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***Francisella* sp. (Family Francisellaceae) causing mortality in Norwegian cod (*Gadus morhua*) farming**

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Abstract In 2004, a new disease was detected in cod (*Gadus morhua*) in western Norway. Affected cod had white granulomas in the visceral organs and skin. A species of *Francisella* was isolated on blood agar plates from moribund cod. The bacterium could be grown at temperatures ranging from 6 to 22°C, but did not grow at 37°C. Challenge experiments showed that *Francisella* sp. was the cause for the new disease. The 16S rDNA gene sequence from *Francisella* sp. showed 99.17% similarity to *F. philomiragia*, and the 16S–23S ribosomal RNA intergenic spacer (249 nt), shows a similarity with that from *Francisella* isolated from tilapia and *F. tularensis* of 96.8 and 35.9%, respectively. The 23S sequence is more similar to *F. tularensis*, 97.7% (2,862 nt), compared to the tilapia isolate 96.8% (2,131 nt). The partial putative outer membrane protein (FopA) sequence (781 nt) from *Francisella* sp. shows a similarity with that from *F. tularensis* and *F. philomiragia* of 77.3 and 98.2%, respectively. Based on sequence data, culturing temperatures and pathogenicity for cod, it is suggested that this *Francisella* sp. from cod could be a new species of *Francisella*, Family Francisellaceae.

Keywords Cod · *Gadus morhua* · *Francisella* · rDNA · FopA · Phylogeny

Introduction

Fish farming is a large industry on a worldwide basis. In the north Atlantic, the major species in production are Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*), Halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*). The production of

Atlantic salmon in Norway was 508,000 tons in 2003 while the newly started cod production amounted to 605 tons only (Agnalt et al. 2004). A major problem in cod production is the start feeding phase and mortality associated with this stage in production (Brown et al. 2003; Nylund, University of Bergen, unpublished results). Several species of bacteria have been associated with the problems in the start feeding phase, and during the spring and summer of 2004, the first major disease caused by bacteria occurred on larger cod at different production sites in western Norway. During the spring and summer of 2005, this disease apparently spread to a larger area (Nylund, University of Bergen, unpublished results).

In the autumn of 2004, we received material from a cod farm in Rogaland county, western Norway. The cod, 2–3 kg, showed loss of appetite, reduced swimming performance, and dark pigmentation. There were few other external signs of disease, but subdermal white granulomas could be found on gills and in the mouth cavity. The most prominent internal signs were a swollen spleen, kidney and heart where these blood rich organs were covered with and penetrated by white granulomas. These granulomas contained, in most cases, a transparent liquid. Sections of these tissues show necrosis, degeneration, inflammation and proliferation of cells, and a few bacteria could be present in these granulomas.

PCR, using primers targeting the 16S from proteobacteria, gave a positive result, and sequencing of the PCR products showed that this bacterium was closely related to *Francisella philomiragia* (cf. Jensen et al. 1969; Hollis et al. 1989). Blasting the 16S sequence showed that similar *Francisella*-like sequences had been previously found in tilapia (*Oreochromis* spp.) in Taiwan and Isaki (*Parapristipoma trilineatum*) in Japan. Several studies have also showed that *Francisella* spp. and bacteria with 16S sequences similar to that from *Francisella* species are present in ticks, amoebas, fish and environmental samples (Suitor and Weiss 1961; Niebylski et al. 1997; Noda et al. 1997; Anda et al. 2001; Scoles 2004; Kamaishi et al. 2005; Barns et al. 2005; Sjøstedt 2005a; Tomaso et al. 2005).

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The present study describes culturing conditions, morphology, and sequences of the rRNA genes, the 16S–23S ribosomal RNA intergenic spacer and the partial sequence of a putative outer membrane protein (FopA) from this *Francisella* isolate. A challenge experiment on cod has been performed to show that this bacterium is the causative agent of a new disease, francisellosis, described from farmed cod. The histopathology is described from cod with chronic francisellosis.

Materials and methods

Sample collection

Included in this study are 20 cultured Atlantic cod (*Gadus morhua*) collected in 2004 and 2005 from four different farms in western Norway located in the counties; Rogaland, Hordaland and Møre og Romsdal. The cod came from farms experiencing mortalities associated with white granulomas on skin, gills and internal organs. Tissues; gills, skin, heart, spleen, liver, head kidney and kidney were collected from all fish included in this study. Tissues were stored at (80°C or fixed for later processing for histology and electron microscopy.

Histopathology and transmission electron microscopy (TEM)

All tissues, gills, skin, heart, spleen, liver, head kidney and kidney, from cod collected from farms and from challenged cod (see below), were fixed by immersion, at 6°C, in a modified Karnovsky fixative where the distilled water was replaced by a Ringers solution (Nylund et al. 1995). The fixative contained 4% sucrose. Before embedding in EPON 812, the tissues were stained/post fixed in 2% OsO₄. Semi and ultrathin sections were cut on Reichert-Jung Ultracut E. The ultrathin sections (30–40 nm) were stained for 1.5 h in 2% aqueous uranyl acetate solution and then stained with lead citrate. Semithin sections, 1.5 µm, were stained in toluidine blue.

Isolation, media and growth conditions

The first isolation of the organism was made by streaking tissues (spleen and head kidney) from cod (*Gadus morhua*) on blood agar plates added 0.1% cysteine and 1% glucose (BCG-plates). Stock cultures were maintained on these plates.

