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Title: *Moritella viscosa* in lumpfish (*Cyclopterus lumpus*) and Atlantic salmon (*Salmo salar*)

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Conflict of Interest

The authors have no conflicts of interest.

Abstract:

Winter ulcer disease, caused by *Moritella viscosa*, is a significant problem in cold water salmonid farming, although the bacterium can infect and cause disease in a number of other fish species, such as lumpfish (*Cyclopterus lumpus*). Lumpfish are used as cleaner fish, to eat sea lice from Atlantic salmon (*Salmo salar*) in sea pens. It remains to be established whether *M. viscosa* can be transmitted between the fish species. In this study, we examined whether a salmon isolate of *Moritella viscosa* could infect and cause disease in lumpfish. We further examined whether a lumpfish isolate of *Moritella viscosa* could infect and cause disease in salmon. Finally, we examined whether vaccination of salmon with a salmon isolate of *M. viscosa* conferred protection against a lumpfish isolate. The data indicate that while lumpfish appeared to be resistant to a salmon isolate of *M. viscosa*, the salmon could be infected with a lumpfish isolate of *M. viscosa.* Vaccination protected the salmon against the salmon isolate of *M. viscosa* but did not confer sufficient protection to prevent infection with the lumpfish isolate.

Keywords:

Moritella viscosa; Atlantic salmon; Lumpfish; Experimental infection; Vaccine.

1. Introduction

Moritella viscosa, the etiological agent of winter ulcer disease, is a significant problem in salmonid farming in cold regions. Disease generally occurs where seawater temperature drops below 8-10°C (Benediktsdottir, Helgason, & Sigurjonsdottir, 1998; T. Lunder, Eversen, Holstad, & Håstein, 1995) and outbreaks are most common in post-smolts in the first year at sea (Coyne et al., 2006). Winter ulcer disease primarily manifests as superficial skin lesions that can develop into chronic skin and muscle ulcers which may be followed by terminal septicemia, although mortalities are typically low (Benediktsdottir et al., 1998; Grove, Reitan, Lunder, & Colquhoun, 2008; Tunsjø et al., 2011). Several virulence factors and putative virulence factors have been identified, and clinical symptoms can be induced using extracellular proteins (Bjornsdottir, Gudmundsdottir, & Gudmundsdottir, 2011; Karlsen et al., 2014). Coinfection with other bacterial species in winter ulcers is common, most notably *Tenacibaculum* spp. (Olsen et al., 2011) and *Aliivibrio wodanis* (T Lunder et al., 2000), although data by Hjerde *et al.* indicates that *A. wodanis* may inhibit growth of *M. viscosa* (Hjerde et al., 2015).

In addition to salmonids, *M. viscosa* has also been isolated from Atlantic cod (*Gadus morhua,* (Colquhoun, Hovland, Hellberg, Haug, & Nilsen, 2004)), plaice (*Pleuronectes platessa,* (T Lunder et al., 2000)), and both asymptomatic and diseased Atlantic lumpfish (*Cyclopterus lumpus,* (Benediktsdottir, Verdonck, Sproer, Helgason, & Swings, 2000) and unpublished data). It has further been shown that turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*) can be experimentally infected with *M. viscosa* (Bjornsdottir, Gudmundsdottir, Bambir, Magnadottir, & Gudmundsdottir, 2004; Gudmundsdottir, Bjornsdottir, Gudmundsdottir, & Bambir, 2006), although naturally infected turbot and halibut have not been observed. Experimental infection of Atlantic salmon with *M. viscosa* strain F57, isolated from a healthy wild-caught lumpfish in 2000 (Benediktsdottir et al., 2000), indicated that the isolate was avirulent or low virulent in salmon (Bjornsdottir et al., 2011) and it was speculated that lumpfish were resistant to winter ulcer disease. Once lumpfish were shown to be a suitable biological method to constrain sea lice (*Lepeophtheirus salmonis*) infestations in salmon in cold waters (Imsland et al., 2014), the demand for lumpfish by salmon farmers increased dramatically. Lumpfish farming became a fast growing industry, with a production of 11.8 million farmed juvenile fish in Norway alone in 2015 (Powell et al., 2017). Inevitably, when a new fish species is introduced to aquaculture, new diseases and infectious agents emerge (Alarcon et al., 2016). A growing number of infectious agents have been isolated from lumpfish (reviewed by Powell *et al.* (Powell et al., 2017)), ranging from known pathogens that were likely transmitted from other fish species, such as *Piscirickettsia salmonis* (Marcos-Lopez et al., 2017), to novel agents such as *Cyclopterus lumpus* virus (*Flaviviridae*) (Skoge, Brattespe, Økland, Plarre, & Nylund, 2018), lumpfish rana virus (*Iridoviridae*) and a new subtype of viral hemorrhagic septicemia virus (*Rhabdoviridae*) (unpublished data). Lumpfish have also been shown to be able to transmit *Neoparamoeba perurans*, the parasite causing amoebic gill disease, to Atlantic salmon (Haugland, Olsen, Rønneseth, & Andersen, 2017). Interestingly, while the salmon developed severe disease, the disease progression in lumpfish was significantly slower. Unlike salmon, lumpfish activity appears to be a poor indicator of their health status (Alarcon et al., 2016; Haugland et al., 2017). The lack of clear disease symptoms and decrease in appetite may increase the risk of lumpfish infections going unnoticed.

