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First isolation, identification and characterisation of *Tenacibaculum maritimum* in Norway, isolated from diseased farmed sea lice cleaner fish *Cyclopterus lumpus* L

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ABSTRACT

The use of cleaner fish as biological controls of salmon lice (*Lepeophtheirus salmonis*) has increased exponentially in the last decade in Norwegian Atlantic salmon (*Salmo salar*) production. This alternative to chemical treatments has resulted in the emergence of lumpsucker (*Cyclopterus lumpus*) hatcheries and culture facilities in Norway. It has been shown that the use of lumpsuckers can be an effective, biological approach for the removal of salmon lice, but it has also been shown that there are a number of biological challenges (i.e. parasites and bacteria) with the production and use of these fish. This study describes the first case of isolation of *Tenacibaculum maritimum*, a significant fish pathogen worldwide, in cultured juvenile lumpsuckers in Norway. The fish were lethargic and showed skin lesions characterised by increased mucus production and presence of whitish necrotic tissue especially in the head region. Skin scrapings revealed large amounts of bacteria dominated by rod-shaped *Tenacibaculum*-like bacteria, which were shown to be closely related to *T. maritimum* type strain through genetic and phenotypic characterisation. Histopathological analysis showed that the bacteria was closely associated with the pathology and therefore could be contributing to the disease and/or mortality.

Statement of relevance: This is the first isolation of *Tenacibaculum maritimum* in Norway and in lumpsuckers, a major aquaculture pathogen worldwide. There is a need for increased knowledge of the biological challenges facing cultured lumpsuckers, as this species is being used in increasing number by the Norwegian salmon industry.

1. Introduction

Cleaner fish (i.e. wrasse, *Labridae* spp. and lumpsucker, *Cyclopterus lumpus*) have become an important non-chemical sea lice control for the Norwegian salmon farming industry. The use of lumpsuckers has become more popular recently and is now being farmed to meet the growing demands of the industry. It is estimated that 15–20 million lumpsuckers will be produced during 2016 (Bornø et al., 2016). The use of lumpsuckers as cleaner fish is advantageous in that they show greater temperature range tolerance over wrasse (Sayer and Reader, 1996; Imsland et al., 2014). As with all species of fish, lumpsuckers have health and welfare issues that are continually being discovered. During 2015 several cases of acute mortalities were reported during late summer and autumn, mainly following sea transfer (Bornø et al., 2016). Causes of reported mortalities are often unclear, but bacterial infections are the most common diagnostic finding (Nilsen et al., 2014; Bornø et al., 2016). So far, the following bacterial fish pathogens have been associated with disease in lumpsuckers: atypical *Aeromonas salmonicida*, *Vibrio anguillarum*, *Pasteurella* sp., *Vibrio ordalii*, *Moritella viscosa*, *Tenacibaculum* spp. and *Pseudomonas anguilliseptica* (Bornø and Lie, 2015; Gulla et al., 2015b; Alarcón et al., 2016; Bornø et al., 2016). The importance of other frequently recovered bacteria such as *Aliivibrio logei*, *Aliivibrio wodanis*, *Vibrio tapetis* and *Vibrio splendidus* is still unclear (Gulla et al., 2015a; Bornø et al., 2016).

Tenacibaculum maritimum is a Gram-negative, gliding bacterium that causes tenacibaculosis, an ulcerative disease of marine fish (Wakabayashi et al., 1986) and is a major cause of economic loss in mariculture worldwide. Tenacibaculosis is associated with lesions such as ulcers, necrosis, eroded mouth, frayed fins and tail-rot (Santos et al., 1999; Toranzo et al., 2005). *T. maritimum* has been isolated from a variety of marine species including red sea bream *Pagrus major* and black sea bream *Acanthopagrus schlegeli* in Japan (Wakabayashi et al., 1986), yellowtail *Seriola quinqueradiata* in Japan (Baxa et al., 1988), Dover sole *Solea solea* in Scotland (Bernardet et al., 1990), turbot *Scophthalmus maximus* in Spain (Alsina and Blanch, 1993), wedge sole *Dicologoglossa*

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cuneate in Spain (López et al., 2009), sea bass *Dicentrarchus labrax* in France (Bernardet et al., 1994), Atlantic salmon *Salmo salar* and barramundi *Lates calcarifer* in Australia (Soltani et al., 1996), Pacific sardine *Sardinops sagax* in Western USA (Chen et al., 1995), Atlantic salmon in Western Canada (Ostland et al., 1999), and puffer fish *Takifugu rubripes* in Japan (Rahman et al., 2014). The bacteria has also been isolated from sea lice *Lepeophtheirus salmonis* found on Western Canadian Atlantic salmon (Barker et al., 2009). Even though *Tenacibaculum* spp. have been isolated from diseased lumpsuckers and other fish species in Norway, *T. maritimum* has never been isolated (Olsen et al., 2011).