The cod from the fish farms were examined bacteriologically by inoculation of kidney and spleen tissues on: (a) BCG-plates, which were incubated for 30 days at 10°C, and (b) on blood agar plates containing 1.5% NaCl which were incubated for 30 days at 15°C. After the isolation of *Francisella* sp. from farmed cod, several different culturing temperatures were tested; 6, 10, 15, 19, 22, 29 and 37°C.

In addition to the isolation of *Francisella* sp., two other bacteria were isolated on the blood agar plates, *Virbio logei* and *Chryseobacter* sp. These two bacteria were identified by sequencing of the 16S rRNA gene; *V. logei* (Accession no.: DQ318955) and *Chryseobacter* sp. (Accession no.: DQ318956). A mycoplasma (Accession no.: DQ318957) was also identified, by PCR on spleen tissues from one farmed cod using the general eubacterial primers, EubA(1518R) 5'-AAG GAG GTG ATC CAN CCR CA and EubB(27F) 5'-AGA GTT TGA TCM TGG CTC AG (Giovannoni 1991).

It was also attempted to culture *Francisella* sp. in cell cultures established from salmonids (cf: Devold et al. 2000; Dannevig et al. 1997). *Francisella* sp. from BCG-plates were suspended in PBS, diluted 1:100, and incubated at 10°C in cell culture flasks with a mono-layer of salmon head-kidney (SHK-1) cells and Atlantic salmon kidney (ASK) cells. The cells were incubated for 30 days or until cytopathic effect (CPE) could be observed. Negative control cell cultures were always included.

DNA extraction and amplification

DNA was extracted using the DNeasy DNA Tissue kit (Qiagen) as recommended by the manufacturer. Elution was performed twice in 50 µl 10 mM Tris-HCl, pH = 8.5 to increase the overall DNA yield, and the DNA was stored at (20°C). DNA was extracted from 20 farmed cod and from different tissues (spleen and kidney) collected from the cod included in the challenge trial. Extraction of DNA from bacterial colonies was achieved by heating the bacteria at 95°C for 5 min followed by centrifugation at 14,000 rpm for 1 min. The supernatant was stored ((20°C) for later use in PCR.

The PCR reaction mixture (50 µl) contained 10 × PCR buffer with 1.5 mM MgCl₂ (Amersham Pharmacia Biotech Inc.), 25 mM of each dNTP (Promega), 0.2 µM of each primer (Invitrogen), 1 U Taq DNA polymerase (Amersham Pharmacia Biotech Inc.) and 300 ng DNA. Primers used for amplification of the rDNA genes, the complete 16S–23S ribosomal RNA intergenic spacer and the putative outer membrane protein (FopA) are described in Table 1. Amplification was performed in a GeneAmp PCR System 9700 machine (Applied Biosystems) at 95°C for 5 min; 35 cycles of 94°C for 30 s, X C (dependent on the primer set, see Table 1) for 45 s, 72°C for 1 min followed by extension at 72°C for 10 min and a short storage at 4°C.

DNA sequencing and analysis

PCR products were purified with QIAquick PCR purification kit (Qiagen) as described by the manufacturer. Sequencing was then performed with ABI PRISM Big-Dye terminator chemistry (version 2) according to Applied Biosystems (ABI). All sequences were assembled using the Vector NTI Suite 7.0 program (InforMax

Table 1 Overview of primers used in for sequencing the rDNA genes, the complete 16S–23S ribosomal RNA intergenic spacer, and the putative outer membrane protein (FopA) from *Francisella* sp. The location of the primers is given in relation to Accession no.: DQ309246 (rDNA genes) and DQ333226 (FopA)

Primer	Sequence	Location
rDNA gene forward		
FC-F1	5'-CCT GGC TCA GAT TGA ACG CTG G	Outside
FC-F2	5'-ACA GGT CTT CGG ATG CTG ACG	42–62
FC-F3	5'-AGA GAT AGA TTG GTG CCT TCG G	973–994
FC-F6	5'-TTC AGA GGC GAT GAA GGA C	844–1,862
FC-F4	5'-GCA AAA GTA TGG GAT GAG CTG TGG	53–2,576
FC-F10	5'-TTC AGA GGC GAT GAA GGA C	675–3,694
Reverse		
FC-R20	5'-GGA AAA AGA TGG CGA CTA CC	Outside
FC-R6	5'-TGT GAT GAG CCG ACA TCG	310–4,293
FC-R7	5'-CAC TGC ATC TTC ACA GCG	839–3,822
FC-R2	5'-CGA CAA GGA ATT TCG CTA CC	747–3,728
FC-R5	5'-TCG CTC GCC ACT ACT ATG GG	54–2,035
FC-R1	5'-TCA CTC CGT GGT AAA CGC C	432–1,414
FopA forward		
FMP-F1	5'-GTC TCA ATG TAC TAA GGT TTG CCC	Outside
FMP-F2	5'-CAA GAT AGA ACT GGY CAG TGG	393–413
Reverse		
FMP-R1	5'-RSC ACC AAT CAT RTT AGT ACC	outside
FMP-R2	5'-CAC CYA AAC CAG CAA ATA CTC	561–541

Inc.). Sequences obtained in the present study were submitted to the GenBank.

Phylogenetic analysis

The Vector NTI Suite software package was used for the multiple alignments of nucleotide sequences. To perform pair wise comparisons between the different sequences of the 16S rRNA gene, the multiple sequence alignment editor GeneDoc was used (Nicholas 1997). Sequences already available on the EMBL nucleotide database were also included in the comparisons.

