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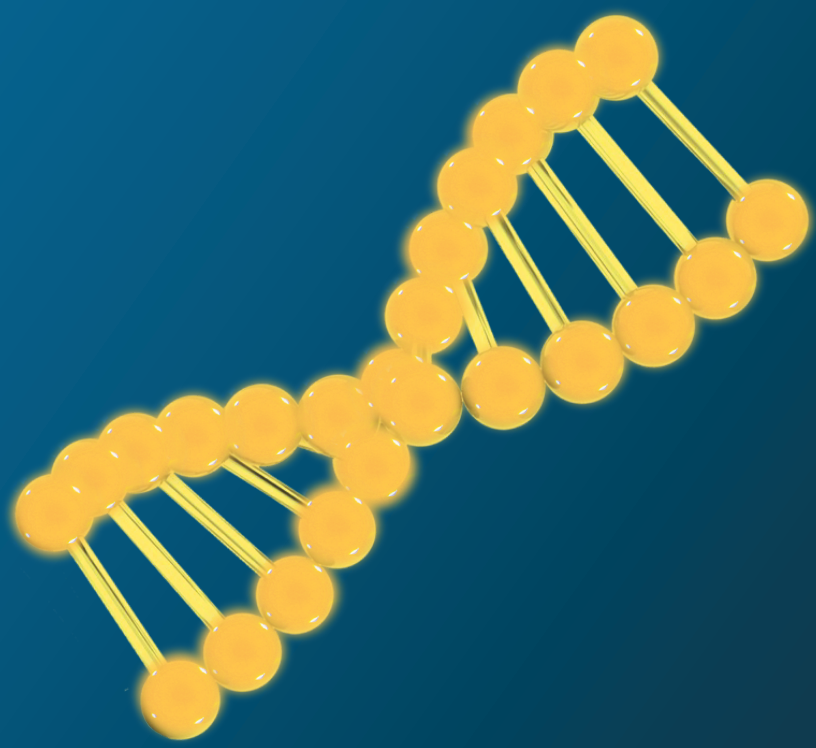

From genotype to phenotype: diversity and population structure of *Flavobacterium psychrophilum*

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ABSTRACT

Flavobacterium psychrophilum is the etiological agent of bacterial cold-water disease (BCWD) causing high fish mortalities and significant economic losses to the freshwater salmonid aquaculture industry around the world. Today BCWD outbreaks are mainly treated with environmentally hazardous antimicrobial agents and alternative preventative measures are urgently needed in order to ensure the well-being of animals and the sustainability of aquaculture. The diversity of pathogenic bacteria challenges the development of universal control strategies and in many cases the pathogen population structure, i.e. the total genetic diversity of the species must be taken into account. This work integrates the tools of modern molecular biology and conventional phenotypic microbiology to gain knowledge about the diversity and population structure of *F. psychrophilum*. The present work includes genetic characterization of a large collection of isolates collected from diverse origins and years, from aquaculture in a whole region including different countries, and provides the first international validation of a universal multilocus sequence typing (MLST) approach for unambiguous genetic typing of *F. psychrophilum.* Population structure analyses showed that the global *F. psychrophilum* population is subdivided into pathogenic species-specific clones, of which one particular genetic lineage, clonal complex CC-ST2, has been responsible for the majority of BCWD outbreaks in rainbow trout (*Oncorhynchus mykiss*) in European aquaculture facilities over several decades. Genotypic and phenotypic population heterogeneity affecting antimicrobial resistance in *F. psychrophilum* within BCWD outbreaks was discovered. Specific genotypes were associated with severe infections in farmed rainbow trout and Atlantic salmon (*Salmo salar*), and in addition to high adherence, antimicrobial resistance was strongly associated with outbreak strains. The study brought additional support for the hypothesis of an epidemic *F. psychrophilum* population structure, where recombination is an important force for the generation and maintenance of genetic diversity, and a significant contribution towards mapping the genetic diversity of this important fish pathogen. Evidence indicating dissemination of virulent strains with commercial movement of fish and fish products was strengthened.

Keywords: *Flavobacterium psychrophilum*, diversity, genotype, phenotype, population structure

SAMMANFATTNING

Bakterien *Flavobacterium psychrophilum* förorsakar kallvattensjuka hos laxfisk i sötvattensodlingar med kraftig fiskdöd och stora ekonomiska förluster som följd. För behandling av kallvattensjuka brukas en stor mängd antibiotika och alternativa förebyggande metoder är nödvändiga både för att trygga den odlade fiskens hälsa och för att främja den ekologiska hållbarheten av fiskodling. Utvecklingen av universella preventiva metoder kräver en kartläggning av bakteriens genetiska diversitet och fenotypiska egenskaper. I denna avhandling integreras moderna molekylärgenetiska metoder med klassisk mikrobiologi för att undersöka diversiteten och populationsstrukturen hos *F. psychrophilum*. I arbetet genomfördes en genetisk undersökning av en omfattande samling av isolat från olika geografiska områden och tidsperioder och en internationell validering av MLST (eng. multilocus sequence typing) för genetisk karaktärisering av *F. psychrophilum*. Populationsgenetiska analyser visade att den globala *F. psychrophilum* populationen är indelad i värdspecifika epidemiska kloner, av vilka en grupp närbesläktade genotyper, klonalkomplexet CC-ST2, förorsakat de flesta utbrotten av kallvattensjuka hos regnbågslax (*Oncorhynchus mykiss*) i europeiska fiskodlingar under flera decennier. Specifika genotyper av *F. psychrophilum* förknippades med allvarliga infektioner hos regnbågslax och lax (*Salmo salar*) medan antibiotikaresistens samt en hög förmåga att adherera var starkt associerade med isolat från sjukdomsutbrott. Vid analys av diversiteten hos *F. psychrophilum* inom enskilda sjukdomsutbrott upptäcktes både genetisk och fenotypisk variation som kan försvåra behandling av kallvattensjuka med antibiotika. Avhandlingen bidrar med en omfattande genetisk kartläggning av *F. psychrophilum* och stöder hypotesen om en epidemisk populationsstruktur, där rekombination är den drivande kraften för uppkomsten och upprätthållandet av genetisk diversitet hos den fiskpatogena bakterien. Resultaten styrker bevisen för att spridningen av virulenta *F. psychrophilum*-genotyper skett via internationell handel av fisk och rom.

Nyckelord: Diversitet, fenotyp, *Flavobacterium psychrophilum*, genotyp, populationsstruktur

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on four original publications, which are referred to in the text by Roman numerals I–IV. The original publications have been reprinted with the kind permission of ASM journals (**paper I**), Elsevier Ltd. (**paper II**), Inter-Research Science Center (**paper III**) and John Wiley & Sons Ltd. (**paper IV**).

I. Nilsen H*, **Sundell K***, Duchaud E, Nicolas P, Dalsgaard I, Madsen L, Aspán A, Jansson E, Colquhoun DJ & Wiklund T (2014) Multilocus sequence typing identifies epidemic clones of *Flavobacterium psychrophilum* in Nordic countries. *Applied and Environmental Microbiology* 80, 2728–2736.

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II. **Sundell K** & Wiklund T (2015) Characteristics of epidemic and sporadic *Flavobacterium psychrophilum* sequence types. *Aquaculture* 441, 51–56.

III. **Sundell K**, Heinikainen S & Wiklund T (2013) Structure of *Flavobacterium psychrophilum* populations infecting farmed rainbow trout (*Oncorhynchus mykiss*). *Diseases of Aquatic Organisms* 103, 111–119.

IV. **Sundell K** & Wiklund T (2011) Effect of biofilm formation on antimicrobial tolerance of *Flavobacterium psychrophilum*. *Journal of Fish Diseases* 34, 373–383.

1 INTRODUCTION

Aquaculture is the fastest growing food producing industry in the world (FAO 2014). The mass productive nature of fish farming subjects animals to living in high population densities and makes conditions ideal for bacterial pathogens to take hold. In cases of disease outbreaks, high mortalities can often only be avoided by the administration of environmentally hazardous antimicrobial agents. An increase in antimicrobial resistance of pathogenic aquatic bacteria is an emerging problem and alternative preventative measures are urgently needed for ensuring the wellbeing of animals and for improving the sustainability of aquaculture. The inadequate information on the biology, diversity and population structure, i.e. the genetic variability, of pathogenic bacteria in the aquatic environments hampers the design of strategies for disease prevention.

Flavobacterium psychrophilum has been one of the most detrimental pathogens for freshwater reared salmonid species for several decades. For many years since its initial discovery in the 1940s, the bacterium seemed limited to North America, but over time with the growth of the fish farming industry, the pathogen has spread throughout the world. *F. psychrophilum* has been considered ubiquitous in the aquatic environment causing infections in fish under stressful conditions, but the pathogen has rarely been isolated from outside the fish host (Bernardét & Bowman 2006). Some strains of *F. psychrophilum* found in fish farm environments show a high degree of pathogenicity or virulence, while others seem less harmful to fish (Madetoja *et al.* 2002). These observations raise the question whether instead of being endemic in the environment, virulent *F. psychrophilum* strains are spread to new areas with the transport of fish causing high mortalities when introduced into fish farms.