This study describes the first isolation of *T. maritimum* associated with disease in Norwegian aquaculture. Several identical isolates, designated *Tenacibaculum* sp. strain NLF-15, were recovered from diseased lumpsuckers. Lumpsuckers hatched in mid-April 2015, were transported to an on-growing farm in Norway in September and kept in indoor tanks with running sea water at a temperature around 12 °C. Signs of disease first appeared in mid-September when the fish showed loss of appetite, became lethargic and skin lesion in the areas around the eyes, on the head and bone nodules emerged. The lesions were characterised by increased mucus production and presence of whitish necrotic tissue. The disease spread to all tanks, the mortality was high, and >150,000 fish died in the period of September to November.

2. Material and methods

2.1. Examination of diseased fish and isolation of bacteria

Skin scrapings were collected from freshly diseased fish and smears from affected parts of the skin were stained using the color rapid-set from Lucerna-Chem (Lucerne, Switzerland) and examined under a light microscope at 100× magnification. Bacterial samples were collected from the head region of diseased fish and grown on Marine Agar (MA) (Difco 2216) plates. Sub-cultivation was performed on MA plates at 16 °C for 72 h and the isolates were preserved in CryoTube™ vials (Thermo scientific) at −80 °C.

The skin, kidney and liver of the fish were tested using real time RT-PCR by an accredited Norwegian commercial diagnostic laboratory for the presence of *Tenacibaculum finnmarkense*, *Pasteurella* sp., *Vibrio anguillarum*, atypical *Aeromonas salmonicida*, *Pseudomonas anguilliseptica*, *Nucleospora cyclopteri* and *Paramoeba* spp.

Additional bacterial sampling was performed from frozen lumpsuckers from the same outbreak using MA plates and blood agar (BAS) plates containing 1.5% NaCl.

2.2. Genetic and phylogenetic analysis

Genomic DNA was extracted using the DNeasy® blood and tissue kit (Qiagen) following the cultured cells Quick-start protocol. PCR was performed using the 16S rRNA primers 27F and 1518R (Giovannoni et al., 1996), the *T. maritimum* specific primers: MAR1 and MAR2 (Toyama et al., 1996), and Mar1 and Mar2 (Bader and Shotts Jr, 1998), and primers designed for 11 Housekeeping (HK) genes (Habib et al., 2014). The target genes and primer sequences are listed in Table 1. Amplification was based on a standard reaction mixture containing 2.5 μL Extra buffer, 1.25 mM deoxyribonucleotide triphosphates, 0.75 units (0.15 μL) *Taq* DNA polymerase (BioLabs, New England), 5 μM (1 μL) of forward and reverse primers; DNase-RNase free water was added to a final volume of 25 μL (16.85 μL H_2O). Amplification using the 16S rRNA primers B27F and A1518R was performed at 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 90 s, followed by 72 °C for 10 min. Amplification using the primer pairs MAR1 and MAR2 and Mar1 and Mar2 was performed as described by Toyama et al. (1996) and Bader and Shotts Jr (1998), respectively. Amplification using the HK genes primers listed in Table 1 was performed at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C (50 °C for primers: *glyA*, *infB*, *tgt*, *tuf* and *yqfO*) for 30 s, and 72 °C for 1 min, followed by 72 °C for 10 min.

Table 1
List of PCR primers used in present study.