Phylogenetic trees were constructed using maximum likelihood analysis to describe the phylogenetic history of the bacteria based on the 16S nucleotide sequence. Analyses were performed using TREE-PUZZLE 5.0 (Available at: <http://www.tree-puzzle.de>). Parallel trees were constructed using PAUP v4.0 (Swofford 1998) with maximum likelihood as optimality criterion and the heuristic search option, in order to verify that the two methods produced a similar tree topology. For the PAUP analysis, Modeltest 3.6 (Posada and Crandall 1998) was used to identify the models best suited for the datasets. The PAUP tree was not bootstrapped due to lack of computer capacity. Phylogenetic trees were drawn using TreeView (Page 1996).

Challenge experiment

Bacteria, *Francisella* sp., *Vibrio logei* and *Chryseobacter* sp., grown on blood agar were used in a challenge experiment on Atlantic cod (*Gadus morhua*). The bacteria were scraped of blood agar plates and suspended in a phosphate buffer solution (PBS) to a concentration of about 1.0×10^8 .

The cod was supplied by a hatchery close to Bergen. Initial weight and length of fish were about 15 g and

12 cm. The fish were acclimatised for 7 days to particle (20 µm) and UV-filtered (Katadyn J1/P, effect: 50 mWs cm²) seawater (34‰), kept at 10°C in 0.15 m³ tanks (flow rate = 5 l min⁻¹; 50 specimens in each tank), and fed commercial pellets twice a day.

The challenge experiment included five different groups kept in separate tanks, (30 cod in each tank). The control group was injected intraperitoneal (i.p.) with 0.2 ml PBS. The second group received an i.p. injection of filtered (0.45 µm) supernatant from tissue homogenate (spleen) of cod with internal white granulomas. The last three groups were challenged by an i.p. injection (0.2 ml) of *V. logei*, *Chryseobacter* sp. and *Francisella* sp., respectively. The fish were challenged on 4 February 2005. The experiment was terminated after 122 days (6 June 2005). To determine if *Francisella* sp. could be transmitted from cod challenged by i.p. injections to disease-free cod, 20 fish were added to the tank, 19 days after challenge. These co-habitants were tagged by marking the pectoral fins.

Mortality was registered in all tanks and all fish were examined bacteriologically by inoculation of kidney tissues on: (a) BCG-plates which were incubated for 30 days at 10°C, and (b) on blood agar plates containing 1.5% NaCl which were incubated for 30 days at 15°C. Tissues were sampled from the skin, gills, heart, kidney, liver and spleen from all fish and; (a) stored at (80°C for later RNA extraction and (b) fixed in a modified Karnovsky fixative for histological and transmission electronmicroscopical (TEM) studies.

Results

Clinical signs and histopathology

Atlantic cod (*Gadus morhua*) suffering from infection with *Francisella* sp. shows loss of appetite, reduced swimming performance, and dark pigmentation. There

are few other external signs of disease, but white granulomas can be found in the skin, gills and in the mouth cavity. Internal signs may range from just slightly swollen spleen and kidney to most *viscera* covered with white granulomas. In the terminal stages of the disease, the spleen may have grown to three times the normal size being completely covered with and penetrated by granulomas (Fig. 1). Heart, kidney and liver are also among the organs/tissues most strongly affected by degeneration and presence of granulomas. At the terminal stages, there are few bacteria present within the granulomas which may be filled with a transparent liquid. The cell types, organization, and ultrastructural features were similar in granulomas from spleen, kidney, skin and heart tissues.

The target cells for *Francisella* sp. seem to be phagocytes and cells with phagocyte functions (reticulo-endothelial system, RES) (Fig. 2). In the early stages of the disease, bacteria are always present in phagocytes in the spleen and kidney, but can also be found in endothelial cells lining the heart chambers and in leucocytes attached to the blood vessel walls in the liver, pseudo-branch and gills. In the later stages of the disease, the granulomas consist mainly of host cells (phagocytes, fibroblasts and lymphocytes) organized in concentric cellular layers and with little or no bacteria present

(Fig. 3). In the terminal stages of granulomas, there is a prominent development of necrosis in the core, i.e. the cells in the center dies and is replaced by a transparent liquid, and no bacteria can be detected by microscopy in the core vacuole.

Growth temperature and morphology

Francisella sp. grows on BCG-plates at 6, 10, 15 and 19°C. The growth at 22°C is slow compared to the growth at 15 and 19°C. No growth was observed at 29 and 37°C.

Francisella sp. grown on blood agar, are coccoid with a diameter of about 500 nm (range from 300 to 700 nm) (Fig. 4). More elongated forms, as long as 1.0 µm, may also be present. *Francisella* sp. present in cells and tissues have more or less the same size and morphology as that seen on blood agar plates (Fig. 4). However, they are slightly more polymorphic and less coccoid shaped, and may be as long as 1.5 µm.

Francisella sp. grows in both SHK-1 and ASK cells at 10°C, but the growth is best in SHK-1 cells. A visible production of *Francisella* sp. in SHK-1 cells could be observed 11 days after inoculation. Most of the SHK-1 cells were lost 26 days after inoculation with *Francisella* sp. due to replication of the bacterium.

