated group was minimal (0.02%, RPS (relative per cent of survival) 99.80) and significantly lower compared to 10.13% mortality in the control group and 2.51% mortality (RPS 75.2) in the bath vaccinated group. Specific disease outbreaks were not observed during the four months. Growth was significantly enhanced in the injection vaccinated group compared to both the unvaccinated control and the bath vaccinated group. The humoral antibody response to the various bacteria was significantly elevated in the injection vaccinated group showing 4-5 fold titre increases three and four months after immunization. In contrast, no increase of titres was seen in the bath vaccinated and untreated groups. Marked cellular reactions in the abdominal cavity of injected fish were registered. A total of 3000 fish have been tagged and released to evaluate the effect of vaccination on the recapture rate. The implications of immunoprophylactic measures in the restocking programme with Baltic salmon are discussed. (C) 1997 Elsevier Science B.V

General information
State: Published
Organisations: National Veterinary Institute, Section for Fish Diseases, National Institute of Aquatic Resources, Section for Aquatic Protein Biochemistry, Royal Veterinary and Agricultural University
Authors: Buchmann, K. (Intern), Dalsgaard, I. (Intern), Nielsen, M. E. (Intern), Pedersen, K. (Intern), Uldal, A. (Ekstern), Garcia, J. (Ekstern), Larsen, J. (Ekstern)
Pages: 335-348
Publication date: 1997
Main Research Area: Technical/natural sciences

Publications
Journal: Aquaculture
Volume: 156
Issue number: 3-4
ISSN (Print): 0044-8486
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.294 SNIP 1.542 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.151 SNIP 1.394
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.941 SNIP 1.263
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 0.909 SNIP 1.173
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.019 SNIP 1.318
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.008 SNIP 1.689
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.915 SNIP 1.236
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.016 SNIP 1.627
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.121 SNIP 1.926
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.992 SNIP 1.418
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.049 SNIP 1.317
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.908 SNIP 1.113
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.049 SNIP 1.251
Original language: English
Electronic versions:
Vaccination_improves_survival_Aquaculture_1997.pdf
DOIs:
10.1016/S0044-8486(97)00097-5
Vibrio damsela associated with diseased fish in Denmark
A total of 26 Vibrio damsela strains were isolated in Denmark. Fifteen strains were isolated from the head kidney of rainbow trout (Oncorhynchus mykiss) in aquaculture, eight were from organs of two stingrays (Dasyatis pastinaca) held in captivity, two were from organs and pathological material of a nurse shark (Orectolobus ornatus) held in captivity, and one was from the water in the aquarium where the rags and shark were kept. This is the first report on the isolation of V. damsela in Denmark and the first report on this organism associated with diseased farmed rainbow trout. Ecological and epizootiological aspects of the observation are discussed on the basis of phenotypic and genotypic properties of the isolated strains.

General information
State: Published
Organisations: National Veterinary Institute, Section for Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark
Authors: Pedersen, K. (Intern), Dalsgaard, I. (Intern), Larsen, J. (Ekstern)
Pages: 3711-3715
Publication date: 1997
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 63
Issue number: 9
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Characterization of atypical Aeromonas salmonicida isolates by ribotyping and plasmid profiling

A total of 38 strains of atypical Aeromonas salmonicida, three oxidase-negative but otherwise typical Aer. salmonicida, three typical Aer. salmonicida, and two reference strains, isolated from several countries and fish species were examined with respect to rRNA gene restriction patterns (ribotypes) and plasmid profiles. Most epidemiologically unrelated strains had different ribotypes, whereas isolates from the same outbreak were identical. All strains, except one, carried one or more large plasmids (>55 kbp) and all strains, except two, additionally carried one or more smaller plasmids. Many strains isolated from the same outbreak showed different plasmid profiles although some plasmids were identical. The results suggest the existence of several atypical Aer. salmonicida. It also seems that ribotypes are stable properties for these bacteria while the plasmids are more labile.

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Pedersen, K. (Intern), Dalsgaard, I. (Intern), Larsen, J. (Ekstern)
Pages: 37-44
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Bacteriology
Volume: 80
Issue number: 1
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Comparison of a commercial biochemical kit and an oligonucleotide probe for identification of environmental isolates of Vibrio vulnificus

Methods for the identification and isolation of environmental isolates of Vibrio vulnificus were evaluated. Alkaline peptone water supplemented with polymyxin B and colistin-polymyxin B-cellobiose agar were employed for the isolation of suspected V. vulnificus from water, sediment and shellfish samples. When comparing the identification of putative V. vulnificus obtained with the API 20E assay and an oligonucleotide probe, 29 API 20E profiles were obtained with only four profiles (representing 20 isolates) reaching the identification threshold of V. vulnificus among a total of 66 isolates hybridizing with the probe. The results indicated that, compared with colony hybridization, the API 20E assay was not adequate for the identification of environmental isolates of V. vulnificus.

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, A. (Ekstern), Dalsgaard, I. (Intern), Høi, L. (Ekstern), Larsen, J. (Ekstern)
Pages: 184-188
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Letters in Applied Microbiology
Volume: 22
Issue number: 2
ISSN (Print): 0266-8254
Forbedret overlevelse af laksesmolt i Østersøen efter vaccinering

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, Section for Aquatic Protein Biochemistry
Authors: Buchmann, K. (Ekstern), Dalsgaard, I. (Intern), Garcia, J. (Ekstern), Heuer, O. (Ekstern), Larsen, J. (Ekstern), Nielsen, M. E. (Intern), Pedersen, K. (Ekstern), Uldal, A. (Ekstern)
Number of pages: 52
Publication date: 1996
Fortsatte undersøgelser af yngeldødeligheds syndromet (YDS) hos regnbueørred. Delrapport B: Undersøgelse vedrørende årsagerne til deformiteter hos fisk

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Madsen, L. (Intern), Dalsgaard, I. (Intern)
Number of pages: 33
Publication date: 1996