Salmon vaccination against *M. viscosa* is general practice in cold regions, although the components of the vaccine may differ depending on the needs in each region. Lumpfish vaccines are less standardized, and *M. viscosa* is not always included. While sequencing of the 16S rRNA gene is typically used for identification of *M. viscosa*, the gyrase B (*gyrB*) gene is more variable and has been used to distinguish subtypes of *M. viscosa* (Grove et al., 2010). Karlsen *et al*. sequenced the genomes of twelve *M. viscosa* from four different fish species, including one isolate from lumpfish (Karlsen, Hjerde, Klemetsen, & Willassen, 2017). Based on the data, the lumpfish isolate grouped with a cod isolate, but the authors could not associate subgroups with the host type. As lumpfish and salmon are in close proximity in sea pens, it is important to establish whether *M. viscosa* can be transmitted between the fish species, and whether vaccination with a salmon isolate can confer protection against lumpfish isolates.

In this study, we examined whether *M. viscosa* isolated from an Atlantic salmon could infect and cause disease in lumpfish. We further examined whether *M. viscosa* isolated from a lumpfish could infect and cause disease in Atlantic salmon vaccinated with a salmon isolate of *M. viscosa*.

2. Materials and methods

2.1 Fish

The experimental procedures were approved by the Icelandic Food and Veterinary Authority (permit 2017-03-08) and were carried out in accordance with EU Directive 2010/63/EU for animal experiments.

While the data were combined for analysis, the lumpfish were kept in 3 separate groups per condition during the experiment, with 10 fish per container. Salmon were kept in 3-4 separate groups per condition, with 6-8 fish per container. Fish were kept in approximately 8°C water with at least 80% oxygen saturation and a drip flow of water at about 0.2L*min^{-1}*kg of fish.

2.1.1 Atlantic salmon (*Salmo salar*)

Fifty gram pre-smolt Atlantic salmon were used in this study. The fish had been vaccinated with Alpha ject 5-3 (Pharmac, Norway) 41 days previously, which contains inactivated *Aeromonas salmonicida* subsp. *salmonicida*, *Vibrio salmonicida*, *Vibrio anguillarum* subsp. O1, *Vibrio anguillarum* subsp. O2a, and *M. viscosa*, isolated from Atlantic salmon. The fish were kept in 25 liter plexiglass containers and fed 1.1mm pellets (Biomar, Denmark) two times per day. The light was set to a cycle of 15 hours darkness to 9 hours of light. After acclimatization for 7 days in freshwater, the fish were transferred to full salinity sea water (35ppt) in which they were acclimatized for an additional 7 days before infection.

2.1.2 Lumpfish (*Cyclopterus lumpus*)

Five gram lumpfish were used in this study. While lumpfish in Iceland are routinely vaccinated against *V. anguillarum* O1, *Pseudomonas anguilliseptica* and *A. salmonicida* subsp. *salmonicida*, the fish in this study had not been vaccinated. The fish were kept in 12 liter white plastic buckets and fed 1.1mm pellets two times a day. The light was set to a cycle of 15 hours darkness to 9 hours of indirect light. Black plastic discs were placed in the buckets to give the lumpfish a dark, vertical surface to adhere to. The fish were acclimatized for 7 days before infection.