Target gene	Name	Sequence (5′–3′)	Source
16S rRNA	B27F	AGAGTTTGATCMTGGCTCAG	Giovannoni et al. (1996)
16S rRNA	A1518R	AAGGAGGTGATCCANCCRCA	Giovannoni et al. (1996)
16S rRNA	MAR1	AATGGCATCGTTTTAAA	Toyama et al. (1996)
16S rRNA	MAR2	CGCTCTCTGTTGCCAGA	Toyama et al. (1996)
16S rRNA	Mar1	TGTAGCTTGCTACAGATGA	Bader and Shotts Jr (1998)
16S rRNA	Mar2	AAATACCTACTCGTAGGTACG	Bader and Shotts Jr (1998)
atpA	fwd	ATTGGWGAYCGTCAAACWGG	Habib et al. (2014)
atpA	rev	CCAAAYTTAGCRAAHGCTTC	Habib et al. (2014)
dnaK	fwd	GGWACYACNAAYTCDTGTGT	Habib et al. (2014)
dnaK	rev	TCWATCTTMGCTTTYTCAGC	Habib et al. (2014)
glyA	fwd	CAYTTAACWCAYGGWTCDCC	Habib et al. (2014)
glyA	rev	ACCATRTTTTTRTTTACHGT	Habib et al. (2014)
gyrB	fwd	AGTATYCARGCRCTRGAAGG	Habib et al. (2014)
gyrB	rev	GTWCCTCCTTCRTGYGTRTT	Habib et al. (2014)
ileS	fwd	CCWACHTTTGGWGCHGAYGA	Habib et al. (2014)
ileS	rev	GAATCRAACCAWACATCAAT	Habib et al. (2014)
infB	fwd	ATGCCDCAAACWAAAGARGC	Habib et al. (2014)
infB	rev	GTAATHGCTCCAACYCCTTT	Habib et al. (2014)
rlmN	fwd	GCKTGTGTDTCDAGYCARGT	Habib et al. (2014)
rlmN	rev	CCRCADGCDGCATCWATRTC	Habib et al. (2014)
tgt	fwd	GAAACWCCWATWTTYATGCC	Habib et al. (2014)
tgt	rev	TAYAWYTCTTCNGCWGGTTC	Habib et al. (2014)
trpB	fwd	GTWGCNCGWATGAAAATGYT	Habib et al. (2014)
trpB	rev	CCWGGRTARTCYAATCCTGC	Habib et al. (2014)
tuf	fwd	AGAGAWTTATTRTCTTTCTA	Habib et al. (2014)
tuf	rev	GTTACCTGACCWGCWCCWAC	Habib et al. (2014)
yqfO	fwd	GCBGAARRTTTTGAYAAYGT	Habib et al. (2014)
yqfO	rev	AYTTCRTARGCDACYTCTTC	Habib et al. (2014)

The PCR product was confirmed by gel electrophoresis and enzymatically purified using ExoStar 1-Step® (GE Healthcare Bio-Sciences Corp) in an Arktik Thermal Cycler (Thermo Scientific) at 37 °C for 15 min and at 80 °C for 15 min. The sequencing reaction was performed using a BigDye® version 3.1 reaction in an Arktik Thermal Cycler, at 96 °C for 5 min, 25 cycles of 96 °C for 10 s, 58 °C for 5 s, and 60 °C for 4 min. The reaction was composed of a mixture of 5.5 μL deionised water, 1 μL BigDye® Terminator 3.1 version sequencing buffer, 1 μL BigDye Terminator 3.1 version Ready Reaction Premix (2.5X) (Invitrogen), 3.2 pmol (1 μL) forward and reverse primers and 1.5 μL purified PCR product. Sequencing was carried out by the Sequencing Facility at Høyteknologisenteret i Bergen (http://www.uib.no/seqlab). Samples were cleaned with Agencourt CleannSeq (Beckman Coulter, Inc.) before being sequenced in a 96-capillary 3730xl DNA Analyzer (Applied Biosystems). Vector NTI® (Invitrogen) was used to assemble and align the obtained sequences. *T. maritimum* NCIMB 2154^T was used as a positive control in all *T. maritimum* specific PCR and *Tenacibaculum ovolyticum* NCIMB 13127^T was used as a negative control in the MAR1 and MAR2, and Mar1 and Mar2 PCR.

In the current study, alignments of 16S rRNA genes (1351 base positions) and concatenated HK gene sequences of 5811 positions (*atpA* 1-567, *dnaK* 568-1140, *glyA* 1141-1698, *gyrB* 1699-2295, *ileS* 2299-2841, *infB* 2842-3405, *rlmN* 3406-3954, *tgt* 3955-4440, *trpB* 4441-4809, *tuf* 4810-5364 and *yqfO* 5365-5811) were constructed for phylogenetic analysis. The 16S rRNA gene sequence alignment included the sequence of the *Tenacibaculum* sp. strain NLF-15 and sequences from all type strains in genus *Tenacibaculum*. The HK gene sequence alignment included concatenated sequences of *Tenacibaculum* sp. strain NLF-15 and 18 type strains in genus *Tenacibaculum.* Concatenation of the HK gene alignments was performed using Kakusan4 (Tanabe, 2011). All alignments were constructed in AlignX in Vector NTI® (Invitrogen) before sequences were adjusted to equal length and correct reading frames in GeneDoc (Nicholas et al., 1997). The best fitted evolutionary model for each alignment was calculated using Mega 6 (Tamura et al., 2013). The BEAST package v1.8 (Drummond and Rambaut, 2007) was used for Bayesian analysis of the 16S rRNA gene sequence dataset using the K2 + G + I model, a relaxed lognormal molecular clock and a mcmc of 100,000,000 generations. *Kordia algicida*^{T} (GenBank