Due to the lack of efficient vaccines, control of *F. psychrophilum* infections today mainly relies on antimicrobial therapy. The widespread use of antimicrobial agents has resulted in the emergence of resistant *F. psychrophilum* strains (Barnes & Brown 2011), which may hamper the control of recurrent disease outbreaks. Even though *F. psychrophilum* isolated from disease outbreaks may show *in vitro* susceptibility towards antimicrobial agents, medicated fish do not always respond to treatment *in vivo*. Whether relapses in infections are a consequence of undetected mixed infections with multiple strains, or involve phenotypic changes in the bacterium contributing to a decreased antimicrobial susceptibility in part of the infective *F. psychrophilum* population have not been investigated properly.

Whereas the phenotype describes the observable characteristics or traits of an organism, the genotype refers to the actual set of genes carried by it. The development of molecular fingerprinting techniques has opened a new dimension for studies in microbial diversity. Whole-genome sequencing of bacterial isolates provides the highest discriminatory power, but is at the time both impractical and uneconomic for use in population genetic studies which require comparative analysis of bacterial populations rather than individual isolates.

Due to the cosmopolitan nature of *F. psychrophilum*, there has been a need for a validated genotyping method for epidemiological and population genetics studies. Exploring the population structure is a prerequisite for the description of life-style, niche adaptation and pathogenesis of bacterial pathogens. Bacterial population structure analysis aims to describe the total genetic variation within a species and requires isolation and genetic characterization of a large number of strains representative of the whole bacterial population. Population structure analyses reveal whether a pathogenic bacterial species consists of a single homogeneous population, or include one or several genetically distinguishable subpopulations differing in host range and pathogenicity. An understanding of the population structure is critical, not only to understand the biology of a pathogen, but also for the development of control measures.

This thesis aims to contribute with increased knowledge on the diversity and population structure of *F. psychrophilum*. This work integrates the tools of modern molecular genetics and conventional phenotypic microbiology in an attempt to associate important phenotypic characteristics of *F. psychrophilum* with the genotype, and with factors relating to the epidemiology and control of the pathogen.

1.1 *Flavobacterium psychrophilum*

1.1.1 Geographic and host range

Today *F. psychrophilum* is a common pathogen in fish farm environments all around the world, but its original geographic distribution and host range prior to the development of the fish farming industry and the international trade of fish and fish eggs is unknown (Nicolas *et al.* 2008). Initially, the pathogen seemed limited to North America (Borg 1948, 1960), until the mid-1980s, when it was described on the European continent (Austin & Stobie 1991, Balta 1997, Bernardét *et al.* 1988, Sarti *et al.* 1992, Toranzo & Barja 1993, Weis 1987) and subsequently in the Pacific region (Wakabayashi *et al.* 1991, Bustos *et al.* 1995, Lee & Heo 1998, Schmidtke & Carson 1995). In the Nordic countries, *F. psychrophilum* was first reported in Denmark in 1985 (Dalsgaard & Hørlyck 1990, Lorenzen *et al.* 1991) and in Sweden and Finland in 1986 and 1993, respectively (Wiklund *et al.* 1994).

In Norway, the largest salmon producing country in the world, *F. psychrophilum* was only considered a minor problem in brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) hatcheries until 2008 when the pathogen was causing high mortalities in several rainbow trout (*Oncorhynchus mykiss*) farms (Nilsen *et al.* 2011). Most species of salmonids are believed to be susceptible to *F. psychrophilum* and the bacterium has also been isolated from several non-salmonid species (Barnes & Brown 2011, Austin & Austin 2012). High mortalities occur most frequently in rainbow trout and coho salmon (*O. kisutch*), which are considered particularly susceptible to *F. psychrophilum* (Borg 1948). The pathogen is also known to cause considerable losses in farmed and natural populations of ayu (*Plecoglossus altivelis altivelis*) (Wakabayashi *et al.* 1991).

1.1.2 Pathogenesis

F. psychrophilum is the etiological agent of bacterial cold-water disease (BCWD), a condition that has been referred to as rainbow trout fry syndrome, fry mortality syndrome, low-temperature disease and peduncle disease (Nematollahi *et al.* 2003b, Barnes & Brown 2011). In spite of extensive studies, it is still not known how *F. psychrophilum* enters the fish. Horizontal transmission from fish to fish seems most likely, but vertical transmission through eggs and sexual fluids is also highly suspected (Brown *et al.* 1997, Taylor 2004). Although BCWD causes high mortalities in rainbow trout in farm environments, mortality with *F. psychrophilum* is difficult to achieve under laboratory conditions unless the pathogen is injected through the fish skin. The lack of a natural waterborne

challenge model has hindered the acquisition of knowledge on the specific virulence factors employed by *F. psychrophilum* and their roles in the pathogenesis of BCWD. However, mechanisms involved in the adhesion to host tissue, protease production, iron acquisition and production of lipopolysaccharides have been linked to virulence of the bacterium (Dalsgaard 1993, Nematollahi *et al.* 2003a, Álvarez *et al.* 2008). Putative virulence genes have also been identified in whole-genome analysis of *F. psychrophilum* (Duchaud *et al.* 2007). More recent studies by Högfors-Rönnholm (2014) have implied that phase variation (see 1.4.2) in *F. psychrophilum* might be involved in the pathogenesis of BCWD, but this phenomenon has not yet been shown to occur *in vivo*.

1.1.3 Clinical signs

Infections with *F. psychrophilum* usually occur at temperatures below 15°C and the clinical signs vary depending on the virulence of the strain and on the age and species of the fish affected. High mortalities are most common in fry, where infections appear to develop fast without clinical signs and result in an acute systemic infection with spleen hypertrophy (Nematollahi *et al.* 2003b, Starliper 2011). Infected fingerlings can appear lethargic or anemic and show exophthalmia, dark skin pigmentation and sometimes skin lesions as the first signs of infection. Clinical signs in juveniles are usually anemia, exophthalmia and larger necrotic skin lesions and ulcers. In fingerling and juvenile populations infections are less acute and the mortalities are not as high as in fry populations (Dalsgaard 1993, Nematollahi *et al.* 2003b). In less susceptible salmonid species, such as Atlantic salmon and brown trout, and in adult fish, infections often manifest as fin or tail rot, or ulcerations without significant mortalities.

1.1.4 Prevention and control

Despite the significant negative economic impact of *F. psychrophilum* on aquaculture, no commercial vaccines are available against BCWD today although some experimental vaccines have shown promising results (Sundell *et al.* 2014). Chemical baths have limited preventive effect on BCWD because *F. psychrophilum* mainly causes systemic infections in fry. Therefore, control of *F. psychrophilum* infections mainly relies on orally administered antimicrobial agents. Oxytetracycline and oxolinic acid have been widely used for treating *F. psychrophilum* infected fish in European aquaculture, but the increasing resistance towards these antimicrobials has led to therapeutic treatments with florfenicol (Barnes & Brown 2011). *F. psychrophilum* is also able to survive routine iodophore egg disinfection practices used in hatcheries (Cipriano *et al.*

1995, Brown *et al.* 1997, Kumagai *et al.* 1998), complicating control of a potential vertical transmission from broodfish to offspring. Alternative approaches such as the use of probiotics, immunostimulants or phage therapy have not provided sufficient protection against *F. psychrophilum* infections and hence proper management strategies are the only means for prevention of disease outbreaks (Pérez-Pascual *et al.* 2015).

1.2 Strain typing of *F. psychrophilum*

Biochemical and physiological analyses have showed that *F. psychrophilum* strains infecting fish are biochemically highly homogeneous regardless of the host species affected (Faruk 2000, Valdebenito & Avendaño-Herrera 2009). Classic characterization techniques based on enzymatic reactions, such as API ZYM, have been limited in distinguishing between *F. psychrophilum* phenotypes (Hesami *et al.* 2008). The antigenic variation in *F. psychrophilum* has been used to serologically distinguish between strains and a number of different typing categories have been proposed (Lorenzen & Olesen 1997, Izumi & Wakabayashi 1999, Mata *et al.* 2002). However, no direct association between serotypes and isolate virulence, geographical origin or the host fish species has been found (Faruk *et al.* 2002).

To obtain a higher discriminatory power for studying the diversity of the fish pathogen *F. psychrophilum*, strain typing has shifted from phenotypic and antigenic characterization to molecular genetic techniques based on diversity and phylogenetic analysis of DNA sequences in bacterial isolates. Thus far the genotypic diversity in *F. psychrophilum* has been analysed by random amplification of polymorphic DNA (RAPD) (Chakroun *et al.* 1997), restriction fragment length polymorphism of PCR products (PCR-RFLP) (Izumi *et al.* 2003), ribotyping (Cipriano *et al.* 1996, Chakroun *et al.* 1998, Dalsgaard & Madsen 2000, Madsen & Dalsgaard 2000, Madetoja *et al.* 2002), reciprocal suppression subtractive hybridization by microarray analysis (Soule *et al.* 2005a), polymorphism in 16S rRNA genes (Soule *et al.* 2005b), and more recently by pulsed-field gel electrophoresis (PFGE, see 1.2.1) (Arai *et al.* 2007, Chen *et al.* 2008, Del Cerro *et al.* 2010, **paper III**) and multilocus sequence typing (MLST, see 1.2.2) (Nicolas *et al.* 2008, Siekoula-Nguedia *et al.* 2012, Apablaza *et al.* 2013, Fujiwara-Nagata *et al.* 2013, Strepparava *et al.* 2013, Avendaño-Herrera *et al.* 2014, **paper I**). Alone or in combination with other genotyping methods, plasmid profiling has also been used as a tool for genetic characterization of *F. psychrophilum* isolates (Lorenzen *et al.* 1997, Chakroun *et al.* 1998, Izumi & Aranishi 2004a, Kim *et al.* 2010, **paper III**).