Prevalence and characterization of Vibrio cholerae isolated from shrimp products imported into Denmark
A total of 3,555 metric tonnes of warm water shrimp were imported into Denmark from December 1994 to July 1995. V. cholerae O1 was not detected in any of the 748 samples analyzed. Non-O1 V. cholerae was found in a single (0.1%) cooked frozen shrimp product and in five (0.7%) raw frozen products, all originating from shrimp produced in aquaculture. Six isolated strains agglutinated in polyvalent O antisera, but did not agglutinate in Ogawa or Inaba antisera. The six strains were resistant to colistin and sulfisoxazole; three strains also showed resistance to ampicillin. None of the strains contained plasmids or genes encoding cholera toxin (CT) or heat-stable enterotoxin (NAG-ST). The absence of V. cholerae O1 and the low number of samples containing CT and NAG-ST negative non-O1 strains in imported shrimp suggest that V. cholerae in such products may not constitute a public health problem.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases, Technical University of Denmark
Authors: Dalsgaard, A. (Ekstern), Bjergskov, T. (Ekstern), Jeppesen, V. (Ekstern), Jørgensen, L. V. (Intern), Echeverria, P. (Ekstern), Dalsgaard, I. (Intern)
Pages: 694-697
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Food Protection
Volume: 59
Issue number: 7
ISSN (Print): 0362-028X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.68 SJR 0.759 SNIP 0.82
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.96 SNIP 1.031 CiteScore 2.03
Web of Science (2015): Indexed yes
Undersøgelse af forekomst og betydning af Vibrio vulnificus i badevand, ål og importerede skaldyrsprodukter

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, A. (Ekstern), Høi, L. (Ekstern), Dalsgaard, I. (Intern), Larsen, J. (Ekstern)
Pages: 244-245
Publication date: 1996
Main Research Area: Technical/natural sciences
A multidisciplinary danish research program on rainbow trout (Oncorhynchus mykiss) farming

A new research programme involving eight Danish institutions is described. The programme started in 1993 and is expected to run for 5 years. The primary objective of the research initiative is to exploit and integrate the knowledge of several institutions and disciplines for the benefit of the production of rainbow trout. The programme includes several projects with aspects of disease prevention, genetics, and nutrition. In most of the projects, the work has been divided into stages of 2 and 3 years, respectively. During a 2 year period, production, management and health status are recorded at the participating fish farms, and all data are organized in a database. Diseases cause major problems in rainbow trout production, therefore a great deal of the effort in this programme deals with diseases caused by viruses, bacteria and parasites. On the basis of the database, epidemiological examinations are carried out as well as investigations of the possibilities of preventive measures and cost-benefit analyses. In the genetic studies, polymorphic genetic markers will be developed and used for analysis of the genetic structure of selected fish stocks. Microsatellites will be developed and introduced in the study. Primarily genetic differences between lines/strains and their crossings will be estimated with the purpose of describing the genetic level and the importance of additive and non-additive genetic effects. In the nutritional area the product quality and pollution questions will be in focus.

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, National Veterinary Institute, Section for Aquaculture
Authors: Berg, P. (Ekstern), Eggum, B. (Ekstern), Møller, S. (Ekstern), Holm, L. (Ekstern), Jørgensen, P. (Ekstern), Olesen, N. (Ekstern), Buchmann, K. (Ekstern), Larsen, J. (Ekstern), Dalsgaard, I. (Intern), Møllegaard, S. (Intern), Jeppesen, V. (Ekstern), Frier, J. (Ekstern), McLean, E. (Ekstern), Horlyck, V. (Ekstern), Graver, C. (Ekstern), Kristensen, T. (Ekstern), Birk, E. (Ekstern), Pedersen, P. B. (Intern)
Pages: 257-260
Publication date: 1995
Main Research Area: Technical/natural sciences

Publication information
Journal: Water Science and Technology
Volume: 31
Issue number: 10
ISSN (Print): 0273-1223
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Atypical Aeromonas salmonicida associated with ulcerated flatfish species in the Baltic Sea and the North Sea

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Wiklund, T. (Ekstern), Dalsgaard, I. (Intern)
Pages: 218-224
Publication date: 1995
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Aquatic Animal Health
Volume: 7
ISSN (Print): 0899-7659
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.472 SNIP 0.583 CiteScore 1.09
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.549 SNIP 0.698 CiteScore 1.06
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.518 SNIP 0.783 CiteScore 1.24
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.447 SNIP 0.883 CiteScore 1.23
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.663 SNIP 1.185 CiteScore 1.58
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.628 SNIP 0.644 CiteScore 0.96
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.485 SNIP 0.68
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.538 SNIP 0.797
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.478 SNIP 0.649
Scopus rating (2007): SJR 0.655 SNIP 0.983
Scopus rating (2006): SJR 0.47 SNIP 0.609
Scopus rating (2005): SJR 0.501 SNIP 0.701
Scopus rating (2004): SJR 0.524 SNIP 0.697
Scopus rating (2003): SJR 0.687 SNIP 0.992
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.658 SNIP 0.914
Scopus rating (2001): SJR 1.073 SNIP 1.126
Scopus rating (2000): SJR 0.614 SNIP 0.728
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.607 SNIP 0.901
Original language: English
Source: orbit
Source-ID: 227797
Publication: Research - peer-review › Journal article – Annual report year: 1995
Forekomst og betydning af Vibrio vulnificus i kystnære områder. Evaluering af AI 20E og DNA probe til påvisning af V. vulnificus

**General information**
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources
Authors: Dalsgaard, A. (Ekstern), Dalsgaard, I. (Intern), Høi, L. (Ekstern), Larsen, J. (Ekstern)
Pages: 495-500
Publication date: 1995
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Dansk Veterinaridsskrift
Volume: 10
ISSN (Print): 0106-6854
Ratings:
BFI (2018): BFI-level 1
BFI (2017): BFI-level 1
BFI (2016): BFI-level 1
BFI (2015): BFI-level 1
BFI (2014): BFI-level 1
BFI (2013): BFI-level 1
ISI indexed (2013): ISI indexed no
BFI (2012): BFI-level 1
ISI indexed (2012): ISI indexed no
BFI (2011): BFI-level 1
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
BFI (2009): BFI-level 1
BFI (2008): BFI-level 1
Original language: Danish
Source: orbit
Source-ID: 225214
Publication: Research › Journal article – Annual report year: 1995