accession nr: AB681152) was used as the outgroup. Kakusan4 was used for calculation of substitution rate and best fit model for the individual loci and codon positions for Bayesian analysis of the concatenated HK gene alignment. The Bayesian phylogenetic analysis of the HK gene dataset was conducted in MrBayes (Ronquist et al., 2012) using the data block with the proportional codon proportional model from Kakusan4 and a mcmc of 100,000,000 generations. The Effective Sample Size values (ESS) in the Bayesian analysis were inspected using Tracer ver. 1.6 (Rambaut et al., 2014). All ESS values were within the recommended range (above 200) for all parameters. A maximum clade credibility tree was obtained using a 10% burn-In in Tree-Annotator and viewed using FigTree (Drummond et al., 2012). For 16S rRNA gene sequence similarity analysis, Percent Nucleotide Identity (PNI) was calculated using the distance matrix option in BioEdit. In the Average Nucleotide Identity (ANI) calculations, the sequences of *T. maritimum* NCIMB 2154^T and *Tenacibaculum* sp. strain NLF-15 from the concatenated HK alignment was uploaded and analyzed using the Average Nucleotide Identify option in EzGenome (Kim et al., 2012). The 16S rRNA sequences and the HK gene sequences of the type strains were obtained from GenBank. All sequences obtained in the current study are available in GenBank with accession numbers KU885458 to KU885469.

For determining if *Tenacibaculum* sp. strain NLF-15 belonged to a known sequence type (ST) of *T. maritimum*, the MLST profile that consisted of seven HK gene sequences (*atpA*, *gyrB*, *dnaK*, *glyA*, *infB*, *rlmN* and *tgt*), were uploaded and analyzed in the *Tenacibaculum* MLST databases found at the Multi Locus Sequence Typing website (http://pubmlst.org/tenacibaculum/) (Jolley and Maiden, 2010).

2.3. Histology and scanning electron microscopy (SEM)

Collected tissues (gills and skin) were fixed by immersion, at 4 °C, in a modified Karnovsky fixative where the distilled water was replaced by a Ringers solution. The fixative contained 4% sucrose. For histology, semithin sections (1.0 μm) were cut on a Reichert-Jung Ultracut E and stained using toluidine blue. The fixed tissues were also post-fixed in 2% OsO_4 for 60 min, washed in PBS, dehydrated through acetone, and critical-point dried using liquid CO_2 as the transitional fluid. The dried tissues were mounted by means of double-stick carbon tape on SEM stubs and sputter-coated with gold/palladium alloy. Specimens were examined at 15 kV with a ZEISS Supra 55VP scanning electron microscope.

2.4. Phenotypic analysis

All phenotypic tests were performed on cultures incubated at 16 °C for 72 h on MA unless otherwise stated. The colony morphology and ability to stick to agar was investigated on MA. The cell morphology was investigated using light microscopy. Presence of flexirubin type pigments was determined by the bathochromic shift test using a 20% (w/v) KOH solution (Fautz and Reichenbach, 1980). The Gram reaction was performed with a Fluka 77730 Gram Staining Kit (Fluka® analytical) following the manufacturer's protocol and the non-staining KOH method (Buck, 1982). The oxidase reaction was determined using BBL™ DrySlide Oxidase, following the manufacturer's protocol. Catalase activity was examined using the slide (drop) method following the protocol by Reiner (2010). Production of H_2S was detected by taping a lead acetate impregnated paper strip (Sigma) to the inside of the lid of MA plates, using Parafilm M® to seal lid and plate. The plates were incubated at 16 °C for 7 days. Growth at temperatures of 8, 16 and 19 °C was determined on MA plates incubated for 7 days.

3. Results

3.1. Examination of diseased fish and isolation of bacteria

The examined freshly diseased lumpsuckers had skin lesions in the areas around the eyes and on the head that were characterised by large amounts of mucus and the presence of whitish necrotic tissue. Examination of skin scrapings from the lesions revealed large amounts of bacteria dominated by long (5–8 μm) and thin rod-shaped, *Tenacibaculum*-like, bacteria (Fig. 1). Colonies grown on MA were pale and translucent. They had uneven edges and adhered to each other. All MA plates showed identical *Tenacibaculum*-like colonies, which constituted most of the bacterial growth present. The examined fish showed no gross pathology internally.

The results from the qPCR screening were negative for the presence of *Tenacibaculum finnmarkense*, *Pasteurella* sp., *Vibrio anguillarum*, atypical *Aeromonas salmonicida*, *Pseudomonas anguilliseptica*, *Nucleospora cyclopteri* and *Paramoeba* spp. The following bacteria were recovered on MA and/or BAS from frozen material: *Nonlabens marinus*, *Loktanella salsilacus*, *Psychrobacter* sp., and *Polaribacter* sp.