2.2 Bacteria

Two recent virulent *M. viscosa* isolates were used to infect fish in this study; F112/17 from farmed Atlantic salmon and F6/15 from lumpfish. The bacteria were cultured on blood agar (Oxoid CM 55 (Oxoid, UK), 5% defibrinated sheep blood and 2% NaCl (Sigma Aldrich, Denmark)) at 15°C, and in salt-brain heart infusion medium (BD-Difco, France) and 2% NaCl overnight with shaking at 150rpm at room temperature. Bacterial density was measured by optical density (OD600m, GeneQuant pro, UK) and confirmed by plating on agar.

In addition, two previously published Icelandic isolates, K58 (salmon isolate, virulent) and F57 (lumpfish isolate, low virulence in salmon) and unpublished recent clinical isolates from farmed Atlantic salmon (F69/17), Arctic char (*Salvelinus alpinus,* F3/16 and F27/17) and rainbow trout (*Oncorhynchus mykiss,* F39/14, F3/15 and F192/16) were included for comparison in genetic studies (Benediktsdottir et al., 1998; Benediktsdottir et al., 2000).

2.3 Infection of fish

2.3.1 Bath infection

Fish were transferred to aerated containers with $1*10^5$ CFU/ml bacteria added to the seawater. After 60 minutes, the fish were transferred to tanks with clean seawater. Lumpfish were infected with a lumpfish isolate (F6/15) or a salmon isolate (F112/17), and salmon were infected with F6/15 or F112/17. Mock-infected fish that were kept in containers with seawater were used as controls.

2.3.2 Intraperitoneal infection

Fish were transferred to aerated containers with anesthesia (70mg/L MS-222, Sigma Aldrich, China). Once the fish were non-responsive, 0.1ml of $2.9*10^5$ CFU/ml F6/15 or $1.2*10^5$ CFU/ml F112/17 bacteria were injected into the abdominal cavity of both lumpfish and salmon. The fish were then transferred to tanks with clean seawater. Mock-infected fish that were injected with a 2% NaCl solution were used as controls.

2.4 Quantitative PCR

To examine whether fish were infected with *M. viscosa*, wound swabs and kidney swabs were inoculated on blood agar plates (with 2% NaCl) and incubated at 15°C. Bacterial colonies were placed in RLT buffer, and DNA extracted using the QIAamp DNA mini kit in a QIAcube (Qiagen, Germany). Quantitative PCR specific for *M. viscosa* was performed, as previously described by Grove *et al.* (Grove et al., 2008), with 2X TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 300 nM primers (Fw: 5′-CGTTGCGAATGCAGAGGT-3′, Rev: 5′-AGGCATTGCTTGCTGGTTA-3′) and 150 nM Probe (FAM-5′-TGCAGGCAAGCCAACTTCGACA-3) with Black Hole quencher 1. The amplification was performed in a StepOnePlus (Applied Biosystems, Singapore) at 50°C for 2 min, 95°C for 10 min, 45 cycles of 95°C for 15 sec and 60°C for 60 sec. Data were analyzed using StepOne software v2.3. All primers and probes used in this study were from TAGC (Denmark).

2.5 PCR and sequencing

DNA was extracted from bacterial cultures using the Gene JET genomic DNA purification kit (Thermo Fisher Scientific, Lithuania).

For the PCR reactions, Taq 2X Master mix (New England Biolabs, USA) and 2μM primers (shown in Table 1) were used in a Veriti thermal cycler (Applied Biosystems, Singapore). The PCR conditions for amplification were 95°C for 3 min, followed by 35 cycles of 95°C for 1 min, 52°C for 1 min and 72°C for 1 min, and final elongation at 72°C for 5 min. The PCR products were electrophorized in a 1% agarose gel (AppliChem, Germany) in a BioRad electrophoresis tank (BioRad, Sweden), and imaged using an InGenius Bio-imaging system (Syngene, UK).

For phylogenetic analysis, the 16S rRNA and *gyrB* PCR products were excised, purified using GeneJET Gel Extraction kit (Thermo Fisher Scientific) and sequenced by GeneWiz Europe (UK) using the 8F, 1544R and gyrBR primers (Table 1).

In addition to phylogenetic analysis, 16S sequence data was used to confirm that the bacteria that were recovered from the fish were the same isolates that were used in the trial.