Fig. 1 **a** Atlantic cod (*Gadhus morhua*), from a fish farm, showing the typical signs of francisellosis, including white granulomas on the spleen, kidney, and heart. **b** Atlantic cod challenged with *Francisella* sp. Note the presence of white granulomas in kidney, liver and spleen

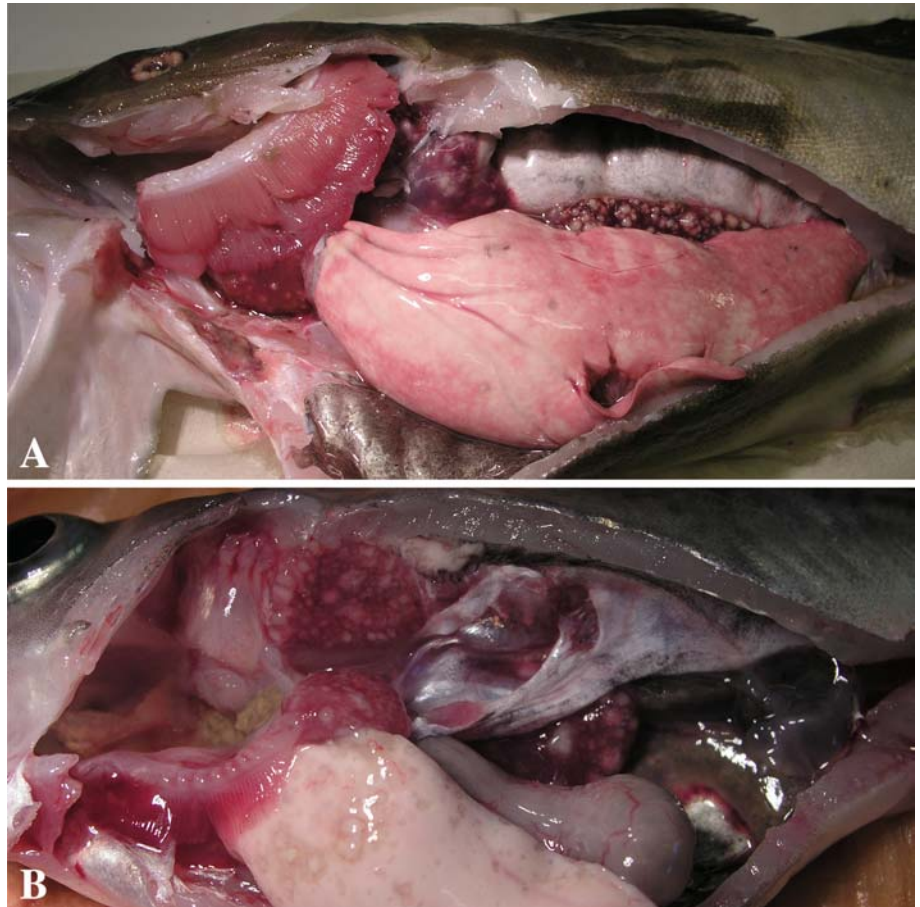
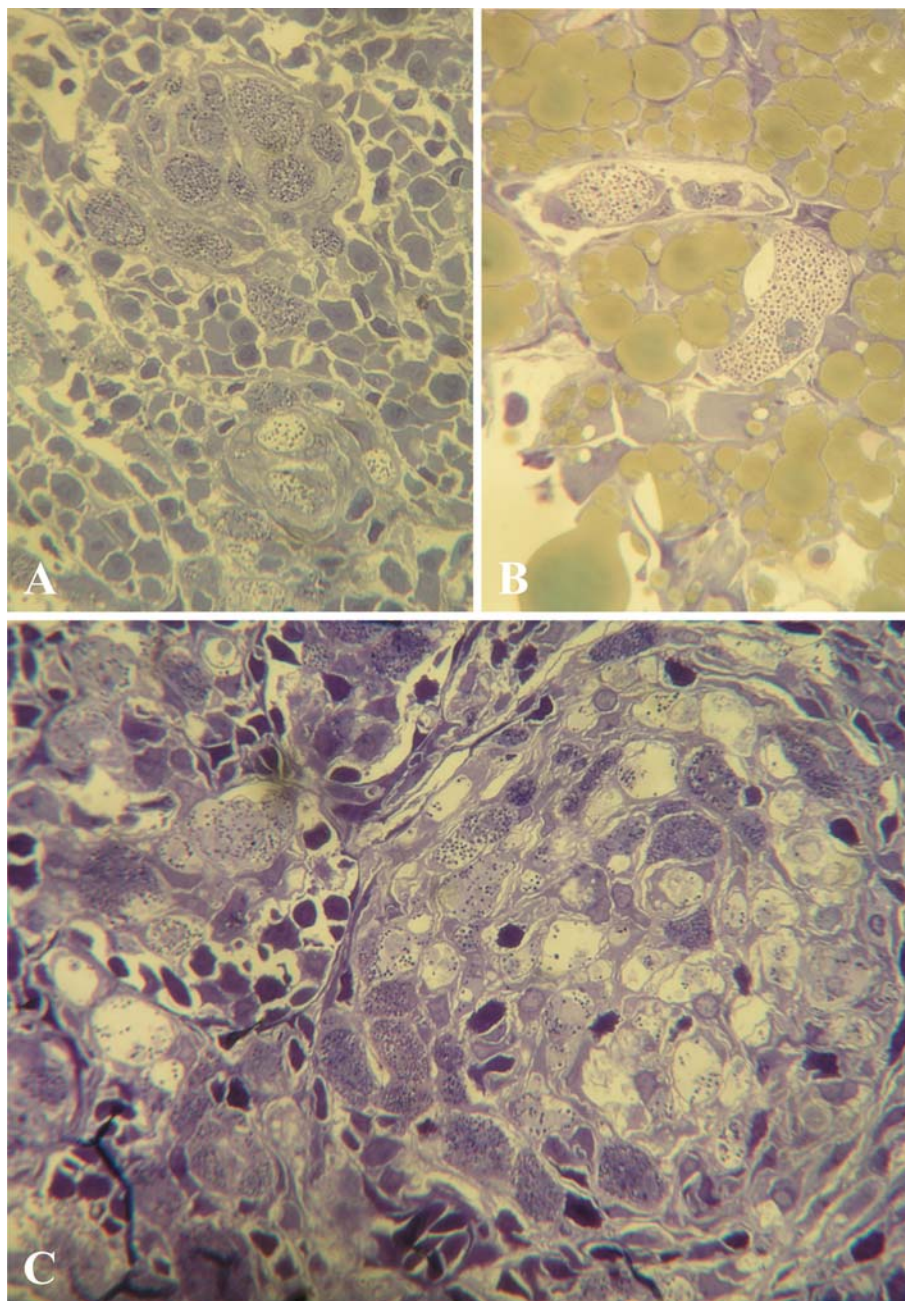