The choice of a proper typing approach is crucial in various kinds of epidemiological studies because typing methods for outbreak analyses (local epidemiology) are not necessarily suitable for studying the relatedness among strains from different geographic regions (global epidemiology) (Maiden *et al.* 1998).

It is already well documented that multiple genotypes of *F. psychrophilum* can coexist in fish farm environments (Madsen *et al.* 2005, Del Cerro *et al.* 2010) and even in the same fish individual (Chen *et al.* 2008), and that these can differ markedly in their virulence in fish (Madetoja *et al.* 2002). Although the mechanisms leading to a change in virulence are not known, a genetic feature underlying differences in virulence phenotypes can be assumed. Phylogenetic and population structure analyses enable us to associate specific genetic lineages with phenotypic characteristics, such as pathogenicity and host range, and in combination with clinical data, specific genotypes responsible for high mortalities in fish can further be identified. For this purpose, a universal strain typing scheme for *F. psychrophilum* applicable for both acute outbreak investigations and long-term epidemiological surveillance would enable the prediction of phenotypic traits from genotypic analyses and facilitate the development of molecular markers for rapid strain differentiation. Below a brief introduction to the two main molecular typing methods used in this thesis; PFGE (see 1.2.1) and MLST (see 1.2.2), and their application in phylogenetic and population genetic studies.

1.2.1 PFGE

Pulsed-field gel electrophoresis (PFGE) is a DNA banding-based subtyping method (Schwartz & Cantor 1984), which differentiates genomes by infrequent cutting of bacterial DNA with restriction enzymes. This produces large DNA fragments of different sizes, which can be separated by gel electrophoresis under a pulsed-electric field. Variations are translated into unique patterns of DNA fragments and allow specific identification of strains. PFGE has been considered the gold standard for bacterial subtyping and epidemiological tracking because of its high discriminatory ability and has even proven to be a successful method for typing of *F. psychrophilum* (Arai *et al.* 2007, Chen *et al.* 2008, Del Cerro *et al.* 2010, **paper III**). The high discriminatory power of PFGE makes it particularly suitable for outbreak investigations. Standardized typing protocols have been developed for several human pathogens (Ribot *et al.* 2006), but so far PFGE analyses of *F. psychrophilum* have involved different restriction enzymes and protocols making interlaboratory comparison of typing results unreliable.

1.2.2 MLST

Multilocus sequence typing (MLST) is a sequence-based genotyping method based on polymorphisms in (usually) seven housekeeping genes (loci) which are amplified by PCR and sequenced. Each unique locus is given an arbitrary allele number and the combined 7-numbered allelic profile defines the overall genotype or sequence type (ST) of the isolate. In MLST, each allelic change is counted as a single genetic event, regardless of the number of nucleotide polymorphisms involved due to mutation or recombination. This is an important consideration when studying highly recombining bacterial species, when differences between closely related strains are more likely explained by a recombination event, involving changes at multiple sites, than by a single point mutation (Maiden *et al.* 1998).

The ability of the MLST analysis to give high strain discrimination relies on the choice of genes or loci to be analysed. The advantage of MLST is that it produces nucleotide sequence data that can readily be exchanged and compared between different laboratories and studies. Additionally, the allelic profiles and sequence data can be stored in public web databases to be accessed by researchers around the world. Compared to DNA band-based typing methods MLST provides typing results more amenable to further population genetic analyses (see 1.3). A MLST scheme for *F. psychrophilum* which characterizes isolates based on the allelic profile of the seven housekeeping genes *atp*A, *dna*K, *fum*C, *gyr*B, *mur*G, *trp*B and *tuf* was proposed by Nicolas *et al.* (2008) and further optimized by Siekoula-Nguedia *et al.* (2012). The chosen loci were considered typical core genome genes that are highly discriminatory and likely to be relatively neutral, i.e. not experiencing frequent adaptive selection. A dedicated MLST database presenting the total known diversity of *F. psychrophilum* has been made publicly available (pubmlst.org/fpsychrophilum).

1.2.3 Phylogenetic analysis of genotyping data

The ability to determine the genetic relatedness of pathogenic bacterial strains is fundamental to both epidemiological and evolutionary studies. For grouping bacterial isolates with similar genetic patterns or DNA sequences and for characterizing the relationships among isolates, hierarchical cluster analysis with the unweighted pair group method with arithmetic mean (UPGMA) (Sneath & Sokal 1973) is most widely used in microbial epidemiology. The tree-like dendrogram constructed by UPGMA provides a convenient means of identifying genetically indistinguishable and closely related isolates that can be assigned to the same genetic cluster. However, UPGMA is not considered a good algorithm

for construction of phylogenetic trees in the study of bacterial population genetics as it relies on the rates of evolution among different lineages to be approximately equal. When studying the population structure of bacterial species that show evidence of significant recombination, dendrograms provide essentially no information on the relationships between distantly related genotypes and on the patterns of evolutionary descent (Feil *et al.* 2004). In these cases, alternative tools such as eBURST (see 1.3.1) and split networks (see 1.3.2) are more suitable for displaying the evolutionary relationships between isolates.

1.3 MLST-based inference of diversity and population structure

MLST data enables a variety of analyses either based on the allelic profiles or on the sequence data of each gene. To reduce the effect of recombination, MLST data is frequently analysed utilizing the allelic profiles instead of the nucleotide sequences. Genetic diversity is highly variable between different bacterial species and generally measured as the variation in the sequence polymorphism of genes shared between all individuals in the population under study. The simplest measure of genetic diversity is that two genes drawn at random from a population are of different allelic types. In practice, the genetic diversity should increase when epidemiologically unrelated strains are analysed.

Bacterial population structure analysis aims to describe the total genetic variation within a species and requires genetic characterization of preferably a large collection of strains from different origins representative of the global bacterial population. Population structure analyses reveal whether the pathogen species consists of a single homogeneous population, or include one or several genetically distinct subpopulations confined to separate niches exhibiting a more or less virulent lifestyle. Reflective of their lifestyle, pathogenic bacterial species can display a variety of population structures ranging from strictly clonal to freely recombining (Smith *et al.* 2000).

The first data resulting from the application of MLST data of *F. psychrophilum* for population genetic analyses have suggested an epidemic structure where recombination is an important force for the generation and maintenance of genetic diversity (Nicolas *et al.* 2008, Siekoula-Nguedia *et al.* 2012, Vos & Didelot 2009). However, the number of *F. psychrophilum* isolates analysed by MLST has so far been limited and more data is needed to provide a reliable picture of the *F. psychrophilum* population structure.

1.3.1 eBURST

The eBURST (based upon related sequence types) algorithm is convenient for use in population structure analysis of MLST data as it makes use of the allelic profiles to group closely related STs into a clonal complex (CC). In eBURST, a CC is defined as a group of STs in a population that share 6 out of 7 alleles with at least one other ST in the group. Within each CC, the ST which demonstrates the greatest number of single-locus variants (SLVs) is assigned as the predicted founder from which the most parsimonious patterns of descent is displayed (Feil *et al.* 2004). CCs are typically composed of a single predominant genotype or ST with a number of close relatives of this genotype. The simplest biological explanation for this is that the founding genotype increases in frequency in the population as a consequence of a fitness advantage or of random genetic drift and becomes a predominant clone. As the clone increases in frequency in the population, the founding genotype gradually diversifies, to result in a CC. The CCs and founder STs identified by eBURST are dependent on the sample used for analysis. Therefore, researchers should make use of all the available MLST data for clustering identified STs into CCs.

Compared with tree-like dendrograms, eBURST aids in understanding the evolutionary relationships and patterns of descent among strains. Although recombination may over the long term preclude the recovery of the true relationships between distantly related isolates of a species, even a high rate of recombination is unlikely to prevent the recognition of CCs in epidemiological studies, which are typically concerned with disease outbreaks or the spread of highly pathogenic strains between countries that occur over a relatively short evolutionary timescale (Feil *et al.* 2004). Hence, eBURST is particularly suitable for exploring how CCs diversify and can provide evidence concerning the emergence of clones of particular clinical relevance.

1.3.2 Split networks

Split networks are suitable for representing the relationship between MLST gene sequences suspected to have been affected by recombination. In these instances, standard phylogenetic trees, which only display single relationships between isolates, do not provide an equal representation for all possible evolutionary relationships. Split networks, such as neighbor-net (Bryant & Moulton 2004), are capable of displaying conflicting results and produce a tree-like structure when the descent appears to be clonal and a network-like graph when recombination plays a significant role in the evolutionary history of the genes.

1.3.3 Tests of recombination

Estimating the significance of recombination is important for understanding the evolution and origin of pathogenic bacterial strains. Because the detection abilities of different tests can vary markedly between samples or data sets (Posada 2002) it is important to use a combination of methods for recombination analyses. The measure of linkage disequilibrium (LD) between alleles at different loci provides a means to determine to which extent the bacterial population can be considered clonal due to LD and linkage among loci, or recombinant, when LD is not detected.