**Aeromonas salmonicida subsp. salmonicida: correlation of protein patterns, antibiotic resistance, exoprotease activity, haemolysis and pathological lesions produced in vivo**
A collection of 130 strains of the bacterial fish pathogen Aeromonas salmonicida subsp. salmonicida isolated from diseased salmonids in Denmark, Norway, North America and Scotland has been characterized with regard to protein patterns, antibiotic resistance and exoprotease activity. Whole cell and outer membrane protein profiling could distinguish three different profiles in A. salmonicida. Eight outer membrane proteins were demonstrated (49, 40, 38, 37, 33, 31, 30 and 29 kDa). One protein profile was deficient in a 38 kDa outer membrane protein and instead contained an outer membrane protein of 37 kDa which was not detectable among the other protein profiles. Strains with the 37 kDa outer membrane protein showed multiple low-level antibiotic resistance towards cephalothin, penicillin, chloramphenicol, tetracycline and quinolones. In addition, these strains were exoprotease deficient. Strains with the 37 kDa protein were unable to degrade cattle and trout serum proteins and displayed a delayed degradation of casein. Haemolysis on cattle blood agar plates was similarly delayed. In vivo examination of extracellular products from a normal protein profile strain and one with the 37 kDa outer membrane protein demonstrated major differences in pathological effects in rainbow trout. The strain possessing the 37 kDa outer membrane protein produced almost no pathological effects while the normal protein profile strain produced typical furuncles.

**General information**
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Nielsen, B. (Ekstern), Brown, D. J. (Ekstern), Larsen, J. L. (Ekstern)
Pages: 387-397
Publication date: 1994
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Journal of Fish Diseases
Volume: 17
A multi-disciplinary Danish research programme on rainbow trout (Oncorhynchus mykiss) farming.

General information
State: Published
Organisations: Section of Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Section for Fish Diseases, National Institute of Aquatic Resources, Section for Aquaculture
Authors: Berg, P. (Ekstern), Eggum, B. (Ekstern), Møller, S. (Ekstern), Holm, L. (Ekstern), Jørgensen, P. (Ekstern), Olesen, N. J. (Intern), Buchmann, K. (Intern), Larsen, J. (Ekstern), Dalsgaard, I. (Intern), Møllergaard, S. (Intern), From, J. (Ekstern), Frier, J. (Ekstern), McLean, E. (Ekstern), Hørløj, V. (Intern), Graver, C. (Intern), Kristensen, T. (Ekstern), Birk,
Characteristics of "atypical", cytochrome oxidase-negative Aeromonas salmonicida isolated from ulcerated flounders (Platichthys flesus (L.))

"Atypical", cytochrome oxidase-negative variants of the fish pathogen Aeromonas salmonicida, isolated from ulcerated flounder (Platichthys flesus), were studied using different methods. Two of the strains possessed a protein that corresponded to the A-layer protein of Aer. salmonicida. The strains reacted with antibodies against the A-layer and monoclonal antibodies against the O-antigen of typical Aer. salmonicida. These tests confirm that the isolates from flounder should be classified as Aer. salmonicida. Analysis of the fatty acids showed that the isolates were rather homogenous but the values of the guanine plus cytosine content of the DNA of the bacteria varied too much for any conclusion to be drawn on their taxonomic location. The strains examined exhibited several biochemical characters that differed from those of the type strains of Aer. salmonicida subsp. salmonicida and Aer. salmonicida, subsp. achrornogenses. The results suggest that these "atypical", cytochrome oxidase-negative variants may form a new subspecies of Aer. salmonicida.

General information
State: Published
Organisations: Åbo Academy University, Turku University of Applied Sciences, Department of Fisheries and Oceans, Royal Veterinary and Agricultural University
Authors: Wiklund, T. (Ekstern), Dalsgaard, I. (Intern), Eerola, E. (Ekstern), Olivier, G. (Ekstern)
Pages: 511-520
Publication date: 1994
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 76
Issue number: 5
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.56
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.69
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.51
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.55
ISI indexed (2011): ISI indexed yes
Characterization of Aeromonas salmonicida subsp. salmonicida: A comparative study of strains of different geographic origin

A total of 130 strains of the fish pathogen Aeromonas salmonicida isolated in Denmark, Norway, Scotland, Canada and the USA were examined. The strains originated from farmed salmonid fish. The biochemical, physiological and serological characteristics, antibiotic resistance patterns and cell surface-related properties were compared. Aeromonas salmonicida was found to be remarkably consistent in general cultural and biochemical characteristics. It is noteworthy that the strains were positive in the fermentation of L-arabinose and were negative in the fermentation of D-arabinose. All the strains were highly proteolytic. It was observed, however, that 5% of the strains did not digest calf and trout serum and the production of haemolysin and degradation of casein by the same strains were delayed compared with the other strains. Common to all of the rough strains were auto-aggregation and ability to bind the dyes Coomassie brilliant blue and Congo red and the majority of these strains were highly hydrophobic. The strains were tested for their susceptibility to 22 antibacterial agents. Antibiotic resistance profiles of Aer. salmonicida indicated that resistance to the quinolones and oxytetracycline was increasing and that multi-resistant strains were found in several countries. The variation found in antibiograms could have potential as epidemiological markers in certain geographic areas.

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Nielsen, B. (Ekstern), Larsen, J. L. (Ekstern)
Pages: 21-30
Publication date: 1994
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 77
Issue number: 1
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Diseases and injuries associated with mortality of hatchery reared Baltic cod (Gadus morhua L.) larvae

A cod hatching plant was established in 1992 on the island of Bornholm in the Baltic Sea in order to elucidate the possibilities for restocking of cod fry in this brackishwater system. The disease prevalence in 3 batches of hatchery-reared yolksac larvae from the Baltic cod (Gadus morhua L.) was monitored during the posthatch period. High prevalences of bacteriosis/mycosis, lordosis/scoliosis, injuries and protozoan endoparasitism were recorded. Vibrio sp. and Vibrio anguillarum serovar 04, 06, 08 in addition to nontypable strains and saprolegniaceous fungi were isolated from the larvae. The dinoflagellate-like endoparasites were located in the yolksac of the cod larvae.