Fig. 2 Sections of kidney (a), liver (b), and spleen (c) tissues from Atlantic cod challenged with *Francisella* sp. Note the cells infected with bacteria and the starting development of granulomas



DNA sequences

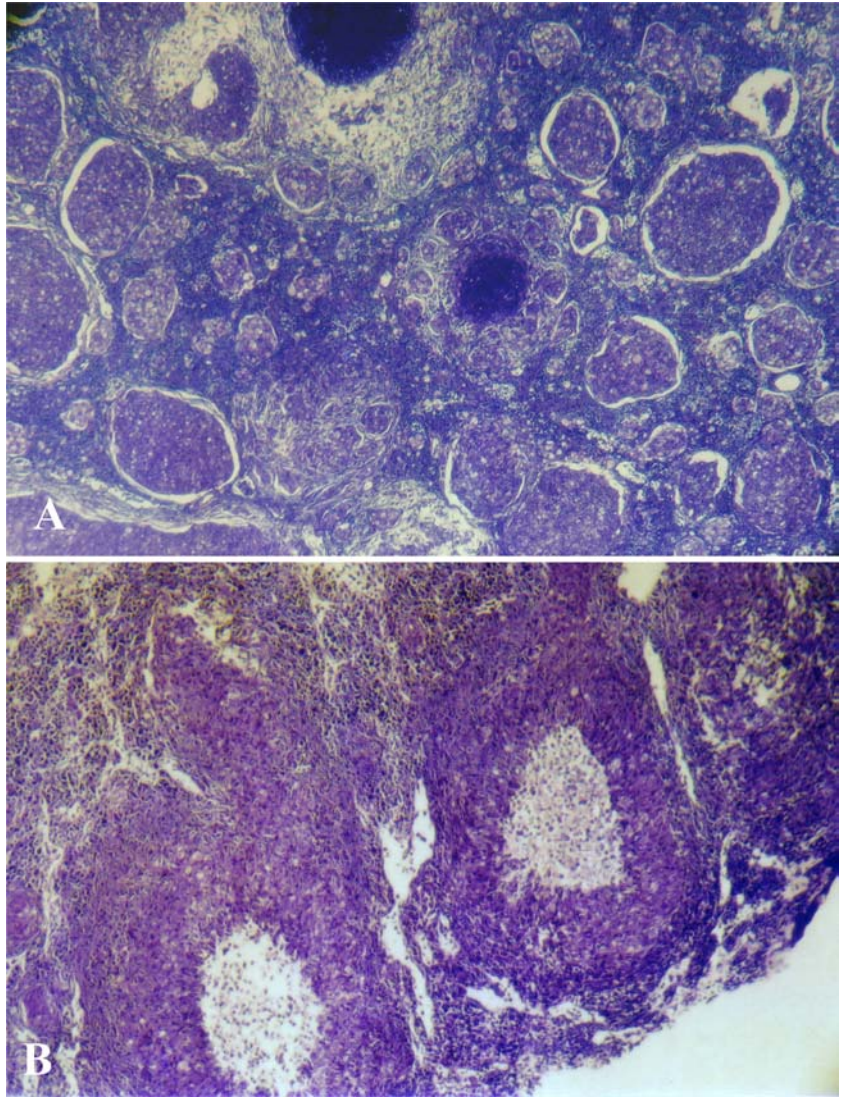
The 16S rRNA gene was sequenced from *Francisella* sp. obtained from 20 different specimens of cod collected in four different farms (DQ309246). The 16S was also sequenced from cod challenged by *Francisella* sp. and cod challenged by tissue homogenate from cod with white granulomas on the internal organs. No variation was observed in the 16S when a partial sequence of 1,430 nt was compared with *Francisella* sp. in the different individual hosts

Comparison of the partial 16S, the complete 16S–23S ribosomal RNA intergenic spacer, and the partial 23S (a total of 3,850 nt) from the cod isolate and a *Francisella* isolate from tilapia (Accession no: AF385857) shows a

similarity of 97.4%. A comparison of the partial 16S sequence from the Atlantic cod isolate and *F. philomiragia* (AJ698862), and the isaki isolate (AB194068) from Asia gives the following similarities, 99.17 and 99.25%, respectively. Comparison of the 16S–23S ribosomal RNA intergenic spacer, from *Francisella* sp. (249 nt) with that from the tilapia isolate (AF385857) and *F. tularensis* (NC006570) give similarities of 96.8 and 35.9%, respectively. The 23S from *Francisella* sp. show a similarity of 97.7% (2,862 nt) and 96.8% (2,131 nt), respectively, with that from *F. tularensis* (AJ749949) and tilapia (AF385857).