LD occurs when alleles at two or more loci are not independent of another, while linkage equilibrium occurs when the allele present at one locus is independent of the alleles at other loci. However, LD in a population should not automatically be considered as evidence of low recombination rates (Smith *et al.* 1993), because it is almost inevitable to some extent in bacteria that divide by binary fission. Hence, LD and an association between alleles observed in large datasets may be due to the recent emergence of successful clones or sampling bias rather than a low rate of recombination. Successful clones which may remain rare in the total population may become vastly over-sampled when isolates are recovered mainly from cases of disease. When analysing MLST data, overrepresentation of particular genotypes can be avoided by restricting the measure of LD to single representatives of the STs identified in the population.

The extent of LD within a bacterial population can be quantified by calculating the standardized index of association (I^S_A) (Haubold & Hudson 2000), which is a measure of the degree of linkage in MLST datasets giving an indication on the population structure. I^S_A is a function of the recombination rate and equal to zero when all alleles are in linkage equilibrium. In contrast, the pairwise homoplasy index (PHI) test, which discriminates between recurrent mutation and recombination, uses the concatenated sequences of the housekeeping genes to find statistically significant evidence for recombination.

1.4 Phenotypic variation

Although the observable traits of bacteria are based upon the expression of underlying genes making up the genotype, the expression of the genes generating the phenotypic characteristics are often heavily influenced by environmental factors. Contrary to the traditional view that infectious bacterial populations are clonal, the generation of phenotypic variants appears to be frequent in bacteria during adaptation to variable environmental conditions, including colonization of the host by pathogenic species (Casadesús & Low 2013). Diversity in a bacterial population may have direct consequences on the pathogenesis of disease and persistence of the bacterial population. A heterogeneous bacterial population including phenotypic variants might also explain why certain bacterial infections are difficult to treat. Phenotypic variation has been shown to affect the virulence (van der Woude & Bäumler 2004) and antimicrobial susceptibility (Costerton *et al.* 1999) in pathogenic bacteria, but depending on the traits that are affected, phenotypic variants may be difficult to detect. Phenotypic antimicrobial resistance, which expresses the ability to grow in the presence of an antimicrobial agent, is a detectable trait under laboratory conditions. The lowest concentration of a specific antimicrobial preventing growth of a strain can be quantitatively measured and is often referred to as the inhibition zone diameter (IZD) on agar medium or the minimum inhibitory concentration (MIC) in nutrient broth. The measured IZD and/or MIC value can then be used to distinguish between susceptible and resistant variants. Relevant examples of phenotypic variation in *F. psychrophilum* with a possible influence on persistence both within and outside the host include biofilm formation (see 1.4.1) and phase variation (see 1.4.2).

1.4.1 Biofilm formation

In aquatic environments, bacteria rarely occur in a free-living planktonic form. Instead, most bacteria are associated with surface attached multicellular communities known as biofilms (Donlan 2002). A biofilm consists of surface-attached bacteria enclosed in a self-produced extracellular polymeric substance matrix where cells differentiate rapidly, forming phenotypically different subpopulations contributing to the ecological fitness of the bacterial population. Biofilm formation can increase the tolerance of the bacterial population to nutrient limitation, antimicrobial exposure and other unfavorable environmental conditions (Costerton *et al.* 1999). Biofilms in the natural environment are likely to consist of several bacterial species and provide thus an ideal niche for rapid exchange of bacterial DNA containing genes for antimicrobial resistance (Donlan 2002). In some cases, a switch from a free living state to a sessile biofilm mode of growth can affect the expression of virulence genes in bacterial strains (Al Safadi

et al. 2012). *F. psychrophilum* has been shown to be capable of forming biofilms on polystyrene surfaces *in vitro* (Álvarez *et al.* 2006, De la Fuente *et al.* 2013), but the clinical and epidemiological significance of biofilm formation of the pathogen has not been evaluated before.

1.4.2 Phase variation

Phase variation is a reversible form of gene expression resulting in phenotypic changes, which increases the diversity within a bacterial population and promotes bacterial fitness (van der Woude & Bäumler 2004). Phase variation is sometimes visible as a change in colony morphology as a result of altered packing of the cells within the colony and provides a source of diversity through ON/OFF switching of genes without the fitness costs of irreversible mutations. Phase variation has been shown to affect biofilm formation, virulence and antimicrobial resistance of pathogenic bacteria. It has also been suggested that phase variation is involved in antigenic changes enabling some pathogens to evade the host immune defense (van der Woude & Bäumler 2004).

Colonies of *F. psychrophilum* can show either a rough (R) or a smooth (S) morphology when grown on solid agar medium and the colony phenotype remains stable even when repeatedly subcultured on the same medium formulation. However, the possibility to convert S *F. psychrophilum* morphotypes to R in liquid broth medium under laboratory conditions is indicative of phase variation *in vitro* (Högfors-Rönnholm & Wiklund 2010). The role of phase variation in the pathogenesis of *F. psychrophilum* infections is not known, but R and S morphotypes have been shown to express different phenotypic characteristics that could be important in host-pathogen interactions. R and S colonies of *F. psychrophilum* have been isolated simultaneously from diseased rainbow trout and both morphotypes have been able to cause disease in experimentally infected rainbow trout (Högfors-Rönnholm & Wiklund 2010). However, genetic homogeneity between R and S morphotypes isolated from infected fish has never been confirmed and their presence in BCWD outbreaks has not been investigated properly.

2 AIMS OF THE THESIS

The aim of this thesis is to bring increased knowledge on the diversity of *F. psychrophilum* populations, not only within individual BCWD outbreaks but also between outbreaks in different countries. An important part of this work assesses the validity of a standardized genotyping method for genetic characterization of *F. psychrophilum* and makes use of a large collection of isolates from Denmark, Finland, Norway and Sweden to provide a reliable picture of the genetic diversity and population structure of the pathogen. MLST of a variety of clinical and environmental isolates covering the period between initial recognition of BCWD in the Nordic countries in the 1980s until present time addresses the question whether *F. psychrophilum* strains causing disease have been equally distributed in the whole population or consisted of one or several genetically distinguishable subpopulations unrepresentative of the bacterial population as a whole (**paper I**). The identification of putatively virulent *F. psychrophilum* lineages, CCs or STs enables the search for distinguishable phenotypic characteristics or molecular markers for differentiation of epidemiologically important genotypes (**paper II**).

Despite heavy mortalities in fish farms due to BCWD outbreaks, mortality in fish has been almost impossible to achieve under laboratory conditions when infecting fish with a single strain or clone of *F. psychrophilum* through the water. This raises the question whether contrary to clonal outbreaks; BCWD in aquaculture environments is caused by heterogeneous *F. psychrophilum* populations (**paper III**). Because heterogeneity within an infective population may have direct consequences on the control of disease, the contribution of within-population heterogeneity is examined with a focus on the effects on phenotypic antimicrobial resistance (**paper III & IV**).

The specific objectives of this thesis were to investigate the:

1. Genetic diversity and population structure of *F. psychrophilum* in Nordic countries (**paper I**)

2. Characteristics of epidemiologically important *F. psychrophilum* genotypes (**paper I & II**)

3. Presence and consequences of within-population heterogeneity in *F. psychrophilum* (**paper III & IV**)

3 MATERIALS AND METHODS

The details of the materials and methods used in the experimental work are described in the original publications (**paper I–IV**). A summary of the materials and methods used is provided below.

3.1 Bacterial isolates and identification

A different set of *F. psychrophilum* isolates was used in each study (**paper I–IV**). More information about the isolates used is listed in the original papers. All isolates used were grown on/in tryptone yeast extract salts (TYES) agar or broth (Holt *et al.* 1993) at 15°C and identified as *F. psychrophilum* by PCR using the primers PSY1 (GTTGGCATCAACACACT) and PSY2 (CGATCCTACTTGCGTAG) (Toyama *et al.* 1994).

Rough (R) and smooth (S) colony types of *F. psychrophilum* were identified under a microscope. All smooth-to-rough converted isolates were prepared by repeated passages in TYES broth (Högfors-Rönnholm & Wiklund 2010) and analysed in parallel with the parent S isolates.

3.2 Phenotypic testing

3.2.1 Antimicrobial susceptibility testing

Susceptibility of selected *F. psychrophilum* isolates to antimicrobial agents was tested by determining the MIC by broth microdilution in round bottomed 96-well microtiter plates (**paper II & IV**) or by measuring the IZD by disc diffusion on TYES agar plates (**paper III**).

3.2.2 Biofilm formation assays

The biofilm formation assays were performed *in vitro* in untreated flat-bottomed 96-well microtiter plates (**paper II & IV**). The effect of biofilm formation on antimicrobial tolerance (**paper IV**) was estimated by comparing the survival of planktonic and biofilm cells following exposure to oxytetracycline and flumequine. The development of resistance in surviving biofilm cells was tested with a repeated broth microdilution susceptibility test. The *in vitro* biofilm forming ability of epidemically and sporadically occurring *F. psychrophilum* STs was measured spectrophotometrically after crystal violet staining (**paper II**).

