Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (Oncorhynchus mykiss, Walbaum)

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Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Huber, I. (Ekstern), Spanggaard, B. (Intern), Appel, K. (Ekstern), Rossen, L. (Ekstern), Nielsen, T. (Ekstern), Gram, L. (Intern)
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In vitro antagonism of the probiont Pseudomonas fluorescens strain AH2 against Aeromonas salmonicida does not confer protection of salmon against furunculosis

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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Løvold, T. (Ekstern), Nielsen, J. (Ekstern), Melchorsen, J. (Intern), Spanggaard, B. (Intern)
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Scopus rating (2012): SJR 1.212 SNIP 1.487 CiteScore 2.32
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Probiotika i akvakultur - en strategi til forebyggelse af fiskesygdom

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Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Spanggaard, B. (Intern), Gram, L. (Intern)
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http://www.difres.dk/dk/publication/files/22122003$FH52.pdf
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Proliferation and location of Vibrio anguillarum during infection of rainbow trout, O. mykiss

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Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene, Technical University of Denmark
Authors: Spanggaard, B. (Intern), Huber, I. (Ekstern), Nielsen, J. (Ekstern), Nielsen, T. (Ekstern), Gram, L. (Intern)
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Scopus rating (2013): CiteScore 1.74
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Scopus rating (2012): CiteScore 1.7
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Scopus rating (2011): CiteScore 2.09
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
The microflora of rainbow trout intestine: a comparison of traditional and molecular identification

The culturability of the intestinal microflora of 48 rainbow trout was detected by comparing direct microscopic counts (4',6-diamidino-2-phenylindole, DAPI) with plate counts (tryptone soya agar, TSA). In general, a high percentage (average 50%) of the microflora could be cultured. The counts of the intestinal microflora varied 3-5 log units between fish within the same sampling point. A total of 504 bacteria were identified by physiologic criteria and 153 strains also by partial sequencing of the 16S rRNA gene. High agreement was found between classical and molecular identification. The dominant intestinal microflora was identified as bacteria belonging to the gamma subclass of Proteobacteria (of the genera Citrobacter, Aeromonas and Pseudomonas), to the Gram-positive bacteria with low G+C-content (of the genus Carnobacterium) and as bacteria belonging to the beta subclass of Proteobacteria. However, the composition of the intestinal microflora showed high variation among three investigated fish farms and also at different time points within one fish farm. (C) 2000 Elsevier Science B.V. All rights reserved.

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State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene, Technical University of Denmark
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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
Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish

To study the possible use of probiotics in fish farming, we evaluated the in vitro and in vivo antagonism of antibacterial strain Pseudomonas fluorescens strain AH2 against the fish-pathogenic bacterium Vibrio anguillarum. As iron is important in virulence and bacterial interactions, the effect of P. fluorescens AH2 was studied under iron-rich and iron-limited conditions. Sterile-filtered culture supernatants from iron-replete P. fluorescens AH2 inhibited the growth of V. anguillarum, whereas sterile-filtered supernatants from iron-replete cultures of P. fluorescens AH2 did not. P. fluorescens AH2 inhibited the growth of V. anguillarum during coculture, independently of the iron concentration, when the initial count of the antagonist was 100 to 1,000 times greater than that of the fish pathogen. These in vitro results were successfully repeated in vivo. A probiotic effect in vivo was tested by exposing rainbow trout (Oncorhynchus mykiss Walbaum) to P. fluorescens AH2 at a density of 10^5 CFU/ml for 5 days before a challenge with V. anguillarum at 10^4 to 10^5 CFU/ml for 1 h. Some fish were also exposed to P. fluorescens AH2 at 10^7 CFU/ml during the 1-h infection. The combined probiotic treatment resulted in a 46% reduction of calculated accumulated mortality; accumulated mortality was 25% after 7 days at 12 degrees C in the probiotic-treated fish, whereas mortality was 47% in fish not treated with the probiont.
An Ichthyophonus hoferi epizootic in herring in the North Sea, the Skagerrak, the Kattegat and the Baltic Sea

An epizootic caused by the internal parasite Ichthyophonus hoferi in herring Clupea harengus was recorded from 1991 to 1993 in the waters around Denmark. A surveillance programme from research vessels and commercial fishing boats was conducted in the North Sea, Skagerrak, Kattegat and Baltic Sea. A total of 15769 hearts of adult herring were examined macroscopically for evidence of infection. The prevalence of the infection in this period decreased from 10.6 to 2.0% in the North Sea, from 8.0 to 5.6% in the Skagerrak, from 12.0 to 1.1% in the Kattegat and from 4.5 to 0.4% in the Baltic Sea. Prevalence rates in research vessel catches were significantly higher (by 1.8 times) than in commercial fishing boat catches. The results show a high degree of temporal and spatial variation of the prevalence of I. hoferi. Infected fish were found to be significantly longer than unaffected fish in the North Sea and the Skagerrak. The majority of the infected fish belonged to the age groups 2+, 3+ and 4+ except in the commercial catches from the North Sea where most of the affected fish belonged to the age groups 5+, 6+ and 7+. Annual mortality in the different areas, based on the observed prevalences was roughly estimated at 12.8 to 36% in 1991, decreasing to a few percent in 1993. The spawning stock biomass of North Sea herring was reduced by 50% during the period 1990 to 1995. This reduction may have been due to a combination of increased fishing intensity and the general effect of the I. hoferi epizootic