Comparison of the partial putative outer membrane protein (FopA) sequence (781 nucleotides, Accession no.: DQ333226) from *Francisella* sp. with that from *F.*

Fig. 3 a and b) Sections through the spleen of cod infected with *Francisella* sp. Note the different stages of development of granulomas. In **b**, the center of the granulomas contains dead cells



tularensis (M93695) and *F. philomiragia* (AF097543) give the following similarities; 77.3 and 98.2%, respectively.

Phylogeny

The genus *Francisella* consists of only two recognized species, *F. tularensis* and *F. philomiragia*, where the former consists of several subspecies. In addition to these recognized species, isolates from ticks and Asian fish (tilapia and isaki) seem to constitute at least two other species (Fig. 5). The cod isolate *Francisella* sp. is positioned between *F. philomiragia* and the Asian fish isolates in the 16S phylogeny. The support values are high and support the distinctness of the Norwegian cod isolate based on the partial 16S sequence.

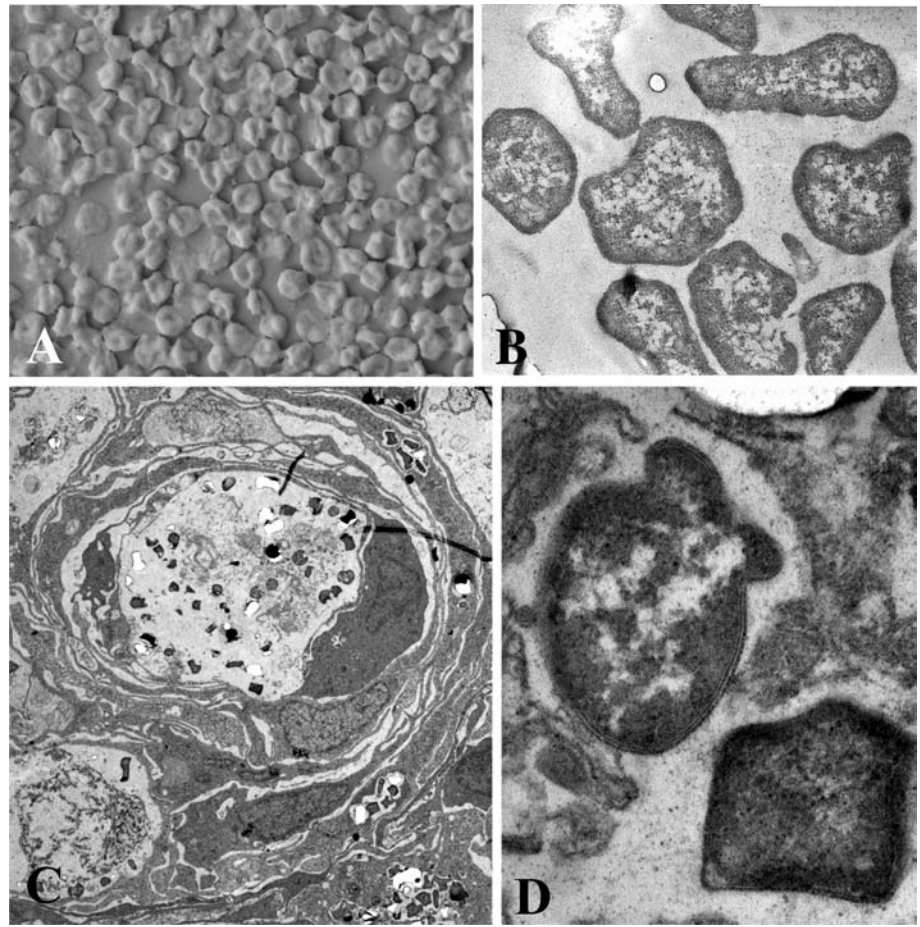
Challenge experiment

During the experimental period of 122 days, 23 cod died after i.p. injection of *Francisella* sp. and 10 co-habitants

died during a period of 103 days. The mortality, in the i.p. challenged cod, started after day 7. Twelve fish died during the period from day 7 until day 12. The rest of the mortality occurred during the period from day 12 until the termination of the experiment. The co-habitants died during the last 30 days of the experimental period. There were no mortalities in the control group and only a low mortality in the groups challenged by *V. logei*, *Chryseobacter* sp. and tissue homogenate from cod with white granulomas, i.e. 2, 3 and 4 specimens, respectively.

Francisella sp. was present in both the i.p. challenged and the cohabitant fish in the tank challenged by *Francisella* sp.. No other bacteria were detected by PCR or isolated from the moribund fish in this tank. The four fish that died in the group challenged by tissue homogenate were all positive for *Francisella* sp., tested by inoculation on blood agar and by PCR on kidney and spleen. *V. logei* and *Chryseobacter* sp. were present in the fish that died in the groups challenged by *V. logei* and *Chryseobacter* sp., respectively.