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Buchmann, K. (Ekstern), Larsen, J. L. (Ekstern)
Pages: 385-390
Publication date: 1993
Main Research Area: Technical/natural sciences

Publication information
Journal: Acta Veterinaria Scandinavica. Supplementum
Volume: 34
Issue number: 4
ISSN (Print): 0065-1699
Isolation of Cytophaga psychrophila (Flexibacter psychrophilus) from wild and farmed rainbow trout (Oncorhynchus mykiss) in Finland

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Wiklund, T. (Ekstern), Kaas, K. (Ekstern), Lönnström, L. (Ekstern)
Pages: 44-46
Publication date: 1993
Main Research Area: Technical/natural sciences

Publication information
Journal: European Association of Fish Pathologists. Bulletin
Volume: 14
ISSN (Print): 0108-0288
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.49 SJR 0.234 SNIP 0.421
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.27 SNIP 0.496 CiteScore 0.64
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.32 SNIP 0.414 CiteScore 0.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.365 SNIP 0.431 CiteScore 0.62
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Virulence mechanisms in Cytophaga psychrophila and other Cytophaga-like bacteria pathogenic for fish

The present knowledge concerning the virulence mechanisms of Cytophaga psychrophila and other Cytophaga-like pathogens for fish, including Cytophaga columnaris, Flexibacter maritimus, and Flexibacter ovolyticus is reviewed as is the taxonomic work defining the genera in the Cytophaga-Flexibacter phylogenetic branch. Although specific adhesins have not been described, many Cytophaga-like bacteria are surrounded by a thin slime layer that attaches the cells to a surface but allows their translational moves as well. Many Cytophaga-like bacteria also exhibit bacteriolytic activity, which seems to be a characteristic of the order Cytophagales, however these activities are not useful in distinguishing pathogenic from nonpathogenic strains. Extracellular proteases produced by Cytophaga columnaris and Cytophaga psychrophila have been identified as have a protease, hemolysin, LPS, and extracellular products (ECP) in Flexibacter maritimus with the ECP and hemolysin possibly being related to its pathogenicity. Four different plasmid profiles have been demonstrated in Cytophaga psychrophila but at present their role, if any, in virulence is not known. In Cytophaga psychrophila there appears to be a common species-specific antigen, and somatic antigen may be used to distinguish pathogenic from nonpathogenic strains. In spite of the importance of Cytophaga psychrophila, its pathogenicity is still poorly understood.

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern)
Pages: 127-144
Publication date: 1993
Main Research Area: Technical/natural sciences

Publication information
Journal: Annual Review of Fish Diseases
Volume: 3
ISSN (Print): 0959-8030
Ratings:
BFI (2008): BFI-level 1
Scopus rating (1999): SJR 0.407 SNIP 0.629
Mycobacteriosis in cod (Gadus Morhua L.) in Danish coastal waters
Cod (Gadus morhua L.) were caught in pond nets at different locations in Danish coastal waters and examined for the presence of nodules in the internal organs. Cod with nodules were found at two carbohydrate-contaminated locations with prevalences up to 20%. Acid-fast bacteria were in many cases observed in the nodules. A procedure for the isolation of mycobacteria is described. The isolation method was developed by investigations of Mycobacterium marinum and Mycobacterium fortuitum injected in rainbow trout (Oncorhynchus mykiss). Fish tissue was decontaminated with sodium hydroxyde and oxalic acid and isolation was done on Löwenstein-Jensen Medium. Applying this method to naturally infected cod, successful isolation of mycobacteria was carried out. Growth conditions and biochemical characteristics of mycobacteria isolated from fish are discussed.

General information
State: Published
Organisations: Danish Institute for Fisheries and Marine Research, Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Mellergaard, S. (Ekstern), Larsen, J. L. (Ekstern)
Pages: 211-219
Publication date: 1992
Main Research Area: Technical/natural sciences

Publication information
Journal: Aquaculture
Volume: 107
Issue number: 2-3
ISSN (Print): 0044-8486
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.294 SNIP 1.542 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.151 SNIP 1.394
Enrofloxacin as an antibiotic in fish

General information
State: Published
Organisations: Unknown
Authors: Dalsgaard, I. (Intern), Bjerregaard, J. (Ekstern)
Pages: 300-302
Publication date: 1991
Main Research Area: Technical/natural sciences

Publication information
Journal: Acta Veterinaria Scandinavica. Supplementum
Volume: 87
ISSN (Print): 0065-1699
Ratings:
Web of Science (2018): Indexed yes
Web of Science (2017): Indexed Yes
Scopus rating (2016): CiteScore 1.01
Scopus rating (2015): CiteScore 0.98
Scopus rating (2014): CiteScore 1.54
Scopus rating (2013): CiteScore 1.41
Scopus rating (2012): CiteScore 1.26
Web of Science (2012): Indexed yes
Scopus rating (2011): CiteScore 1.42
Web of Science (2011): Indexed yes
Plasmids in *Vibrio salmonicida* isolates from The Faroe Islands

**General information**
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Nielsen, B. (Ekstern)
Pages: 206-207
Publication date: 1991
Main Research Area: Technical/natural sciences

**Publication information**
Journal: European Association of Fish Pathologists. Bulletin
Volume: 11
ISSN (Print): 0108-0288
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.49 SJR 0.234 SNIP 0.421
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.27 SNIP 0.496 CiteScore 0.54
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.32 SNIP 0.414 CiteScore 0.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.365 SNIP 0.431 CiteScore 0.62
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.257 SNIP 0.49 CiteScore 0.47
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.236 SNIP 0.364 CiteScore 0.41
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.34 SNIP 0.469
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.292 SNIP 0.459
BFI (2008): BFI-level 1
Preliminary investigations of fry mortality syndrome in rainbow trout

General information
State:Published
Organisations:Danish Veterinary Service, Danish Trout Culture Research Station, Danish Veterinary Laboratory, Danish Institute for Fisheries and Marine Research
Authors:Lorenzen, E. (Ekstern), Dalsgaard, I. (Intern), From, J. (Ekstern), Hansen, E. M. (Ekstern), Hørlyck, V. (Ekstern), Korsholm, H. (Ekstern), Møllergaard, S. (Ekstern), Olsen, N. J. (Ekstern)
Publication date:1991
Main Research Area:Technical/natural sciences

Publication information
Journal:European Association of Fish Pathologists. Bulletin
Volume:11
Issue number:2
ISSN (Print):0108-0288
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.49 SJR 0.234 SNIP 0.421
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.27 SNIP 0.496 CiteScore 0.64
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.32 SNIP 0.414 CiteScore 0.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.365 SNIP 0.431 CiteScore 0.62
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.257 SNIP 0.49 CiteScore 0.47
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.236 SNIP 0.364 CiteScore 0.41
Skin symptoms among workers in the fish processing industry are caused by high molecular weight compounds