3.2.3 Adhesion to mucus coated and uncoated polystyrene

Adhesion to polystyrene, both uncoated and coated with rainbow trout mucus (**paper II**), was examined in flat-bottomed 96-well microtiter plates (Nunclon Δ Surface, Nunc) according to the method described by Högfors-Rönnholm *et al.* (2015).

3.2.4 Protease production

The ability to hydrolyze elastin and gelatin (**paper II**) was studied *in vitro* by streaking each *F. psychrophilum* isolate on TYES agar supplemented with 0.1% and 3% (w/v) of elastin and gelatin (Sigma) respectively. After incubation at 15°C for 5 days, a positive reaction was indicated by a clear zone in the media around the inoculum.

3.2.5 Resistance to iron starvation

Resistance to iron starvation (**paper II**) was tested by measuring the MIC of the iron chelator 2,2′-dipyridyl (DPD) by broth microdilution.

3.3 Genetic testing

3.3.1 MLST

Genomic DNA was extracted from the *F. psychrophilum* isolates (**paper I**) using the Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions. Partial sequences of the seven housekeeping genes *atp*A, *dna*K, *fum*C, *gyr*B, *mur*G, *trp*B and *tuf* genes were amplified and sequenced according to Siekoula-Nguedia *et al.* (2012). Both strands of the amplified sequences were sequenced (Sanger) and all chromatograms manually verified to ensure high quality before assignation of allele types (ATs) and STs using an 'in house' Perl script. Alleles, STs and background information on the isolates were submitted to the *F. psychrophilum* MLST database (pubmlst.org/fpsychrophilum). The *F. psychrophilum* reference strains JIP02/86 and THC02/90 were included as internal controls in all amplification and sequencing analyses.

3.3.2 PFGE

DNA of the tested *F. psychrophilum* isolates (**paper III**) was examined using enzyme restriction and PFGE. For reference, the type strain NCIMB1947 of *F. psychrophilum* isolated from coho salmon was included in the analysis. For PFGE, DNA plugs were prepared according to the protocol described by Ribot *et al.* (2006) with minor modifications. Briefly, the bacterial cells were washed in phosphate-buffered saline (PBS) and suspended to an optical density of 2.0 at

610 nm. Proteinase K was added to the suspensions before mixing with equal volumes of 1% SeaKem Gold agarose (Lonza) and dispensing into plug moulds. Bacterial cells were then lysed overnight with proteinase K and the plugs were washed twice overnight with Tris-EDTA-NaCl buffer at 4°C. DNA from the lysed cells was restricted with 20 units of *Stu*I (Roche) and loaded into 1.2% SeaKem Gold agarose. To separate the DNA fragments the electrophoresis was run in Bio-Rad CHEF DRIII in HEPES buffer with 5 V cm^{-1} for 18 h at 14°C. The pulse times ranged from 0.2 to 5 s for 8 h and from 5 to 15 s for 10 h.

3.3.3 Plasmid profiling

Plasmid DNA from *F. psychrophilum* isolates (**paper III**) grown on TYES agar was isolated with the NucleoSpin Plasmid-kit (Macherey-Nagel) according to the manufacturer's instructions. The plasmid DNA was separated by electrophoresis (4 V cm^{-1}, 60 min) on a 1% agarose-Tris-borate-EDTA gel stained with ethidium bromide and visualized under ultraviolet transillumination (Alpha Innotech Multi Image Light Cabinet). A 1 kb DNA ladder (NEB, N3232) was used for size estimation.

3.3.4 Presence of bacterial insertion sequence IS256

The presence of the bacterial insertion sequence IS256 in *F. psychrophilum* isolates (**paper II**) and the integrity of the collagenase-encoding gene (Duchaud *et al.* 2007) were studied by PCR in a thermal cycler (Arktik™, Finnzymes). The DNA template was prepared by suspending a *F. psychrophilum* colony from a pure culture in a 50 µl volume of double-distilled water (ddH_2O). Each reaction contained 1×Phire® reaction buffer (Thermo Scientific), 0.5 µM of the primers coll_fw AAATTGCTGCCGATGCTTGG and coll_rev CATATCCTGTTTTACCATCCCA (Duchaud *et al.* 2007), 200 µM of each dNTP, 0.4 µl Phire® HotStart II DNA polymerase (Thermo Scientific), 1 µl DNA template and ddH_2O to a total volume of 20 µl. Samples were initially denaturated for 30 s at 98°C, followed by 30 amplification cycles including denaturation for 5 s at 98°C, annealing of primers for 5 s at 60°C, and extension for 30 s at 72°C. After the last cycle, the PCR mixture was incubated at 72°C for 60 s.

The amplicons were electrophorezed (4 V cm^{-1}, 30 min) on a 1% agarose-Tris-borate-EDTA gel stained with ethidium bromide and detected with ultraviolet transillumination. The presence or absence of IS256 was indicated by a 2418 or 1046 bp amplicon respectively (Duchaud *et al.* 2007).

3.4 Analysis of genotyping data

3.4.1 PFGE data analysis

The PFGE profiles (**paper III**) were analysed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) and a similarity dendrogram was constructed using UPGMA and Dice similarity coefficient.

3.4.2 MLST data analysis

After assignation of a seven-numbered allelic profile (i.e. ST) clonal complexes (CCs) were identified using eBURST v3 (http://eburst.mlst.net/) (**paper I & II**). The complete allelic profile list from the *F. psychrophilum* MLST database (pubmlst.org/fpsychrophilum) was downloaded (accessed on 28 March 2014) and a population snapshot of the 995 isolates and 166 STs was created using the eBURST v3 algorithm (Fig. 2).

CCs were identified based on sharing 5 (relaxed) or 6 (default) out of 7 alleles and the predicted ancestral genotype (founder) of a CC containing three or more STs was defined as the ST with the highest number of single-locus variants (SLVs). Major complexes included three or more STs and were named according to the ST of the predicted founder. Minor CCs containing only two STs were named after the most represented ST or by default by the ST with the lower numbering. Singletons were defined as an ST not belonging to any CC. Clonal relationships were examined using both relaxed and stringent default settings i.e. five or six shared alleles respectively. Bootstrapping (n=1000) was performed to evaluate model robustness.

3.4.3 Population genetic analyses

Gene diversity (H) and the level of linkage disequilibrium (non-random association) between the ATs found at the 7 MLST loci as measured by the standardized index of association (I^S_A) (Haubold & Hudson 2000) were investigated using LIAN 3.6 (**paper I**). Three datasets were tested: the whole population, single representatives of each ST and founder STs of each CC together with singleton STs. A network representation of the evolutionary relationships between STs was constructed on concatenated sequences using the neighbor-net algorithm (with default settings) available in SplitsTree 4 software (splitstree.org). Recombination within the concatenated sequences of each ST was tested using the pairwise homoplasy index (PHI) test also implemented in SplitsTree 4.

3.5 Statistical analyses

When relevant, statistical analyses were applied on the obtained data (**paper I, II & IV**). Specific details about the analyses are given in the respective publications. Probability values (P-values) of less than 0.05 (two-tailed) were considered statistically significant.

4 MAIN FINDINGS OF THE THESIS

4.1 MLST data analysis

4.1.1 Diversity and population structure of *F. psychrophilum* in Nordic countries

MLST of 560 *F. psychrophilum* isolates (see Appendix, Supplementary Table 1) revealed 81 different STs in the Nordic countries Denmark, Finland, Norway and Sweden (**paper I**). The greatest national variation of genotypes was found in the Finnish collection, with 36 different STs compared to 28, 20 and 17 found in Denmark, Norway and Sweden respectively. The genetic diversity (*H*) in the total dataset was estimated to 0.6127±0.0420. Limiting the analysis to rainbow trout isolates reduced *H* to 0.4589±0.0622 while non-rainbow trout isolates alone resulted in an *H* of 0.8733±0.0108. The rainbow trout isolates from Denmark (*H*=0.3949±0.0501), Finland (*H*=0.5401±0.0626) and Sweden (*H*=0.4594±0.0714) showed a similar range of diversity while corresponding Norwegian isolates showed a much lower diversity (*H*=0.0870±0.0145) due to the dominance of one genotype (ST2) in that country.

Linkage equilibrium of alleles was estimated as I^S_A=0.5419 (P<0.05) for the whole dataset, indicating linkage disequilibrium and therefore a degree of clonality in the population. To avoid overrepresentation of particular strains, analyses were also performed using single representatives of the 81 STs present in the study resulting in I^S_A of 0.232 (P<0.05). When the analysis was limited to one representative (the founder) for each CC (12 STs) and singletons (30 STs), I^S_A fell to 0.125 (P<0.05), close to zero indicating linkage equilibrium (**paper I**). Further investigations of recombination events using the PHI test showed statistically significant evidence for recombination (P=0.0) and a network structure consistent with the presence of recombination events was seen in the split network analysis (Fig. 1).