Fig. 4 Electronmicroscopic pictures of *Francisella* sp. grown on blood agar plates (A and B) and from infected cells in the kidney tissue (C and D). The diameter of *Francisella* sp. is about 0.5 μ m



The cod that died during the first period after infection with *Francisella* sp., acute mortality, showed few gross clinical signs of disease and no macroscopical granulomas. However, sections of tissues showed that most organs and tissues were degenerating containing a high number of cells infected by bacteria. The most prominent sign was a swollen and liquified kidney. Cod suffering from chronic infections with *Francisella* sp., i.e. mortality late in the experimental period, had swollen spleen and kidney and, these and heart were covered with and penetrated by white granulomas (Figs. 1 and 3). Sections of these tissues showed degeneration, inflammation and proliferation of cells (Fig. 3), but few intracellular bacteria were observed in cells from these tissues. Granulomas could also be found on and in the liver. The fish that were killed at the termination of the experiment all showed signs of chronic infection with *Francisella* sp., with white granulomas in heart, spleen and kidney.

Discussion

The induction of chronic granulomatous infections in fish by rickettsia-like organisms (RLO) has been previously described in the literature (Chen et al. 1994;

Chen et al. 2000; Khoo et al. 1995; Chern and Chao 1994; Mauel et al. 2003; Timur et al. 2005). It has not always been possible to detect any members of *Piscirickettsia* by PCR or other specific diagnostic methods (Mauel et al. 2003). However, in 2002 a new *Francisella* isolate was detected in cultured three-line Grunt (*Parapristipoma trilineatum*) suffering from a granulomatous disease and it was shown that this bacterium was the causative agent (Kamaishi et al. 2005). The disease had been present in Japanese aquaculture since 1999. The granulomatous disease detected in Atlantic cod (*Gadus morhua*) culture in Norway in 2004 share much of the same histopathology as that described from tilapia in Hawaii and isaki in Japan, and based on 16S sequences, the causative agent of the cod disease is yet another new isolate of *Francisella* related to *F. philomiragia* and the *Francisella* species from isaki.

The family Francisellaceae, which belongs to the gamma subclass of the class Proteobacteria includes closely related organisms within the genus *Francisella* (Sjöstedt 2005a). There are only two recognized species, *F. philomiragia* and *F. tularensis*, where the former is often associated with the aquatic environment and less virulent for humans compared to *F. tularensis*. However, there is evidence for the existence of several species of