Scratch tests were performed with fish juice containing high and low molecular weight compounds obtained by ultrafiltration and with degradation compounds known to accumulate in fish stored on ice. 75 volunteers were tested. The peptide pattern in raw fish juice and its high and low molecular weight fractions were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and the concentration of protein in the various fractions was determined. Fish products were analysed for bacteria and algae and the concentration of degradation compounds was measured. Mainly high molecular weight compounds (polypeptides) of fish juice were found to be responsible for the skin symptoms.
Study of Vibrio anguillarum strains from different sources with emphasis on ecological and pathobiological properties

A total of 317 Vibrio anguillarum strains were isolated from water, sediment, and diseased as well as healthy rainbow trout at a Danish mariculture farm and from feral fish caught close to the farm. All strains were examined serologically. Ten sera permitted determination of the O group in 66.7% of the strains from diseased rainbow trout. Furthermore, the O group could be determined in 45.1 to 65.4% of the strains from mucus, gills, and intestinal contents of healthy rainbow trout, while only 22.2 to 28.8% of the isolates from water, sediment, and gills or mucus of feral fish were groupable. Serogroup O1 and to some extent O2 appeared to be associated with trout. Strains from these serogroups were selected for analyses of hemagglutinating activity and surface hydrophobicity. Serogroup O1 comprised hemagglutinating as well as nonhemagglutinating strains; from cases of vibriosis, all O1 strains were nonhemagglutinating. The strains belonging to serogroup O2 were generally hemagglutinating. Examinations of surface hydrophobicity by salt aggregation and hydrophobic interaction chromatography suggested that the O1 strains were more hydrophobic than the O2 strains. In pathogenicity tests, O1 strains isolated from gills and mucus of healthy rainbow trout killed all trout in the test groups. A strain from the intestinal contents of healthy rainbow trout did not produce significant mortality. This strain could, however, be frequently reisolated from the pronephros of fish in the test group concerned. After challenge with strains from eel mucus and seawater, mortality was not produced, and furthermore, these strains could not be reisolated from the pronephros.
Vibrio salmonicida isolated from farmed Atlantic salmon in the Faroe Islands

General information
State: Published
Organisations: Fish Disease Laboratory, Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern), Jürgens, O. (Ekstern), Mortensen, A. (Ekstern)
Pages: 53-54
Publication date: 1988
Main Research Area: Technical/natural sciences

Publication information
Journal: European Association of Fish Pathologists. Bulletin
Volume: 8
Issue number: 3
ISSN (Print): 0108-0288
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.49 SJR 0.234 SNIP 0.421
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.27 SNIP 0.496 CiteScore 0.64
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.32 SNIP 0.414 CiteScore 0.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.365 SNIP 0.431 CiteScore 0.62
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.257 SNIP 0.49 CiteScore 0.47
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.236 SNIP 0.364 CiteScore 0.41
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.34 SNIP 0.469
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.292 SNIP 0.459
Disease problems in Danish eel farms

Eel production in recirculation systems has grown rapidly in Denmark and there are now approximately 60 eel farms. Under the intensive culture conditions, fish diseases have caused substantial problems. The most important has been gill infections with monogeneans, Pseudodactylogyrus spp., but at times other parasitic infections as well as fungal and bacterial infections have caused severe problems.

General information
State: Published
Organisations: Danish Institute for Fisheries and Marine Research
Authors: Mellergaard, S. (Ekstern), Dalsgaard, I. (Intern)
Pages: 139-146
Publication date: 1987
Main Research Area: Technical/natural sciences

Publication information
Journal: Aquaculture
Volume: 67
Issue number: 1-2
ISSN (Print): 0044-8486
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.101 SNIP 1.524
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.103 SNIP 1.254 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.002 SNIP 1.34 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.136 SNIP 1.3 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Atypical Aeromonas salmonicida isolated from diseased sand-eels, Ammodytes lancea (Cuvier) and Hyperoplus lanceolatus (Lesauvege)

General information
State: Published
Organisations: Royal Veterinary and Agricultural University, Danish Institute for Fisheries and Marine Research
Authors: Dalsgaard, I. (Intern), Paulsen, H. (Ekstern)
Pages: 361-346
Publication date: 1986
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Fish Diseases
Volume: 9
Issue number: 4
ISSN (Print): 0140-7775
Observation on enteric redmouth in Denmark

General information
State: Published
Organisations: Section for Fish Diseases, National Institute of Aquatic Resources, National Veterinary Institute, Section of Fish Diseases, Division of Poultry, Fish and Fur Animals
Authors: Dalsgaard, I. (Intern), Hørlyck, V. (Intern), Jørgensen, P. (Ekstern), Olesen, N. J. (Intern), From, J. (Ekstern)
Publication date: 1985
Event: Abstract from 2nd International Conference of the European Association of Fish Pathologists, Montpellier, France.
Main Research Area: Technical/natural sciences
First observation of Yersinia ruckeri in Denmark

In August 1983 the Danish Trout Culture Research Station investigated a disease problem in a rainbow trout farm in Mid-Jutland. The following clinical symptoms were observed: haemorrhages in the eyes, ulcerations in the mouth region, and haemorrhages in the mouth cavity. Although the haemorrhages in the eyes could be due to a VHS infection, the overall symptoms suggested a bacterial infection. The affected fish weighed on average 150g and the water temperature at the onset was approximately 16°C. The anterior kidney from 4 fish was sampled aseptically for bacteriological examinations. The initial cultures were made on blood agar incubated at 20°C for 48 h. The inoculated plates showed a pure growth of one colony type. The organism was shown to be a Gram-negative rod, fermenting glucose in O/F medium. Diagnosis was made biochemically. The biochemical reactions were very similar to those of Yersinia ruckeri, the redmouth (RM) bacterium. The trout farm was visited again at the end of September 1983, the water temperature was at that time 12°C. Samples of the anterior kidney from five moribund fish were taken. A pure culture, identified as Yersinia ruckeri, was isolated from all 5 fish. The infection did not cause heavy mortality.
Occurrence of VHS in Danish maricultured rainbow trout