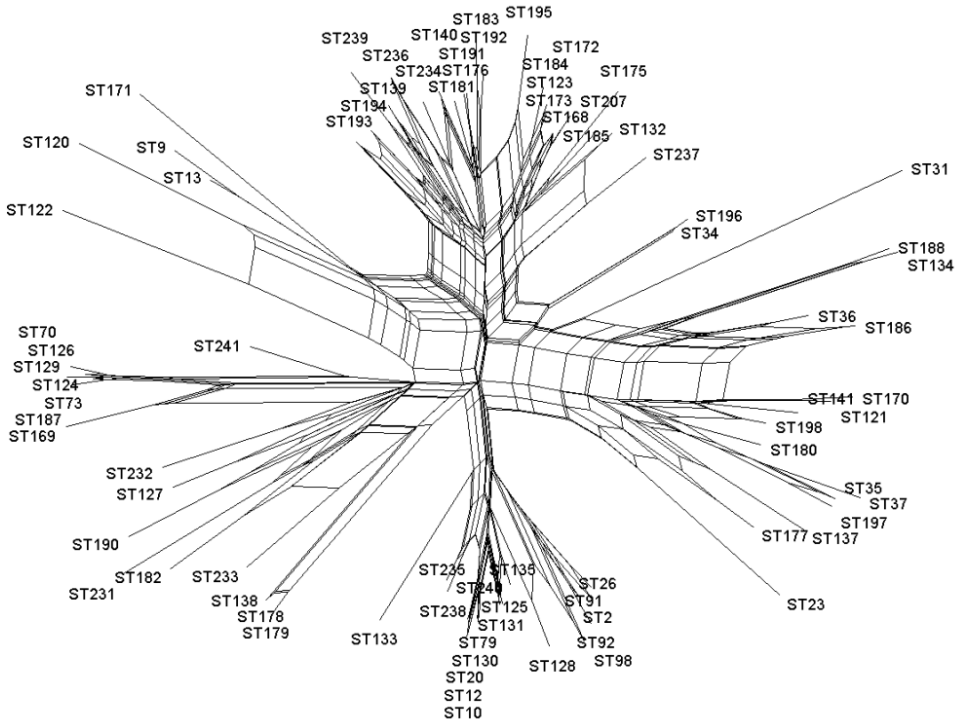


Fig. 1. Split network analysis of the concatenated MLST sequences from the 81 *F. psychrophilum* STs identified in Nordic countries (**paper I**). The reticulated phylogenetic structure of the neighbor-net graph is indicative of extensive recombination of loci providing additional support for the high estimates of recombination in *F. psychrophilum* supported by the PHI test (P=0.0).

4.1.2 Identification of epidemic clones and STs

The eBURST clustering identified seven major and five minor CCs in the Nordic countries (**paper I**). The majority of isolates belonged to CC-ST10, which is presently included in CC-ST2 when the global diversity of *F. psychrophilum* is taken into account (Fig. 2). In Nordic countries, CC-ST2 represented 65% of the total number and 79, 59, 46 and 56% of the Danish, Finnish, Norwegian and Swedish isolates respectively and comprised almost exclusively isolates from rainbow trout. MLST data analysis showed that CC-ST2 has been responsible for the main part of BCWD outbreaks in rainbow trout since the initial recognition of the disease in the Nordic countries in the late 1980s until present time. At the same time, other major CCs (CC-ST124, CC-ST138, CC-ST191 and CC-ST236) shared between countries have been simultaneously present in time and space.

Additional country and species-specific CCs infecting Atlantic salmon (CC-ST123) and rainbow trout (CC-ST125) were found in Norway and in Finland respectively. With relaxed eBURST settings CC-ST124 and the Finnish CC-ST125 were linked to CC-ST2 and can be considered part of the same putatively virulent lineage (Fig. 2). CC-ST125 genotypes have been associated with high virulence (Madetoja *et al.* 2002) and were several times isolated together with CC-ST2 genotypes from the same disease outbreak and even from the same infected rainbow trout individual.

The epidemic clone CC-ST2 comprised several repeatedly isolated STs, particularly ST2 (*n*=157), ST10 (*n*=28), ST79 (*n*=60) and ST92 (*n*=71), connected with severe BCWD outbreaks. In the Nordic countries, the four above-mentioned STs were all initially isolated in Denmark and were later associated with outbreaks in other countries. ST79 was isolated already in 1988, ST2 and ST10 only two years later, and ST92 more recently in 2006. ST92 has been responsible for several BCWD outbreaks since 2008 in several fish farms in Finland and the isolates have been associated with high mortalities and resistance to oxolinic acid. In Norway, ST2 was isolated for the first time in 2004, only a few years before a series of severe disease outbreaks in rainbow trout, and was the only CC-ST2 genotype found in that country. Perhaps significantly, CC-ST2 genotypes were also isolated from eggs and sexual fluids. Isolates orginating from Atlantic salmon were mainly associated with ST70 and the Norwegian CC-ST123. Of the singleton STs (*n*=30), almost 80% were of non-rainbow trout origin (**paper I**).

4.1.3 Characteristics of epidemic STs

To identify phenotypic characteristics discriminating STs related to the genetic lineage CC-ST2 (epidemic) (Fig. 2) from singletons and minor CCs (sporadic) a set of traits was compared between two groups of isolates *in vitro* (**paper II**). Epidemic STs (*n*=16) showed a higher ability to adhere to polystyrene and rainbow trout mucus compared to the sporadic STs (*n*=16). Epidemic STs also showed a higher prevalence of antimicrobial resistance against flumequine, oxolinic acid and oxytetracycline. The two groups showed equal capacities to form biofilms and to resist povidone-iodine and iron starvation. Half of both the epidemic and the sporadic isolates were able to hydrolyze elastin and all isolates were able to hydrolyze gelatin. Six out of the eight CC-ST2 isolates were elastinase producing, while all isolates from the related CC-ST125 (Fig. 2) were unable to degrade elastin. Interestingly, CC-ST2 isolates were distinguished from others by the presence of the bacterial insertion sequence IS256 in the collagenase encoding gene.

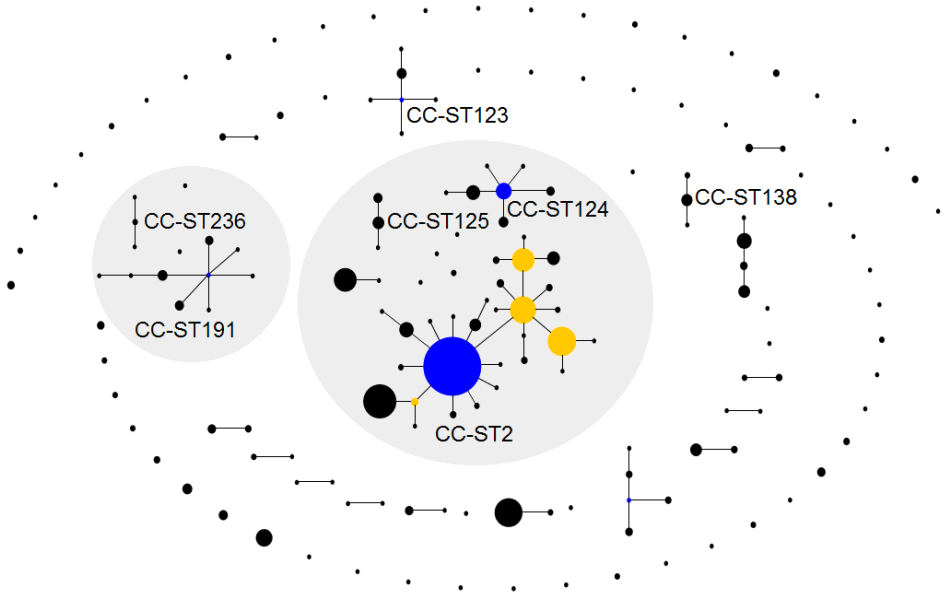


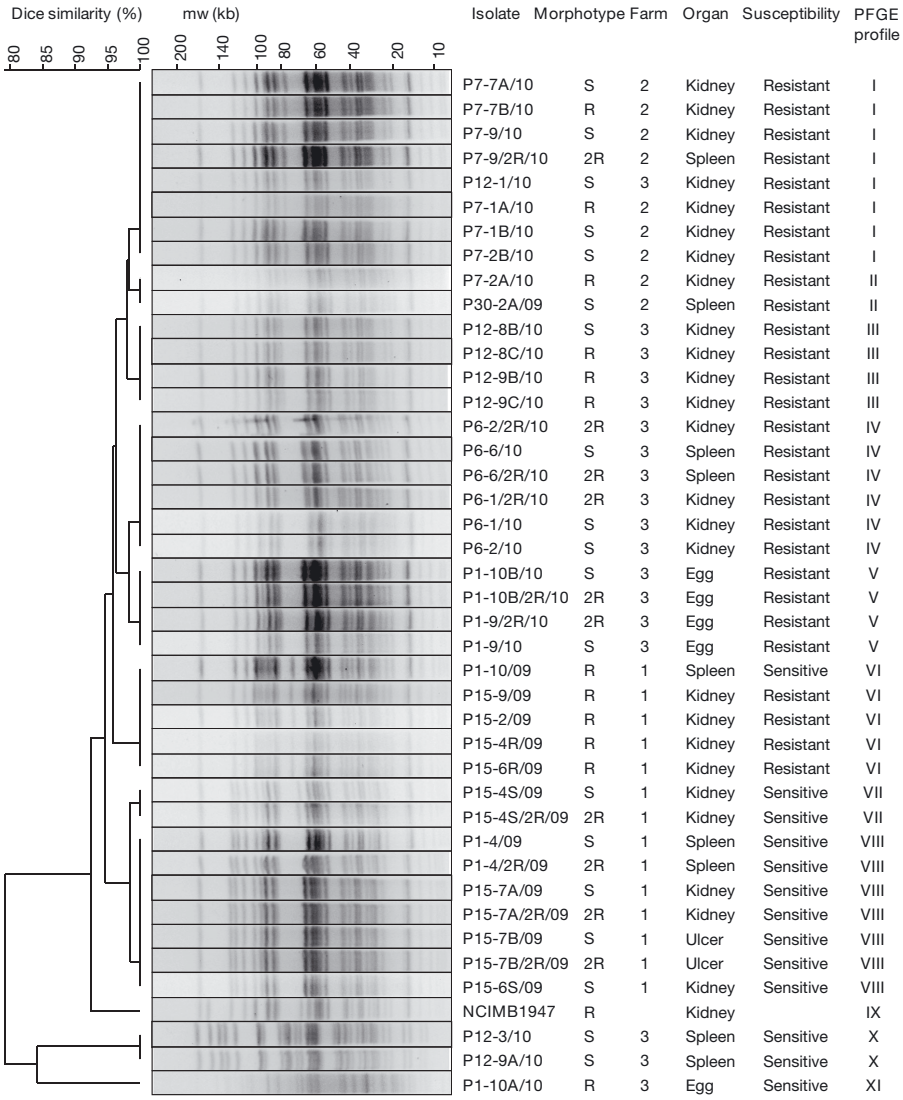
Fig. 2. Population snapshot of the 995 isolates and 166 STs in the global *F. psychrophilum* MLST database with default eBURST v3 settings (last accessed on 28 March 2015). Each dot represents an individual ST and dot size reflects its abundance. Linked clusters represent CCs, singleton STs are unlinked. Clonal founders are shown in blue, subgroup founders in yellow. The major CCs identified in Nordic countries (**paper I**) are indicated and named according to the predicted founder. CCs and STs within the shaded area are linked with the use of a relaxed eBURST group definition.