2.6 Statistical analysis and graphics

Sequence data were aligned using Sequencher version 5.4.1 (Gene Codes, US), and graphed using ClustalX2 (Larkin et al., 2007) and MEGA 6.06 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Distance trees were constructed using the maximum likelihood algorithm in MEGA with 500 bootstraps; the number on each node indicates the percentage with which each topology branch was supported. The *gyrB* sequences used for the dendrogram shown were 926bp. GraphPad Prism 6.07 (GraphPad, US) was used to graph survival of fish and perform statistical analysis using the Mantel-Cox log-rank Chi square test, to analyze the differences in mortality between groups. Results were considered significant if $p<0.05$.

3. Results

3.1 Phylogenetic comparison of isolates

The 16S and gyrB genes were sequenced and compared between the lumpfish isolates (F6/15 and F57), salmon isolates (F112/17, K58 and F69/17), Arctic char isolates (F3/16 and F27/17) and rainbow trout isolates (F39/14, F3/15 and F192/16).

In the 1402bp 16S sequence, we found variation in 2 bases (bases 939C/T and 948 A/G) and of 1087bp gyrB sequence, we found variation in 11 bases (bases 29C/T, 44A/C, 284A/G, 323A/G, 365A/G, 601C/T, 623C/T, 647C/T, 1019C/T, 1061A/T and 1082A/G). The sequences from this study can be accessed as ClustalX2 alignment data files (http://dx.doi.org/10.17632/7c59prvkjz.2). No host-specific pattern was apparent from these data.

Comparison of the *gyrB* sequences to data published by Grove *et al.* (Grove et al., 2010) indicates that all Icelandic *M. viscosa* isolates are "variant type" (Figure 1, upper branch), which is a genetically different cluster from the species type strain (NCIMB 13548, not shown). However, analysis of whole *M. viscosa* genomes by Karlsen *et al.* (Karlsen, Hjerde, et al., 2017) indicates that variability in the *gyrB* gene may not be sufficient to define sublineages.

3.2 Virulence factors

F6/15 and F112/17 were screened for 23 known virulence factors, whose data for K58 (historical salmon isolate) and F57 (historical lumpfish isolate) had been published (Bjornsdottir et al., 2012; Karlsen et al., 2014; Tunsjø et al., 2011). Both F6/15 and F112/17 had the same virulence gene profile as the F57 isolate, while the K58 isolate differed in two virulence genes, for *mat* and *cnf* (Table 2).

3.3 Intraperitoneal infection (i.p.)

3.3.1 Lumpfish

100% mortality was observed in all i.p. infected lumpfish; after 24h and 7 days for the isolates F6/15 and F112/17, respectively (Fig 2A). No external symptoms were visible, but necropsy revealed ascites and organ hemorrhage. Bacteria could be cultured from both ascites and kidneys of all infected fish. No mortality was observed in the mock-infected group and *M. viscosa* could not be isolated from these fish.

While the data show that lumpfish are susceptible to both lumpfish- and salmon isolates of *M. viscosa* when infected i.p., it is not possible to conclude whether the lumpfish were more susceptible to the F6/15 isolate than the F112/17 isolate, as the F6/15 inoculum was higher and the route of infection is not natural.

3.3.2 Salmon

All salmon infected i.p. with F6/15 died within 7 days (Fig 2B). The fish displayed symptoms of lethargy, loss of balance and open wounds at the infection site and cardiac region. Some of the fish had ascites or internal hemorrhaging. Bacteria was cultured from both ascites and kidneys.

Salmon infected i.p. with F112/17 showed symptoms of lethargy and loss of balance. However, while inflammation was visible around the infection site, no open wounds were visible on the fish. Over a 9 day period, the fish all recovered and *M. viscosa* could not be isolated from the fish.

3.4 Bath infection

3.4.1 Lumpfish

No symptoms were visible on the lumpfish until 18 days post infection with F6/15, when moribund fish with external ulceration could be recovered (Fig 2C). Over the course of 10 days, additional lumpfish with external ulcers were recovered. The fish had ascites, but no behavioral changes or decrease in appetite could be observed. *M. viscosa* was isolated from all lumpfish with ulcers, and 47% of kidneys from fish without any external symptoms 27 days post-bath challenge. This indicates that lumpfish can be asymptomatic carriers of *M. viscosa*. Interestingly, none of the lumpfish infected with F112/17 showed ulcers or other symptoms throughout the experiment. We were not able to recover F112/17 isolate from the lumpfish.