In 1982 a severe outbreak of viral haemorrhagic septicaemia (VHS) was observed in four Danish seawater trout farms. Signs of disease were identical to those of VHS in freshwater reared rainbow trout. Characteristics of the isolated virus strains were similar to the "freshwater strains". Source of infection remains obscure and the existence of an Egtved virus reservoir in natural salt water has not been demonstrated.
Bakteriologiske undersøgelser af den naturlige flora i kvæg- og svinegylle

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern)
Number of pages: 130
Publication date: 1982

Publication information
Publisher: Institut for Veterinær Mikrobiologi og Hygiejne, Den kgl. Veterinær- og Landbohøjskole
Original language: English
Main Research Area: Technical/natural sciences
Publication: Research › Ph.D. thesis – Annual report year: 1982

A comprehensive study of environmental and human pathogenic Vibrio alginolyticus strains
An investigation comprising 49 environmental (VAE), 7 human isolates (VAH) and 3 type culture (VAT) strains of Vibrio alginolyticus showed a very high similarity among the strains. By primary isolation a swarming activity on blood agar and a yellow, hemispheric relatively large colony type on TCBS were very conspicuous. Besides the characteristics of Vibrionaceae the most typical and differential diagnostic important features were: sucrose fermentation, lysine decarboxylase activity, acetoine and 2,3 butanediol production, no growth on an electrolyte deficient medium (CLED), arginine and arabinose negative reaction. Swarming on blood agar and growth in 10% NaCl were also important although one of these criteria might be negative. An obvious difference was observed in ornithine where the percentage of positive VAE, VAH and VAT were 51, 100 and 0, respectively. In cellobiose all VAE and VAT but only two VAH were positive. MR positive strains were also found among all categories. The G + C mol% in the strains ranged from 45.9 - 46.8.

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Occurrence of Vibrio parahaemolyticus and Vibrio alginolyticus in marine and estuarine bathing areas in Danish coast

From seventeen sites in three marine and two estuarine bathing areas at the Danish coast water samples were collected during the period from May to November 1979. Total bacterial counts were performed on blood agar. The prevalence of Vibrio parahaemolyticus and Vibrio alginolyticus in 100 ml water samples using the filter membrane technique and TCBS agar plates was investigated with the possible correlation to water temperature and salinity. Only five strains of V. parahaemolyticus were isolated, two from marine and three from estuarine water. V. alginolyticus was isolated in 8.33 and 15.63 per cent, in relation to the total bacterial count/100 ml on TCBS agar, in marine and estuarine water samples, respectively. The maximum was registered in June and July, corresponding with the highest temperatures recorded (12-18 degrees C). V. alginolyticus should be considered a widely distributed bacterium in Danish coastal areas and could be regarded as a bacterial indicator of hygienic and public health importance.

Spring ulcer disease in eels

General information
State: Published
Organisations: Royal Veterinary and Agricultural University
Authors: Dalsgaard, I. (Intern)
Pages: 18-19
Publication date: 1981
Main Research Area: Technical/natural sciences

Projects:

**Bacteriophage based technology to control Flavobacterium pathogens in aquaculture**

National Veterinary Institute  
Period: 01/10/2017 → 30/09/2020  
Number of participants: 3  
PhD Student:  
Donati, Valentina Laura (Intern)
Supervisor:  
Madsen, Lone (Intern)  
Main Supervisor:  
Dalsgaard, Inger (Intern)

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Samfinansieret - Andet  
Project: PhD

**Prevention disease caused by Aeromonas salmonicida**  
National Veterinary Institute  
Period: 01/08/2012 → 30/06/2016  
Number of participants: 6  
Phd Student:  
Bartkova, Simona (Intern)  
Supervisor:  
Kokotovic, Branko (Intern)  
Main Supervisor:  
Dalsgaard, Inger (Intern)  
Examiner:  
Pedersen, Karl (Intern)  
Bojesen, Anders Miki (Ekstern)  
Colquhoun, Duncan John (Ekstern)

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Institut stipendie (DTU) Samf.

**Relations**  
Publications:  
Aeromonas salmonicida - Epidemiology, whole genome sequencing, detection and in vivo imaging  
Project: PhD

**Antibiotikaresistens hos bakterier i dansk fiskeopdræt**  
National Veterinary Institute  
Division of Veterinary Diagnostics and Research  
Bacteriology & Pathology  
Period: 01/10/2011 → 31/03/2013  
Number of participants: 2  
antibiotikaresistent i dansk fiskeopdræt  
Project participant:  
Dalsgaard, Inger (Intern)  
Madsen, Lone (Intern)  
Project

**Minimering af antibiotikaforbrug i akvakultur gennem stikvaccinering af små fisk**  
National Veterinary Institute  
Division of Veterinary Diagnostics and Research  
Bacteriology & Pathology  
Period: 01/07/2011 → 31/12/2014  
Number of participants: 6  
STIKVACC  
Acronym: STIKVACC  
Project ID: 22498  
Project participant:
Fiskesygdomskurser - Udvikling af kursustilbud til akvakulturbrugere om fiskesygdomme, med primær fokud på forebyggelse og behandling samt reduktion af negative miljøeffekter

National Veterinary Institute
Division of Veterinary Diagnostics and Research
Bacteriology & Pathology
Dansk Akvakultur
University of Copenhagen
Period: 01/07/2011 → 31/12/2012
Number of participants: 1
Fiskesygdomskurser
Project ID: 22513
Project participant:
Dalsgaard, Inger (Intern)
Documents:
Ans_gning_Fiskesygdomskurser_maj_2011_Final.pdf

Control Flavobacteriaceae infections in European fish farms
Project aims:
To improve the control of Fp and Tm infections, data at both the epidemiological and molecular levels are urgently needed. This project aims at filling this scientific gap and immediately investing the acquired knowledge to provide the fish-farming community with new tools for diagnostic and prophylactic methods.