3.2. Genetic and phylogenetic analysis

The recovered isolates from the diseased lumpsuckers displaying *T. maritimum* morphology, amplified with the *T. maritimum* specific primers, showed distinct bands at 1088 bp for MAR1 and MAR2 and 400 bp for Mar1 and Mar2 as shown in Fig. 2. Sequencing of these isolates using the 16S rRNA primers B27F and A1518R showed that all sequenced isolates were identical and were designated as *Tenacibaculum* sp. strain NLF-15. The ANI and PNI of strain NLF-15 and *T. maritimum* NCIMB 2154^T were 98.73% and 99.1%, respectively, which means that the isolates belong to the *T. maritimum* species (Stakebrandt and Ebers, 2006; Goris et al., 2007; Richter and Rosselló-Móra, 2009; Kim et al., 2014).

Both the phylogenetic trees based on the 16S rRNA gene sequences (Fig. 3) and HK genes sequences (Fig. 4) shows that *Tenacibaculum* sp. strain NLF-15 forms a distinct branch together with *T. maritimum* NCIMB 2154^T separate from other *Tenacibaculum* type strains. No exact ST match was found for *Tenacibaculum* sp. strain NLF-15 in the MLST database, suggesting that strain NLF-15 is a unique *T. maritimum* strain.

3.3. Histology and scanning electron microscopy

Histology of the skin in the affected areas revealed large amounts of bacteria dominated by two different morphologies, which was also shown in the scanning micrographs (Fig. 5). The bacteria seem to attack the epithelia at the bone nodules in the skin of the lumpsuckers. In the areas around these bone nodules the epithelium was completely lost and substituted by a thick layer of filamentous bacteria (Fig. 6 and Supplementary Fig. 1) in some specimens. Another, rod-shaped bacterium, formed colonies within the layer of the filamentous bacteria (Fig. 7). The gills were not significantly affected, but large patches of mixed bacteria,

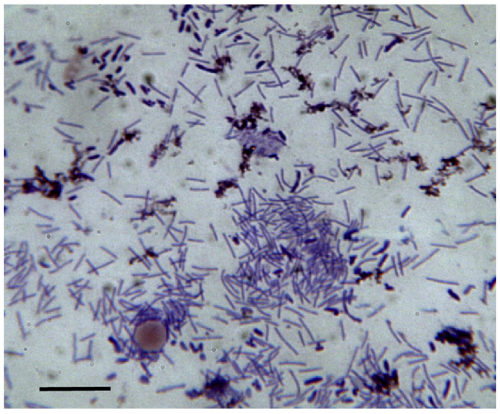

Fig. 1. Skin scrapings from moribund lumpsucker showing presence of different morphologies of bacteria dominated by rod-shaped *Tenacibaculum*-like bacteria. Bar = 10 μm.

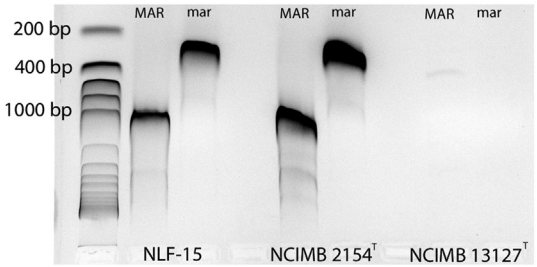

Fig. 2. Gel electrophoresis showing distinct bands at 1088 bp for MAR1 and MAR2 (Toyama et al., 1996) and 400 bp for Mar1 and Mar2 (Bader and Shotts Jr, 1998) for *Tenacibaculum* sp. strain NLF-15 and *T. maritimum* NCIMB 2154^T. A negative reaction is shown for *T. ovolyticum* 13127^T.

mainly consisting of *Tenacibaculum*-like, were infrequently found between the secondary lamellas.

3.4. Phenotypic analysis

All phenotypic characteristics recorded, except growth temperature for *Tenacibaculum* sp. strain NLF-15 are what is described for *T. maritimum* (Wakabayashi et al., 1986; Suzuki et al., 2001). Growth was observed for both the *Tenacibaculum* sp. strain NLF-15 and *T. maritimum* NCIMB 2154^T at all temperatures; however at 8 °C the growth was slow and limited compared to what was observed at 16 °C and 19 °C. Cells were gram-stain negative and were between 2 and 30 μm in size, but most were in the 4–10 μm range. The colony morphology was pale, translucent and circular and adhered to the agar. They were positive for oxidase and catalase, and negative for flexirubin type pigments and production of H_2S.