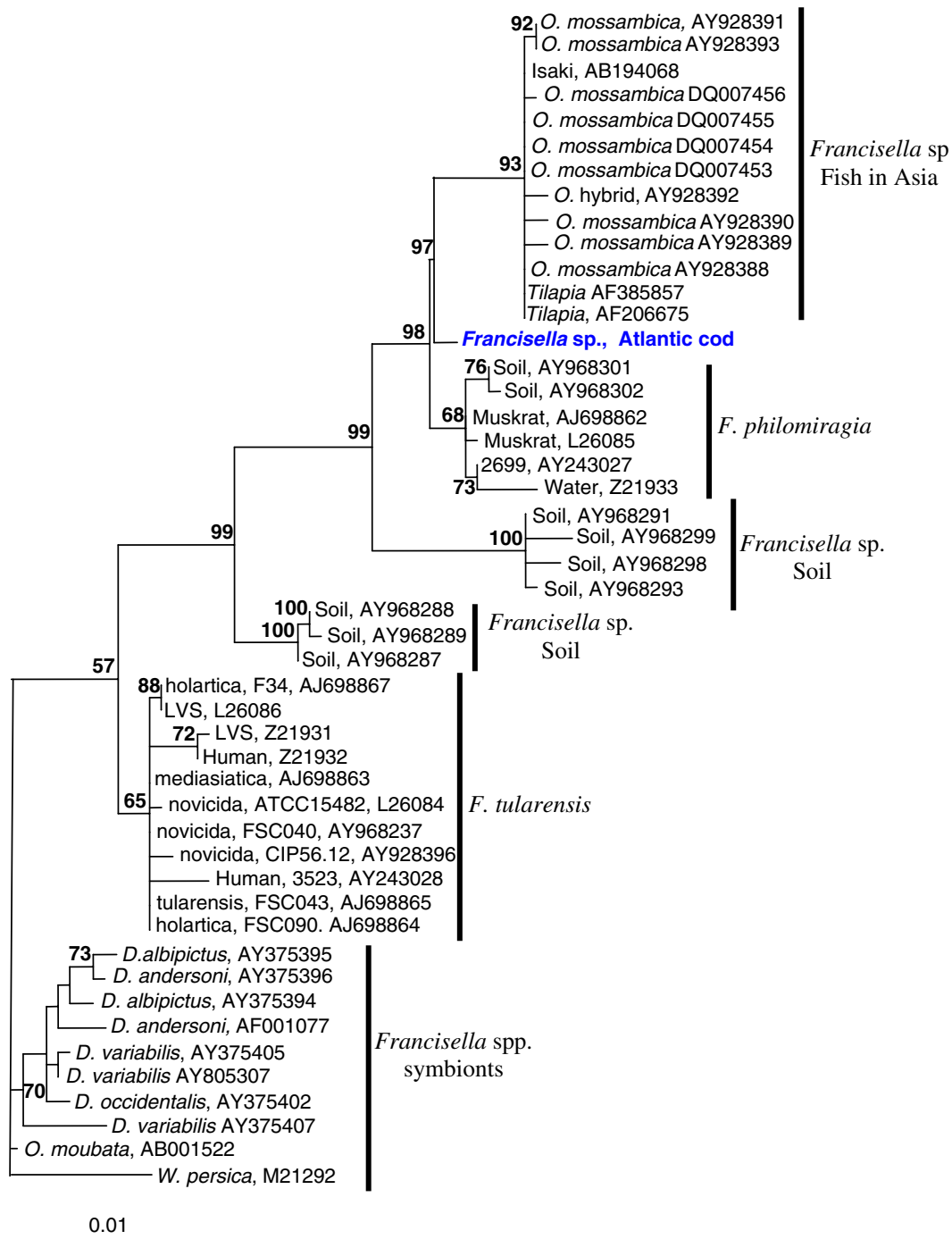


Fig. 5 The phylogenetic relationship of the *Francisella* isolate (DQ309246) from Atlantic cod, *Gadus morhua*, in Norway. The isaki (*Parapristipoma trilineatum*) *Francisella* isolate (AB194068) comes from Japan. *Francisella* spp. isolated from *Tilapia* in Taiwan: AF206675, AF385857, AY928388, AY928389, AY928390, AT928391, AY928392, AY928393, DQ007453, DQ007454, DQ007455, DQ007456, from the environment in

USA; AY968287, AY968288, AY968289, AY968291, AY968293, AY968298, AY968299, AY968301, AY968302, AJ698867, Z21933, AY928396, L26084, AY968237, from mammalian hosts; AJ698862, L26085, AJ698863, AY243027, AJ698864, AJ698865, AY243028, Z21931, L26086, Z21932, as endosymbionts of ticks; AY375405, AY805307, AY375394, AY375395, AY375396, AY375402, AY375407, AF001077, AB001522, M21292

Francisella in the environment, or in association with organisms like ticks, amoeba and fish (Barns et al. 2005). These are yet to be described as full species or members of the genus *Francisella*.

The causative agent of francisellosis in farmed cod, in Norway, differs distinctly, in the rDNA gene sequences, the 16S–23S ribosomal RNA intergenic spacer, the putative outer membrane protein (FopA), and the

optimal culture temperature, compared to the two described species of *Francisella*. It is also distinctly different, in most of the same characters, compared to the *Francisella* isolate from isaki in Japan. Based on phylogenetic analysis of the 16S sequences, the cod *Francisella* is positioned between *F. philomiragia* and the isaki strain. Comparing the 16S–23S ribosomal RNA intergenic spacer and the *FopA* result in a much closer relationship to the isaki strain and *F. philomiragia*, respectively, compared to *F. tularensis*. However, the 23S sequence from the cod *Francisella* is more similar to *F. tularensis* compared to the isaki isolate. Unfortunately, the 23S rDNA gene from *F. philomiragia* is not available in the Genbank preventing a comparison to this species. Based on this, it is not possible to suggest the correct phylogenetic position of the cod *Francisella* since different genes may result in different phylogenetic positions.

Based on the rDNA genes, the 16S–23S ribosomal RNA intergenic spacer, the partial outer membrane protein (*FopA*) gene sequence, the growth temperatures and the phylogenetic position of this new *Francisella* isolate, it could be that this bacterium should be given status as a new and distinct species in the genus *Francisella*, family Francisellaceae.

Little is known about the virulence mechanisms of *Francisella* species in general (Larsson et al. 2005; Sjøstedt 2005b), but the challenge experiment clearly shows that when given a high enough dosage, the *Francisella* isolate from cod may cause a high and acute mortality among infected cod. It is also demonstrated that the bacterium can be transmitted from host to host over short distances. In the present experiment, all challenged fish were strongly affected by *Francisella* sp. which shows that this bacterium, when present, has a high potential to cause problems in cod farms. It is too early to have any strong opinions about how this *Francisella* isolate may enter cod farms, but there are several possibilities. The bacterium may arrive when the fry is delivered from brood fish companies and, so far, the brood fish companies are using wild caught fish as brood fish, which could be naturally infected. Another possibility is natural reservoirs in the vicinity of farms. These reservoirs could be amoeba, wild fish and a long range of marine species (Abd et al. 2003; Barnes et al. 2005). The potential for horizontal transmission between fish farms may also be largely due to the possibility of naturally occurring vectors and due to a long survival time for bacteria in the genus *Francisella* which may allow long distance transmission via sea currents. It has been shown in other *Francisella* species that they may survive for as long as a year in the aquatic environment. Vector transmission and long time survival combined with the fact that the infectious dose necessary to cause a fatal disease can be low (< 10 CFU) in an individual (Oyston et al. 2005; Sjøstedt 2005a, b), point towards this new *Francisella* isolate as a very serious danger for future culture of cod in Norway.

Clinical signs and histopathology indicate that this new *Francisella* isolate may infect and kill a large range of fish species (Chen et al. 1994; Chen et al. 2000; Khoo et al. 1995; Mauel et al. 2003). This, and the fact that other *Francisella* isolates are causing mortality among tilapia and isaki in Taiwan and Japan, suggests that *Francisella* species constitutes a future threat, not only to cod production in Norway, but to all fish farming in the marine environment including salmonids. Future fish farming in Norway and other countries may depend upon the development of an effective control strategy that may involve everything from vaccination to stamping out.

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