National Veterinary Institute
Division of Veterinary Diagnostics and Research
Bacteriology & Pathology
INRA Institut National de La Recherche Agronomique
Instituto Zooprofilattico Sperimentale delle Venezie
PHARMAQ AS
National Fish Disease Laboratory
Phylogene SA
Period: 01/03/2011 → 28/02/2014
Number of participants: 1
Acronym: PathoFish
Project ID: 22479
Project participant:
Dalsgaard, Inger (Intern)

Control Flavobacteriaceae infections in European fish farms
Project Aims: To improve the control of Fp and Tm infections, data at both the epidemiological and molecular levels are urgently needed. This project aims at filling this scientific gap and immediately investing the acquired knowledge to provide the fish-farming community with new tools for diagnostic and prophylactic methods.

Division of Veterinary Diagnostics and Research
National Veterinary Institute
INRA Institut National de La Recherche Agronomique
Instituto Zooprofilattico Sperimentale delle Venezie
Åbo Academy University
National Veterinary Institute
PHARMAQ AS
Phylogene SA

National Fish Disease Laboratory
Period: 01/01/2011 → 28/02/2014
Number of participants: 9
Acronym: PathoFish
Project ID: 22479
Project participant:
Nielsen, Hanne Katrine (Ekstern)
Dalsgaard, Inger (Intern)
Skorski, Gilbert (Ekstern)
Whali, Thomas (Ekstern)

Project Manager, organisational:
Duchaud, Eric (Ekstern)
Nicolas, Pierre (Ekstern)
Amedeo, Manfrin (Ekstern)
Wiklund, Tom (Ekstern)
Brudeseth, Erik (Ekstern)

Financing sources
Source: Forsk. EU - Andre EU-midler
Name of research programme: Forsk. EU - Andre EU-midler
Amount: 3,046,455.00 Danish Kroner

Flavobacterium psychrophilum, forebyggelse og immunforsvar
National Veterinary Institute
Period: 15/06/2010 → 26/02/2014
Number of participants: 8
PhD Student:
Henriksen, Maya Maria Mihályi (Intern)
Supervisor:
Buchmann, Kurt (Ekstern)
Kania, Per (Ekstern)
Lorenzen, Niels (Intern)
Main Supervisor:
Dalsgaard, Inger (Intern)
Examiner:
Olesen, Niels Jørgen (Intern)
Aasted, Bent (Ekstern)
Wiklund, Tom Christer Oskar (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD

Anvendelse af bakteriofagterapi til behandling af fiskesygdomme i akvakultur
Section for Veterinary Diagnostics
Division of Veterinary Diagnostics and Research
National Veterinary Institute
University of Copenhagen
BioMar A/S
Chr. Hansen A/S
Aquasearch farm ApS
Dansk Akvakultur
Period: 01/06/2010 → 31/05/2013
Number of participants: 7
Project ID: 22462
Contact person:
Larsen, Anne Hjørngaard (Ekstern)
Johansen, Eric (Ekstern)
Nielsen, Torben (Ekstern)
Henriksen, Niels Henrik (Ekstern)
Project participant:
Dalsgaard, Inger (Intern)
Madsen, Lone (Intern)
Project Manager, organisational:
Middelboe, Mathias (Ekstern)

Financing sources
Source: Forskningsprojekter - Fødevareministeriet
Name of research programme: Forskningsprojekter - Fødevareministeriet
Amount: 1,442,125.00 Danish Kroner

Danish Fish Immunology Research Network
Division of Poultry, Fish and Fur Animals
National Veterinary Institute
Division of Veterinary Diagnostics and Research
Danish Institute for Fisheries and Marine Research
University of Southern Denmark
Aarhus University
Aquasearch farm ApS
Fishlab
Aquatic Diagnostics Ltd.
BioMar A/S
Norwegian School of Veterinary Science
University of Aberdeen
Marine Laboratory
Friedrich Loeffler Institute
Danish Aquaculture Association
Intervet/Schering-Plough Animal Health
Period: 01/01/2009 → 31/12/2013
Number of participants: 31
Acronym: DAFINET
Project ID: 22454
Contact person:
Skjødt, Karsten (Ekstern)
Henryan, Mark (Ekstern)
Nielsen, Torben (Ekstern)
Sørensen, Kirsten E. (Ekstern)
Adams, Alexandra (Ekstern)
Hjermitslev, Niels (Ekstern)
Evensen, Øystein (Ekstern)
Secombes, Chris (Ekstern)
Collett, Bertrand (Ekstern)
Fischer, Uwe (Ekstern)
Henriksen, Niels Henrik (Ekstern)
Nylen, Jørgen (Ekstern)

Project participant:
Pedersen, Karl (Ekstern)
Jørgensen, Thomas R. (Ekstern)
Vluff, Birgitte Martine (Ekstern)
Salomonsen, Jan (Ekstern)
Aasted, Bent (Ekstern)
Mazaheri, Sanaz (Ekstern)
Einer-Jensen, Katja (Intern)
Rasmussen, Jesper Skou (Intern)
Kjær, Torben (Ekstern)
Troels, Lisbeth K. (Ekstern)
Madsen, Lone (Intern)
Nielsen, Michael Engelbrecht (Intern)
Koch, Claus (Ekstern)
Vitved, Lars (Ekstern)
Hvelplund, Jette (Ekstern)
Reily, Samantha (Ekstern)

Project Manager, organisational:
Buchmann, Kurt (Ekstern)
Lorenzen, Niels (Intern)
Dalsgaard, Inger (Intern)

Financing sources
Source: Forskningsprojekter - Andre ministerier og styrelser
Name of research programme: Forskningsprojekter - Andre ministerier og styrelser
Project

Flavobacterium psychrophilum, epidemiologi og virulens faktorer

National Veterinary Institute
Period: 01/06/2008 → 28/02/2013
Number of participants: 3
Phd Student:
Andersen, Julie Hove (Intern)
Supervisor:
Molin, Søren (Intern)
Main Supervisor:
Dalsgaard, Inger (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD
Improved vaccination strategies in marine aquaculture

Section of Fish Diseases
Division of Poultry, Fish and Fur Animals
National Veterinary Institute
National Institute of Aquatic Resources
University of Copenhagen
Danish Aquaculture Association
Aller Aqua A/S
Fishlab
AquaSearch Vet
Schering-Plough A/S
Period: 01/04/2008 → 30/09/2012
Number of participants: 15
Project ID: 22452
Project participant:
Rasmussen, Jesper Skou (Intern)
Lorenzen, Ellen (Intern)
Olesen, Niels Jørgen (Intern)
Buchmann, Kurt (Ekstern)
Madsen, Simon B. (Ekstern)
Melingen, Geir Olav (Ekstern)