4. Discussion

Based on the isolation of *T. maritimum* from the diseased lumpsuckers and that the bacteria was closely associated with the pathology seen histologically it is likely that the cause of morbidity or mortality observed on the farm was at least partially due to tenacibaculosis. This bacteria is believed to facilitate the entrance of other bacteria, as was seen in both the electron microscopy and histopathology, and is often found in mixed infections with *Vibrio* sp. and motile and non-motile *Aeromonas* sp. (Kimura and Kusuda, 1983; Yardimci and Timur, 2015). In this case, the other bacteria observed were not identified. The molecular screening showed that these tissues were negative for known pathogenic bacteria. This was later confirmed with the additional bacteriology performed on frozen specimens, which showed environmental species not linked to disease in Atlantic salmon or lumpsuckers. However, the freezing of these specimens may have affected the bacterial species recovered.

Skin lesions, tail-rot and white patches on the skin of lumpsuckers are commonly associated with *Pasteurella* sp. infections (Alarcón et al., 2016); however, in one reported high mortality event in Norway

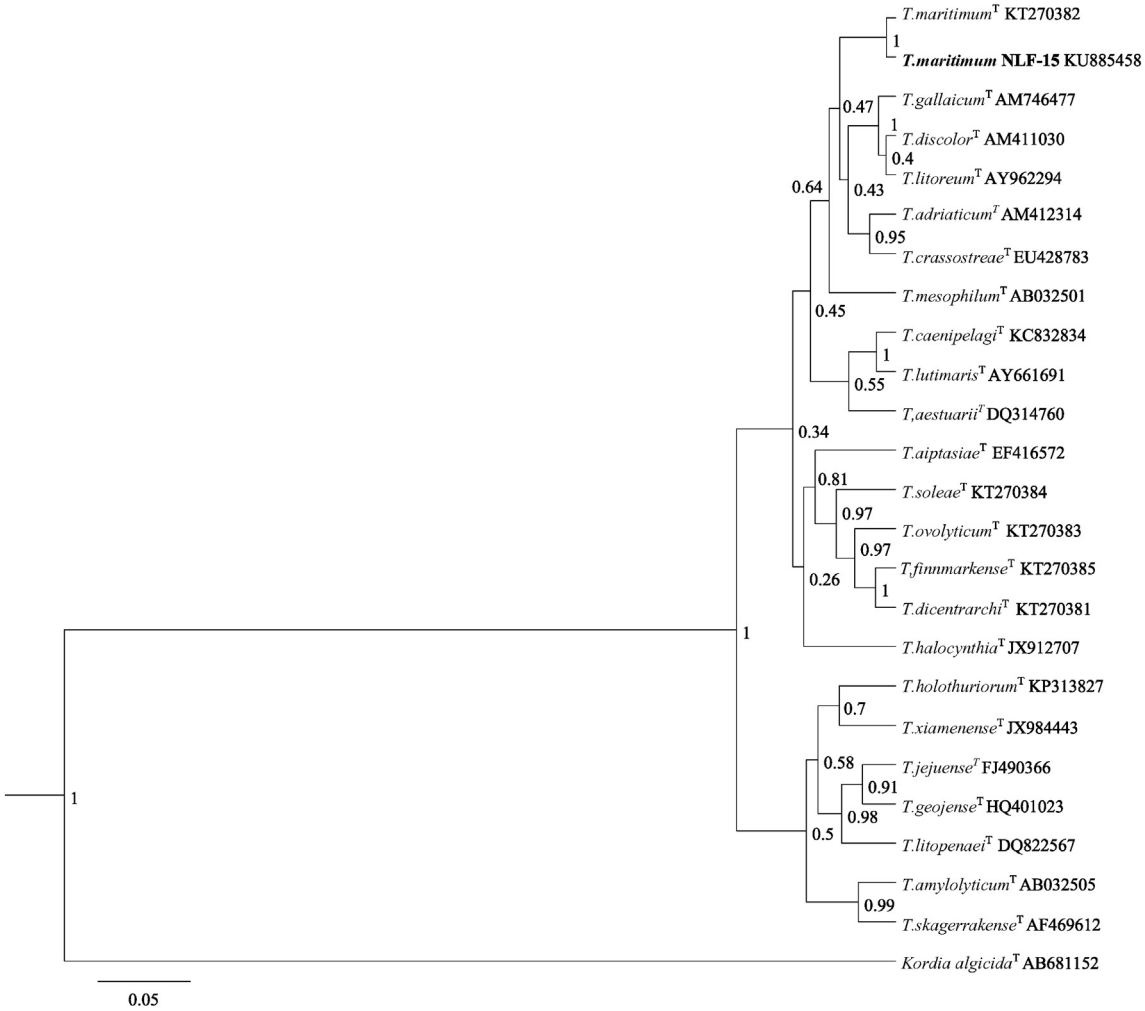

Fig. 3. The phylogenetic position of *T. maritimum* strain NLF-15 and all type strains in genus *Tenacibaculum* based on the 16S rRNA gene sequences. *Kordia algicida*^{T} was used as the outgroup. Accession numbers are shown after the strain name. Scale bar = 0.05 substitutions per site.