4.2 Within-population heterogeneity

PFGE analysis of *F. psychrophilum* isolated from disease outbreaks in three different rainbow trout farms in Finland (**paper III**) showed a high genetic homogeneity between isolates, which was indicated by the band similarity exceeding 94% between 38 of the total 42 isolates examined (Fig. 3). However, most outbreaks (4 out of 6) involved more than one *F. psychrophilum* genotype and both R and S morphotypes (5 out of 6) although the sole isolation of the S form dominated in individual fish. The PFGE analysis showed that the R and S colony types of *F. psychrophilum* isolated from the same BCWD outbreak can be genetically indistinguishable or differ in their genetic profile. Phase variation in *F. psychrophilum in vivo* was indicated by the concurrent isolation of genetically indistinguishable (PFGE, plasmid pattern) R and S morphotypes from the same infected fish individual **(paper III)**. The *in vitro* smooth-to-rough conversion did not change the oxolinic acid susceptibility or the genotypic profile (PFGE, plasmid pattern, MLST) of the isolates (**paper I & III**).

Genotypes common to both fish and fish eggs were identified, but this was not always the case. None of the examined isolates clustered together with the *F. psychrophilum* type strain NCIMB1947, which is considered non-virulent in rainbow trout (Madsen & Dalsgaard 2000). Most outbreak isolates contained from 1 to 3 plasmids each, while isolates without plasmids were also present in infected fish. Isolates with identical plasmid profiles often shared indistinguishable PFGE profiles. Most isolates (74%) from the disease outbreaks were resistant to oxolinic acid. Resistant and sensitive isolates of both colony types could be isolated both from individual disease outbreaks and from individual fish, but no direct associations between colony morphology and oxolinic acid resistance was found.

Biofilm formation was shown to increase the tolerance and resistance of *F. psychrophilum* against flumequine and oxytetracycline *in vitro* (**paper IV**). At high bacterial densities ($>10^7$ CFU mL^{-1}), biofilm cells of *F. psychrophilum* were less susceptible than planktonic to oxytetracycline and flumequine. Most biofilm cells survived a 5-day exposure to antimicrobial concentrations three times the MIC for corresponding planktonic cells. Moreover, when biofilm cells of the type strain NCIMB1947 were exposed to subinhibitory antimicrobial concentrations for five days the MIC of oxytetracycline and flumequine increased by one and two doubling dilutions respectively.



Isolate	Morphotype	Farm	Organ	Susceptibility	PFGE profile
P7-7A/10	S	2	Kidney	Resistant	I
P7-7B/10	R	2	Kidney	Resistant	I
P7-9/10	S	2	Kidney	Resistant	I
P7-9/2R/10	2R	2	Spleen	Resistant	I
P12-1/10	S	3	Kidney	Resistant	I
P7-1A/10	R	2	Kidney	Resistant	I
P7-1B/10	S	2	Kidney	Resistant	I
P7-2B/10	S	2	Kidney	Resistant	I
P7-2A/10	R	2	Kidney	Resistant	II
P30-2A/09	S	2	Spleen	Resistant	II
P12-8B/10	S	3	Kidney	Resistant	III
P12-8C/10	R	3	Kidney	Resistant	III
P12-9B/10	R	3	Kidney	Resistant	III
P12-9C/10	R	3	Kidney	Resistant	III
P6-2/2R/10	2R	3	Kidney	Resistant	IV
P6-6/10	S	3	Spleen	Resistant	IV
P6-6/2R/10	2R	3	Spleen	Resistant	IV
P6-1/2R/10	2R	3	Kidney	Resistant	IV
P6-1/10	S	3	Kidney	Resistant	IV
P6-2/10	S	3	Kidney	Resistant	IV
P1-10B/10	S	3	Egg	Resistant	V
P1-10B/2R/10	2R	3	Egg	Resistant	V
P1-9/2R/10	2R	3	Egg	Resistant	V
P1-9/10	S	3	Egg	Resistant	V
P1-10/09	R	1	Spleen	Sensitive	VI
P15-9/09	R	1	Kidney	Resistant	VI
P15-2/09	R	1	Kidney	Resistant	VI
P15-4R/09	R	1	Kidney	Resistant	VI
P15-6R/09	R	1	Kidney	Resistant	VI
P15-4S/09	S	1	Kidney	Sensitive	VII
P15-4S/2R/09	2R	1	Kidney	Sensitive	VII
P1-4/09	S	1	Spleen	Sensitive	VIII
P1-4/2R/09	2R	1	Spleen	Sensitive	VIII
P15-7A/09	S	1	Kidney	Sensitive	VIII
P15-7A/2R/09	2R	1	Kidney	Sensitive	VIII
P15-7B/09	S	1	Ulcer	Sensitive	VIII
P15-7B/2R/09	2R	1	Ulcer	Sensitive	VIII
P15-6S/09	S	1	Kidney	Sensitive	VIII
NCIMB1947	R		Kidney		IX
P12-3/10	S	3	Spleen	Sensitive	X
P12-9A/10	S	3	Spleen	Sensitive	X
P1-10A/10	R	3	Egg	Sensitive	XI

Fig. 3. Similarity dendrogram of pulsed-field gel electrophoresis (PFGE) results for the *F. psychrophilum* isolates analysed from six BCWD outbreaks in three different fish farms in Finland (**paper III**). The type strain NCIMB1947 was included as a reference. R: rough morphotype, S: smooth morphotype, 2R: smooth-to-rough converted morphotype, mw: molecular weight, 'susceptibility' refers to the level of resistance to oxolinic acid.

5 DISCUSSION

5.1 From genotype to phenotype

Conventional phenotypic characterization methods have been limited in discriminating between *F. psychrophilum* isolates. The application of molecular fingerprinting techniques to *F. psychrophilum* has increased our knowledge on the diversity of the pathogen. Molecular genetic strain typing allows us to establish an association between a strain and a trait and to uncover the genetic basis of phenotypic variation. Since strain typing has direct applications in population genetic analyses, the choice of a proper typing approach is important for epidemiological and population structure analyses.

While considerable effort has been put into the development of genetic characterization techniques for *F. psychrophilum*, proper evaluations of these tools have been limited. In this thesis two different main genotyping methods, MLST and PFGE, were used on a different set of *F. psychrophilum* isolates. Both genotyping methods showed high discriminatory power between isolates and were able to detect multiple genotypes within BCWD outbreaks and even within infected fish individuals. However, due to the challenges with reproducibly between laboratories, sequence based methods, such as MLST, may ultimately replace PFGE for typing of bacterial isolates. A significant part of this work included an international validation of a standardized MLST approach for unambiguous genetic typing of *F. psychrophilum* and represented a successful interlaboratory comparison of results (**paper I**).

5.1.1 Diversity and population structure of *F. psychrophilum*

MLST of 560 *F. psychrophilum* isolates collected from the Nordic countries (**paper I**) over a period of four decades from different sources represented by far the largest study of its type to date. As expected when studying large data sets, a higher overall genetic diversity of *F. psychrophilum* was detected in the Nordic countries (H=0.6127±0.0420) compared to the less comprehensive studies both previously and concurrently performed in other parts of the world (Nicolas *et al.* 2008, Siekoula-Nguedia *et al.* 2012, Fujiwara-Nagata *et al.* 2013, Strepparava *et al.* 2013, Avendaño-Herrera *et al.* 2014). A lower genetic diversity was observed among rainbow trout isolates (H=0.4589±0.0622) compared with non-rainbow trout isolates (H=0.8733±0.0108) and the whole population indicating that BCWD in rainbow trout is caused by a genetically homogeneous group of genotypes. Whether there is an association between diversity and virulence in *F.*

psychrophilum on the species level is not yet known, but indications of evolution from an opportunistic lifestyle to host-specific pathogenicity through a decrease in diversity and increase in virulence has been observed in the fish pathogens *F. columnare* (Pulkkinen *et al.* 2010) and *Aeromonas salmonicida* subsp. *salmonicida* (Reith *et al.* 2008).