Project Manager, organisational:
Lorenzen, Niels (Intern)
Dalsgaard, Inger (Intern)
Pedersen, Karl (Ekstern)
Hansen, Per Juel (Ekstern)
Henriksen, Niels Henrik (Ekstern)
Hørlyck, Viggo (Ekstern)
Sørensen, Kirsten Engell (Ekstern)
Nielsen, Torben (Ekstern)
Nylén, Jørgen (Ekstern)

Financing sources
Source: Forskningsprojekter - Andre ministerier og styrelser
Name of research programme: Forskningsprojekter - Andre ministerier og styrelser
Amount: 1,444,780.00 Danish Kroner

Organic Aquaculture - the linkage between sustainable production and superior products

This project will contribute to the successful establishment of organic trout farming in Denmark. It will develop and optimise new recipes for organic fish feeds with high levels of organic vegetable protein of Danish origin. These will be fed to trout to investigate feed quality as digestibility, effects on the environment, feed conversion, and growth. Effects of the feeds upon general health and welfare, and immunocompetence (vaccination efficacy), will be assessed. Objective sensory and biochemical analyses will provide an overall picture of the eating quality of trout raised with the new organic feeds at an organic farm. Consumer preference for trout with pale coloured meat will be explored, plus other market issues for organic trout (supply chain, traceability, export). Results will be disseminated to industry, consumers and regulatory authorities with open workshops. Guidelines will be prepared for optimal rearing and marketing of organic trout.

National Veterinary Institute
National Food Institute
Division of Seafood Research
Division of Industrial Food Research
Danish Institute for Fisheries and Marine Research
Royal Veterinary and Agricultural University
Danish Technological Institute

Dansk Akvakultur
Period: 01/01/2006 → 31/12/2010
Number of participants: 11
Acronym: ORAQUA
Project ID: 22451
Project participant:
Jokumsen, Alfred (Ekstern)
Pedersen, Lars-Flemming (Ekstern)
Dalsgaard, Inger (Intern)
Nielsen, Henrik Hauch (Intern)
Jacobsen, Charlotte Munch (Ekstern)
Jessen, Flemming (Intern)
Larsen, Erling P. (Ekstern)
Nielsen, Michael Engelbrecht (Ekstern)
Kold, John (Ekstern)
Larsen, Villy J. (Ekstern)

Project Manager, organisational:
McKenzie, David J. (Ekstern)

Financing sources
Source: Forskningsprojekter - Fødevareministeriet
Name of research programme: Forskningsprojekter - Fødevareministeriet
Amount: 548,554.00 Danish Kroner

Project

RESTOCK (38566) (38400 pre-project)
The aim of the pre-project was to explore the potential for restocking the cod stock in the eastern Baltic. A theoretical study was conducted to explore the potential for restocking bringing together scientists from the aquaculture sector, fisheries managers, ecological scientists and scientists with a background in stock enhancement. The ecology, biology and fisheries biology of the eastern Baltic was reviewed and provided the basis for the study. The results indicated a good potential for restocking with first-feeding cod larvae (Støttrup et al. 2008). This was the first example of a study to examine the potential for large-scale restocking prior to the release of fish. A 2-3 month delay in the spawning period compared to 20-30 years ago has altered feeding conditions and predation susceptibility in a way that may have exacerbated the decline in recruitment. Producing and releasing cod larvae during spring would mimic the spawning period recorded in previous times and would coincide with the spring peak in copepod production. An evaluation of 3 different release scenarios showed that a release of 474 million first-feeding larvae over 5 months (covering the historic and present day spawning period) would enhance the average population of 2 year old by 10% and be biologically and economically the most feasible scenario.

Three years of a six year follow up project (RESTOCK) to verify the theoretical findings was funded, but due to political changes, funding for the final three years was not possible and the project was unable to empirically ascertain the potential for restocking. During the three years, 3 cod broodstocks were established with different photoperiods and subsequent spawning periods, together with the development of a technique to determine fish gender non-invasively (McEvoy et al., 2009). Egg and larval incubation techniques were developed and several investigations on temperature, salinity and food impacts on first feeding cod larvae to define the “window of opportunity” for release (i.e. time when the larvae were ready to start feeding to when they began to be too poor in condition to feed) (Støttrup et al., 2008; Overton et al. 2010; Meyer et al 2011a). A release strategy was developed and the first successful release of first-feeding fish larvae at 23 m depth was conducted, but needed further adjustments (Støttrup et al., 2008). An extensive disease monitoring program was established (Støttrup et al., 2008) and the presence of a protistan endoparasite generated a further study (Skovgård et al., 2010). Studies were also conducted to determine explore marking techniques for identification of released fish (Meyer et al., 2011b) and explore growth characteristics in cod larvae (Meyer et al., 2011a).

The project was coordinated by DTU Aqua.

National Institute of Aquatic Resources
Section for Ecosystem based Marine Management
Influence of probiotics and feed on organic rainbow trout health

The aim of OPTIFISH is to optimize growth and survival for organic cultured rainbow trout, the dominant fish species produced in Denmark. A minor part of the rainbow trout is produced as organic fish. Currently there is no production of organic fry, as the classification organic only can be given to fish that have been treated with antibiotics no more than twice in a lifetime. This is hard to achieve as recurrent disease outbreaks, especially with the bacterium Flavobacterium psychrophilum, are seen during the fry stage. A further challenge known from salmon culture is that diets with high plant contents cause enteritis and injury to the intestine, which will affect the absorption of nutrients, affecting the overall health status and welfare of the fish. The result is a higher risk of disease following exposure to pathogenic microorganisms.

OPTIFISH will investigate how organic diet types with varying amounts of fish and plant sources as well as with or without probiotics (lactic acid bacteria) will affect the intestine, the intestinal microbial flora and survival rates of rainbow trout following exposure to pathogens. The use of probiotics for fry are assumed to result in a higher health level. The overall result will be a sustainable production with an optimal utilization of the available organic resources as well as the scope for the industry that they by using the optimal diet type will achieve a robust and healthy fish, something that can be achieved not only in organic but also in traditional farming. A robust and disease-free fry is the most important factor for a higher production in organic aquaculture in the future.