3.4.2 Salmon

Early in the bath experiment, several salmon developed ulcers, regardless of whether the fish had been infected with *M. viscosa* or were uninfected. *Vibrio* species were recovered from these wounds. As the fish were further acclimatized, fewer ulcers were seen. *M. viscosa* was only recovered from the F6/15 bath groups, but not from the control groups or fish bath infected with F112/17. Most of the F6/15 bath challenged fish died without external symptoms (Fig 2D), but *M. viscosa* could be isolated from the kidney. *M. viscosa* was isolated from 26% of all F6/15 bath infected salmon remaining at the end of the experiment. This indicates that salmon are susceptible to the lumpfish isolate. Again, no *M. viscosa* could be isolated from salmon infected with the F112/17 isolate.

4. Discussion

The data in this study indicate that salmon can be infected with a virulent lumpfish isolate of *M. viscosa,* while lumpfish appeared to be resistant to colonization with the salmon isolate.

Vaccination protected the salmon against the salmon isolate of *M. viscosa* but did not confer sufficient protection to prevent infection with the lumpfish isolate. The infected salmon died mostly without symptoms of disease, and while it is possible that the vaccine ameliorated disease, it is also possible that the lack of typical disease symptoms was an artifact of the infection model. Lunder *et al*. reported that experimentally infected fish died of sepsis and organ dysfunction while mortality in field outbreaks was low compared to fish displaying ulcers (T. Lunder, 1992). Experimental infection comparing vaccinated and unvaccinated salmon may further elucidate this.

We did not find any difference in the putative virulence genes carried by the two lumpfish isolates, even though F6/15 was isolated from a lumpfish with severe winter ulcer disease and F57 was isolated from a healthy lumpfish. This could suggest that the difference in virulence between the isolates is environmental rather than genetic, as F6/15 was isolated from a farmed lumpfish and F57 was isolated from a wild lumpfish. The only difference in the virulence genes screened was between the two salmon isolates, isolated approximately 25 years apart in Iceland. Whole genome sequencing may better elucidate the differences between the various isolates.

It also remains to be examined whether lumpfish can transmit *M. viscosa* to salmon in cohabitation. Vaccinating lumpfish may prevent transmission, given that lumpfish isolates are a component of the vaccine. However, vaccination of lumpfish against *M. viscosa* has not elicited high antibody titers against the bacterium (Erkinharju et al., 2017), although antibody levels may not be a good indicator of protection (Karlsen, Thorarinsson, Wallace, Salonius, & Midtlyng, 2017). While it remains to be demonstrated whether vaccination can protect lumpfish against *M. viscosa*, rigorous screening of lumpfish for subclinical infection and screening of dead salmon removed from sea pens may be the most effective preventative measure.