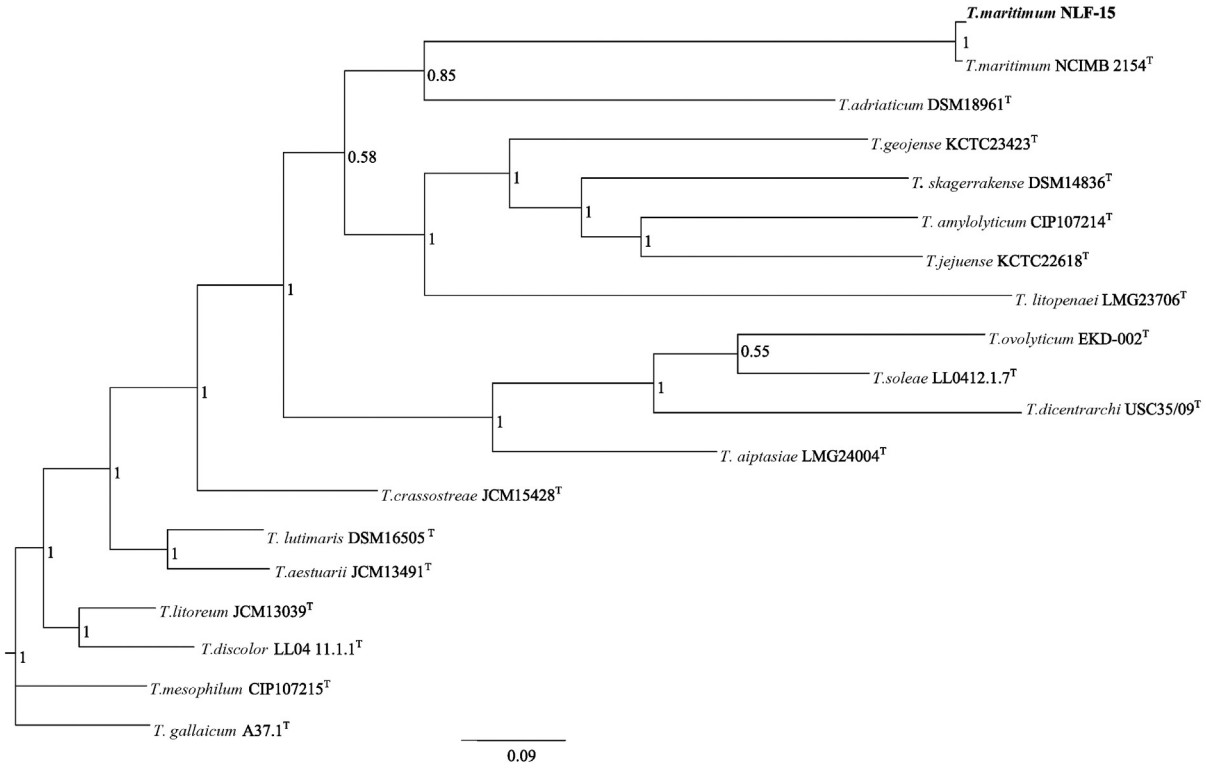

Fig. 4. The phylogenetic relationship of *T. maritimum* strain NFL-15 and 18 type strains in genus *Tenacibaculum* based on the concatenated HK genes sequences. The posterior probability is presented next to each node. Scale bar = 0.09 substitutions per site.

where fish had white patches around the eyes, *Vibrio ordalii* was found to be the associated pathogen (Bornø et al., 2016). White patches on the head and around the eyes were also observed in this case, but the prevalent bacteria present were *T. maritimum* and the tissues were negative for *Pasteurella* sp. with real time RT-PCR. The lack of detection of *T. maritimum* in the mentioned cases may be due to the absence of the bacteria or due to the fact that *T. maritimum* may be difficult to culture and requires the use of agar that contains sea salt (i.e. MA and Flexibacter Maritimus Media (FMM)), which is not routinely used for primary cultures.