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Organisations: National Veterinary Institute, National Institute of Aquatic Resources
Authors: Mellergaard, S. (Intern), Spanggaard, B. (Intern)
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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
Growth of the fish parasite Ichthyophonus hoferi under food relevant conditions

The physical and chemical limits for growth of the internal fish parasite, Ichthyophonus hoferi, have been studied to understand better the ecology of I. hoferi both as a possible food contaminant and a fish pathogen. The effect of temperature (0 degrees-30 degrees C), pH (3-7) and NaCl (0%-10%w/v) on growth were tested in a multi-factorial experiment. Growth was observed at all pH-values, from 0 degrees to 25 degrees C and from 0% to 6% NaCl. No significant differences (P > 0.05) were detected in the growth ability in the temperature range 0 degrees-25 degrees C and from pH 3 to 7. However, increasing the concentration of NaCl significantly decreased the growth of I. hoferi and it is therefore unlikely that I. hoferi will develop and spoil processed products (pickled or salted herring) by continued growth. Hyphal growth could be initiated by incubating spores under CO2, and this may be a potential problem in gas packed fish products.
Ichthyophonus hoferi epidemiology, life cycle, taxonomy and significance in fish products

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State: Published
Organisations: National Institute of Aquatic Resources
Authors: Spanggaard, B. (Intern), Huss, H. H. (Intern)
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Main Research Area: Technical/natural sciences
Phylogenetic relationships of the intercellular fish pathogen Ichthyophonus hoferi and fungi, choanoflagellates and the rosette agent

Ichthyophonus hoferi Plehn and Mulsow, 1911 is thought to be one of the few pathogenic fungal infections of marine fish. The result of an attack is severe epizootics in herring stocks with drastic reduction in the population as a consequence. The exact phylogenetic position of the genus Ichthyophonus is not known. In the present study, a combination of molecular data, ultrastructure and biochemical characters were utilized to investigate the phylogeny of I. hoferi. The genomic DNA encoding the small subunit ribosomal RNA (18S rRNA) was amplified and sequenced. Comparisons with other eukaryotic 18S rRNA sequences indicate that I. hoferi is not a member of the Fungi. In both the parsimony and the neighbor-joining trees, I. hoferi is the sister taxon to the rosette agent. The clade encompassing I. hoferi and the rosette organism is the sister group to the choanoflagellate clade in the neighbor-joining tree, while in the parsimony tree the I. hoferi/rosette clade is equally distant to both the choanoflagellate and animal clades. Transmission electron microscopy showed that I. hoferi has a defined cell wall, an endoplasm that consists of a fine granulated matrix with numerous ribosomes, several nuclei, vacuoles of varying density distributed through-out the cell, and mitochondria with tubular cristae. The cell wall of I. hoferi contains chitin.

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Organisations: National Institute of Aquatic Resources
Authors: Spanggaard, B. (Intern), Skouboe, P. (Ekstern), Rossen, L. (Ekstern), Taylor, J. (Ekstern)
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Morphology of Ichthyophonus hoferi assessed by light and scanning electron microscopy

The morphology of Ichthyophonus hoferi in vitro at pH 3.5 and 7.0 is described using light and scanning electron microscopy. Only vegetative growth was observed. At pH 3.5, hyphal growth was seen. The hyphae of I. hoferi are characterized by evacuated hyphal walls with the cytoplasm migrating to the apex and no septation. In contrast, the growth at pH 7.0 is mainly seen as spherical bodies which vary in size and are uni- to multinucleate. Amoeboid bodies showing slow movements were observed within 3-6 h of transfer to pH 7.0. We propose a life-cycle involving the germination of thick-walled multinucleate spores in the fish stomach as a response to the low pH. The hyphae then penetrate the digestive tract and rupture when they reach a blood vessel (neutral pH), whereby uni- and binucleate bodies and/or amoeboid bodies are released. The small cells are transported in the blood vessels and spread in the organs richly supplied with blood (heart, kidney, spleen, liver and muscle tissue) where they grow to form multinucleate spores.

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Organisations: National Institute of Aquatic Resources
Authors: Spanggaard, B. (Intern), Huss, H. H. (Intern), Bresciani, J. (Ekstern)
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Biological and technological significance of the fish parasite Ichthyophonus hoferi

In 1991 an epizootic of ichthyophoniasis in herring was recorded for the first time in waters around Denmark and Norway causing mass mortality. This Ph.D. study demonstrates how continuously successful subculturing of Ichthyophonus hoferi is possible only at alternating pH (between pH 3-4 and pH7). The morphology of I. hoferi at pH 3.5 and 7.0 was studied using light and scanning electron microscopy. At pH 3.5 only hyphal growth was seen while only growth of uni-to multinucleate spherical bodies was seen at pH 7. These findings were used to explain the lifecycle of this parasite. The phylogenetic position of the genus Ichthyophonus was investigated using a combination of molecular analysis of the genomic DNA encoding the small subunit ribosomal RNA, ultra-structural features and biochemical data. These studies indicated that I. hoferi is not a member of the Fungi, but belongs to the protist Kingdom. Feeding experiments with mice showed that I. hoferi is not a pathogen in mammals. However, the technological significance of I. hoferi infected fish fillets entering processing is severe due to soft texture, unfavourable flavour changes and discolorations of the fish products.
National Institute of Aquatic Resources

Royal Veterinary and Agricultural University
Period: 01/11/1993 → 01/01/1997
Number of participants: 2
Project participant:
Spanggaard, Bettina (Intern)
Project Manager, organisational:
Huss, Hans Henrik (Intern)
Project