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6. Tables

Table 1. PCR primers used in the study

	Gene	Forward primer	Reverse primer	Product size	Reference
Virulence factors	*bfrA*	AACTATGAAAGGCAACAGTAA	AAGCACAACAACAGCCACAG	1011	Karlsen 2014
	mat	GGCTGGAAACAAGTGGTGTT	TCAATATTCCCAGCGAAAGG	1304	Karlsen 2014
	pld	GGCTGGGTTGATTGGAAAT	CCGCAAACCTAAATGGAAAA	1968	Karlsen 2014
	inv	AGGATATTTAAATGCCCGATT	TTCGGACGTTGTTGTCACAT	1257	Karlsen 2014
	aer	CCTCCAAGGTGATGGAAGAA	AACAACTGCCTAAGGGATACCA	1362	Karlsen 2014
	hcp-clpV1	TTCTTGCCATGTGTTACCGA	ATGCTGTTTTTGCGATAGGG	616	Karlsen 2014
	hemG	CATCCTGCCTTCGAATGTTT	ATGGGTTCTAGGTGGTCGTG	484	Karlsen 2014
	cnf	AATGTTAGTTGCCGCCGTAG	CCGAAACCAACGTCAGAAAT	1390	Karlsen 2014
	rtxA	GGCCGGTCAAAATGGTATTA	ATCAAGCTGAACGACCACACT	884	Karlsen 2014
	mitA	ACACATGAAATCAATGTCAA	TCCATTTGAAGGTGTTCATCA	1112	Karlsen 2014
	mitC	CGGAGCAAACCAATACACCT	CAATTTACTGTTGTCCATGCTG	1667	Karlsen 2014
	mts1-M	CAGGTATGGTCGCTATGAGTG	TCTCGAGCTGGTTTGGTAAA	136	Bjornsdottir 2012
	mts2-M	AACTCAAGGTCATATTACTAAAGATGC	GTGGATCTCTTGGCACTGTT	128	Bjornsdottir 2012
	flaA	TGACTTTGCGGCTGAATCG	GATTGCTGATTTGCTTGACTAAGC	90	Tunsjo 2011
	lafA	TCGCAACACGTTTACAAACTCA	GCCTTCTGCAGTTTGCATC	92	Tunsjo 2011
	flpA	GGTGGTTTGTTATCGGTGTTCTATT	CCATAAGGCAAACCACATTCATC	86	Tunsjo 2011
	tapA	ATGTTGGTGTGACATATGCGTTAA	CAGTTTTAGCTCCCCAAGTTAGCT	69	Tunsjo 2011
	protM4	TTGATATTGTCGCCCATGAAGT	CTGCAGTTTCACCTGCCATGT	120	Tunsjo 2011
	protM6	AAATGCAGCGCATGCAAA	CGGCCAGCTTATCCTCATTAAC	62	Tunsjo 2011
	llp	TGGCATCCCTCATGCGTTA	TGCAGAAACCTGAGCAACGT	61	Tunsjo 2011
	plp	AACGTTCGACCCGCGTATTA	CGATGTTTTCACCACCCGATA	62	Tunsjo 2011
	mvp1	CGCCTCGGATTGCAGAGA	GCCGGTGCACTGAGCATT	58	Tunsjo 2011
	vah1	GCAGCCCAGAAGACCCTAATG	TGGTCAGCGGTGCAGGTA	72	Tunsjo 2011
Phylogenetic analysis	*16S*	AGAGTTTGATCCTGGCTCAG	AGAAAGGAGGTGATCCAGCC	1537	Beneduzi 2010
	gyrB	CAGGATCCATGGTTGTTTCC	TATTGGCGATACCGATGATG	2195	This study

Table 2. Screening of virulence factors

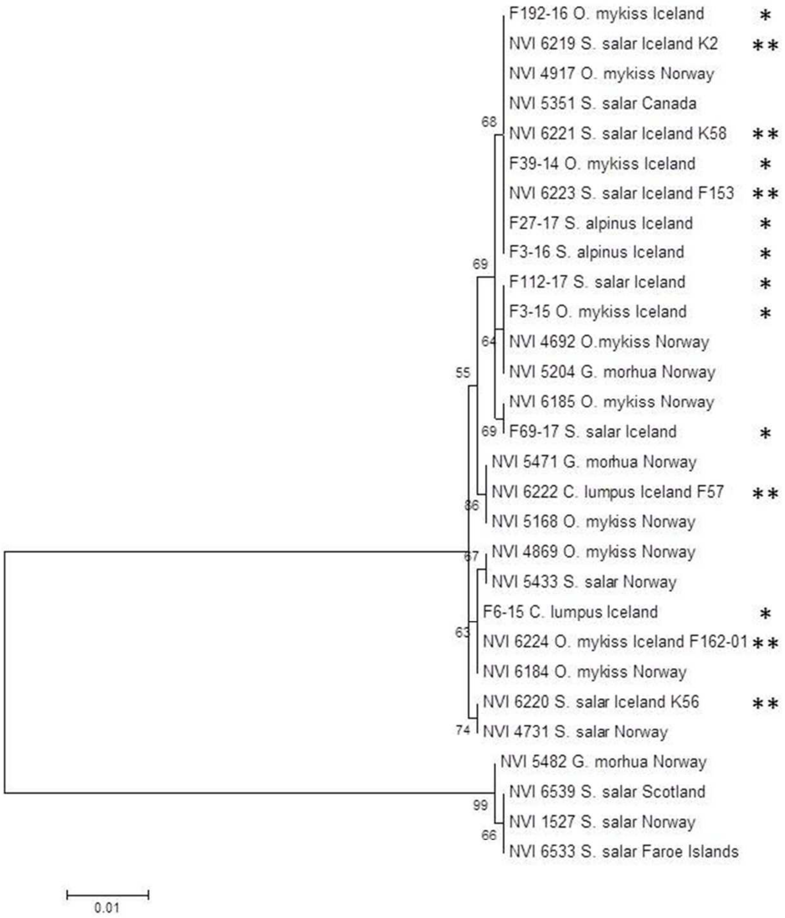
Putative virulence genes	Lumpfish		Atlantic salmon		Expected product size (bp)
	F6/15	F57	F112/17	K58	
flaA	+	+	+	+	90
lafA	+	+	+	+	92
flpA	+	+	+	+	86
tapA	+	+	+	+	69
protM4	+	+	+	+	120
protM6	+	+	+	+	62
llp	+	+	+	+	61
plp	+	+	+	+	62
mvp1	+	+	+	+	58
vah1	+	+	+	+	72
bfrA-B	+	+	+	+	1011
mat	+	+	+	-	1304
pld	+	+	+	+	1968
inv	+	+	+	+	1257
aer	+	+	+	+	1362
hcp-clpV1	+	+	+	+	616
hemG	+	+	+	+	484
cnf	-	-	-	+	1390
rtxA	+	+	+	+	884
mitA	-	-	-	-	1112
mitC	-	-	-	-	1667
mts1	+	+	+	+	136
mts2	+	+	+	+	128