T. maritimum has the potential to cause high mortality in susceptible species with juvenile fish being more predisposed and the prevalence and severity of the disease tends to increase with higher temperatures (>15 °C) (Toranzo et al., 2005). As most salmon production in Norway occurs at lower temperatures than this, the environment may not be optimal for the growth of *T. maritimum* and could be the reason that *T. maritimum* caused tenacibaculosis is not significant in this region. In addition to water temperature, the disease is influenced by environmental and host-related factors (Magariños et al., 1995). Stressful events such as handling and transport in lumpsuckers is therefore likely a major contributing factor to disease susceptibility as high mortality often follows such events (Alarcón et al., 2016). This could be a result of skin abrasion facilitating the entrance of pathogens.

T. maritimum has been isolated from diseased Atlantic salmon both in Western Canada and Australia and other *Tenacibaculum* spp. have been isolated and shown to cause disease in Atlantic salmon in Norway (Olsen et al., 2011; Småge et al., 2015). The same ST of *T. maritimum* has been found to infect multiples species of fish in the same geographical area, which suggest that there is a possibility of cross-species contamination in a farm (Habib et al., 2014). This could therefore have a negative impact, not only on the lumpsuckers, but also to the Atlantic salmon. Transmission of a pathogen from lumpsuckers to Atlantic salmon or *vice versa* was also suggested by Haugland et al. (2016) for *Paramoeba perurans* (syn. *Neoparamoeba*, see Feehan et al. (2013)) the causative agent of amoebic gill disease (AGD) (Crosbie et al., 2012).

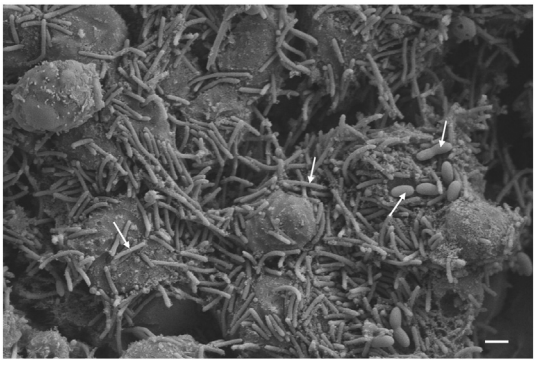

Fig. 5. Scanning micrograph of the skin from a moribund lumpsucker showing the presence of two dominating morphologies of bacteria (arrows). Bar = 2 μm.

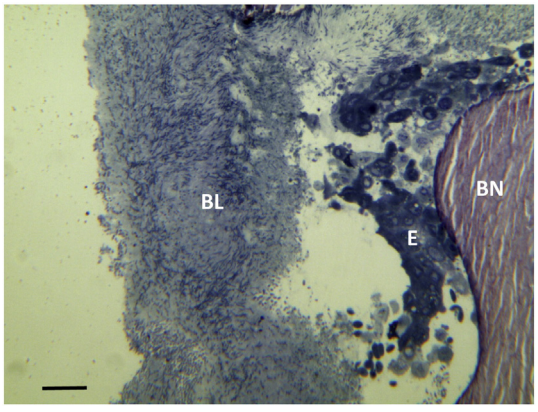

Fig. 6. Loss of skin epithelium (E) in an area around a bone nodule (BN) covered by a thick layer of bacteria (BL). Bar = 50 μm.

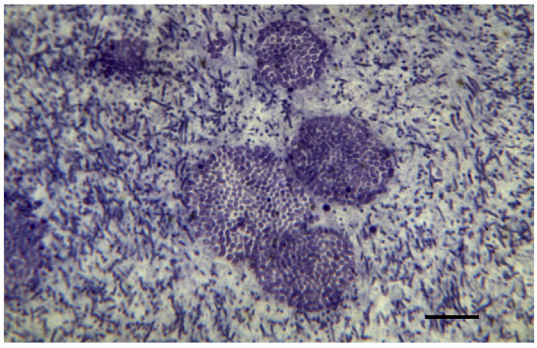

Fig. 7. Bacterial colonies forming inside the layer of *Tenacibaculum*-like bacteria. Bar = 10 μm.

Cross-species contamination could be a bigger issue for Atlantic salmon if the lumpsuckers are found to be less susceptible to *T. maritimum* then Atlantic salmon as they could be asymptomatic carriers as is the case with the amoeba (Haugland et al., 2016). Further studies are required to address this potential issue.

4.1. Conclusions

The outbreak discussed in this paper is the first confirmed isolation of *T. maritimum* in Norway and in lumpsuckers. The findings of this case suggest that lumpsuckers should be screened for *T. maritimum* with molecular diagnostic tests such as real time RT-qPCR in hatcheries and prior to their transfer into Atlantic salmon pens. Also, proper media should be routinely used when investigating high mortality or skin disorders in lumpsuckers. As this case was in a hatchery, another suggestion would be to disinfect intake water to minimise exposure to this pathogen.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2016.06.030.

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