+ = gene present; - = gene not detected

7. Figure legends

Figure 1. Phylogenetic relationship of *M. viscosa* isolated from different fish species. Shown is the phylogenetic relationship of 8 recent isolates from Iceland (labeled with *), and representative data from older Icelandic isolates (**) and other countries (unlabeled) published by Grove *et al*. (Grove et al., 2010), based on the *gyrB* sequence. The tree was generated in MEGA 6.06 using the maximum-likelihood algorithm. Bootstrap values shown at the nodes are derived from 500 replicates and branch lengths corresponding to the divergence of sequences are indicated by the relative scale (scale bar).

Figure 2. Survival rate of *M. viscosa* infected lumpfish and salmon. Figures A) and B) are i.p. infected fish, while C) and D) are bath infected. Figures A) and C) are lumpfish, while B) and D) are salmon. The solid lines are fish infected with F6/15 (lumpfish isolate), while the broken lines are fish infected with F112/17 (salmon isolate). The mortality data includes death caused by *M. viscosa*, as well as other causes, such as asphyxiation after jumping from the containers. Mantel-Cox log-rank chi square test was performed to test significant differences in survival between groups. $p<0.05$ was considered significant (*) and $p<0.005$ was considered highly significant (**).



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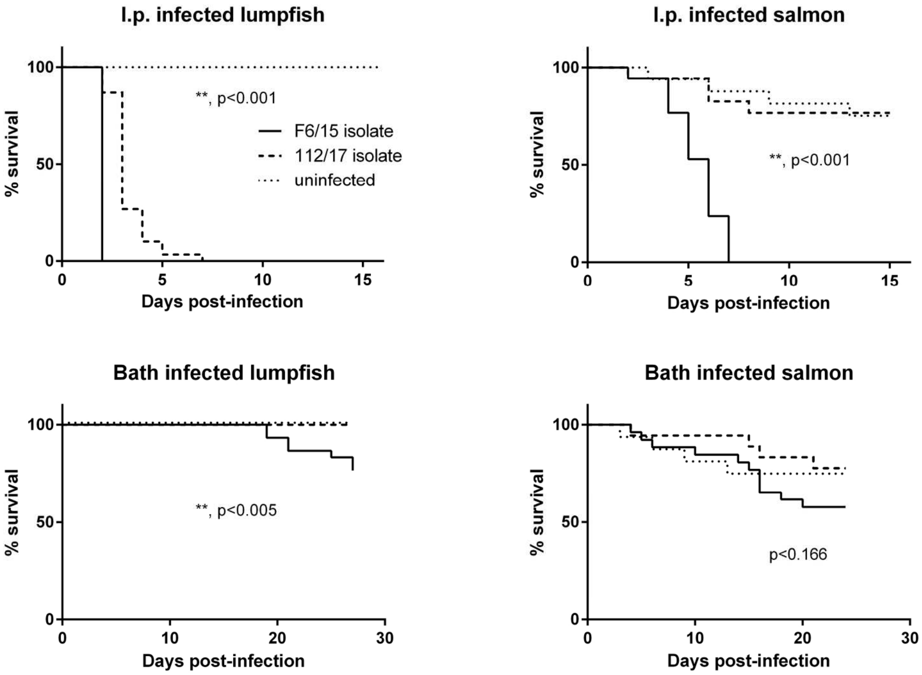
190x254mm (96 x 96 DPI)



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189x137mm (300 x 300 DPI)