The increase in genetic diversity produced by recombination gives advantages over clonal, in particular a faster response to changing environments and selection for genotypes that can escape the host immune response giving rise to epidemic clones. Despite the high estimates of recombination indicated by the PHI test ($P<0.05$) and the reticulated phylogenetic structure depicted by the split network analysis (Fig. 1), clonality was observed in the whole population (I^S_A=0.5419). The observed clonality could be explained by the dominance of clonal lineages unbroken by horizontal gene transmission either as a result of niche specialization, host adaptation, physical barriers or recent introduction. Although *F. psychrophilum* is considered ubiquitous in the aquatic environment, it is rarely isolated from outside the fish host (Bernardét & Bowman 2006). A clonal population structure under a high rate of recombination can also be observed when several particular STs are overrepresented due to sampling bias, as in this study, where *F. psychrophilum* was mainly isolated from disease outbreaks. However, the present study included isolates representing cases in which *F. psychrophilum* was undoubtedly the primary cause of infection as well as isolates from mixed cultures where the bacterium was suspected to have a more opportunistic or accidental role. In addition, the recombination rate in *F. psychrophilum* is high even when compared to other bacterial species (Vos & Didelot 2009). The reason behind the variation in recombination rate between bacterial species and its biological significance is currently unclear. However, in future evolutionary analyses of *F. psychrophilum* genomes the high rate of recombination must be taken into account.

Considering the large number of isolates analysed, the work done in this thesis strongly supports the proposed epidemic population structure of *F. psychrophilum* (Nicolas *et al.* 2008, Siekoula-Nguedia *et al.* 2012, Fujiwara-Nagata *et al.* 2013), where highly successful epidemic clones have arisen from a generally recombinant background population. The global eBURST population snapshot of *F. psychrophilum* (Fig. 2) displaying the presence of CCs against a background consisting of genetically distinct singletons supports the epidemic population structure (Smith *et al.* 2000).

5.1.2 Identification of epidemic clones and STs

Today the global *F. psychrophilum* MLST database (pubmlst.org/fpsychrophilum, last accessed on 28 March 2015) contains genetic information of a total of 995 isolates divided into 166 distinct STs identified in different parts of the world (**paper II**). MLST of *F. psychrophilum* isolates from the Nordic countries (**paper I**) revealed several CCs and STs not identified outside the study area. Of the total 166 *F. psychrophilum* STs identified to date, almost half of them were also identified in the Nordic countries. Despite the high genotypic diversity in the study area, only a limited number of CCs and STs were associated with severe disease outbreaks in different countries. However, the simultaneous presence of genetically divergent clones poses a challenge for future disease prevention and control. The main part of *F. psychrophilum* infections in rainbow trout in the Nordic countries were associated with the globally spread epidemic clone CC-ST2. The reason for the epidemiological success and dominance of CC-ST2 is not known, but this work (**paper I**) brought additional support to the hypothesis that its worldwide distribution is associated with the international trade of fish and associated products. After being introduced, CC-ST2 strains may rapidly be spread throughout a whole country through transport of fish and fish eggs between farms causing negative effects to the aquaculture industry of a whole region (Avendaño-Herrera *et al.* 2014, **paper I**).

So far, limited progress has been made in associating epidemiologically important characteristics with specific *F. psychrophilum* genotypes. In combination with detailed clinical information, MLST of outbreak isolates in the Nordic countries pointed out STs specific to severe *F. psychrophilum* infections in rainbow trout (ST2, ST10, ST79 and ST92) and Atlantic salmon (ST70). Some of them, such as ST10 and ST70, have also been identified from outbreaks in the same host species in Chile and North America (pubmlst.org/fpsychrophilum). *F. psychrophilum* STs associated with severe outbreaks in fish were also isolated from eggs and sexual fluids indicating that vertical transmission might play an important role in the spread of virulent genotypes.

ST2 was the most predominant genotype in the Nordic countries and mainly associated with the rainbow trout host. The epidemiological significance of ST2 was demonstrated by its recent introduction to Norway, most likely through import of contaminated rainbow trout eggs, leading to a series of severe BCWD outbreaks in 2008 with fish mortalities up to 90% in fish farms in a country where *F. psychrophilum* was previously considered only a minor problem (Nilsen *et al.* 2011). In Finland, ST2 was only isolated from two outbreaks in two different fish

farms in 2011, suggesting that ST2 has until recently not been able to establish a foothold in Finnish fish farms. ST2 has frequently been isolated from rainbow trout outside the Nordic countries in France (Siekoula-Nguedia *et al.* 2012), Switzerland (Strepparava *et al.* 2013) and Chile (Avendaño-Herrera *et al.* 2014) and is currently by far the most predominant (25%) ST in the global *F. psychrophilum* MLST database. The apparent affinity of ST2 to the rainbow trout host could be a result of niche specialization or merely due to the intensive monoculture fish farming practiced in European countries (Avendaño-Herrera *et al.* 2014).

The 30 singleton STs identified in the Nordic countries were isolated from a broad host range including isolates from non-salmonid species and the environment. Singleton *F. psychrophilum* STs have been suggested to represent less virulent endemic or environmental strains, acting more like opportunistic pathogens compared to other more host-specific strains (Siekoula-Nguedia *et al.* 2012). It could therefore be expected that *F. psychrophilum* STs appearing as singletons and minor CCs do not pose a direct threat to the farmed salmonid production (**paper I**).

5.1.3 Characteristics of epidemic STs

The mechanisms responsible for high pathogenicity of *F. psychrophilum* in fish are not known, but the repeated involvement of an epidemic lineage in BCWD outbreaks in geographically distinct fish farm environments suggests that changes in virulence phenotypes are related to an increase in transmissibility and fitness. Compared to STs belonging to the epidemic lineage CC-ST2, more sporadically occurring genotypes such as singletons might have a selective disadvantage under aquaculture conditions (**paper II**). Phenotypic characterization between STs belonging to the epidemic lineage CC-ST2 and more sporadically occurring STs consisting mainly of singletons revealed a significantly higher prevalence of antimicrobial resistance against flumequine, oxolinic acid and oxytetracycline among the epidemic genotypes (**paper II**). Antimicrobial-susceptible wild-type isolates were also present among epidemic STs and vice versa, indicating that antimicrobial resistance is not a prerequisite for epidemic STs. However, antimicrobial resistance in pathogen genotypes is affected by the pool of resistance genes and the prevalent selection pressure. Thus, it can be expected that STs within the same CC can possess distinct resistance determinants. Whether the association between antimicrobial resistance and epidemic STs is a result of dissemination of genotypes with intrinsic resistance or of a more

efficient acquisition or expression of resistance determinants remains to be investigated.

Nevertheless, resistance to quinolones appears to be a common feature among *F. psychrophilum* strains isolated from disease outbreaks (Izumi & Aranishi 2004b, Henríquez-Núñez *et al.* 2012, Shah *et al.* 2012, **paper III**). Oxolinic acid-resistant *F. psychrophilum* strains were isolated from each of the six separately investigated BCWD outbreaks in Finland (**paper III**). The prevalence of oxolinic acid-resistant isolates (74%) was surprisingly high because the drug had been banned from use in Finnish aquaculture already in 2001. Since quinolone resistance in *F. psychrophilum* has been associated with heritable chromosomal mutations (Izumi & Aranishi 2004b, Shah *et al.* 2012), it is possible that earlier heavy use of quinolones in aquaculture settings has led to a selection of resistant isolates in the studied farm environments. Interestingly, Shah *et al.* (2012) found a strong association between chromosomal quinolone resistance and *F. psychrophilum* strains epidemiologically linked to the severe BCWD epizootics in Norway in 2008. These findings make it tempting to speculate that there is an association between quinolone resistance and virulence in *F. psychrophilum*.

Epidemic STs also showed a higher ability to adhere to inert and mucus coated surfaces, possibly allowing for a more efficient colonization of fish, tank and equipment surfaces in aquaculture environments (**paper II**). The higher adherence to rainbow trout mucus could also be indicative of specific host adaptation mechanisms in epidemic STs. Although the work does not allow for making associations between adherence *in vitro* and virulence *in vivo*, a positive correlation between *F. psychrophilum* adhesion to gill arches of rainbow trout and virulence has previously been reported (Nematollahi *et al.* 2003a). It is also known that the genome of the virulent *F. psychrophilum* isolate JIP02/86 contains several (n=27) genes encoding proteins related to adhesion (Duchaud *et al.* 2007). Thus, the findings provide a compelling argument for continuing research into genetic factors underlying specific mechanisms of adhesion, since the higher adherence of specific *F. psychrophilum* lineages could be a factor contributing to the potential to cause epidemic BCWD outbreaks.

The proteolytic activity of *F. psychrophilum* has been studied as a potential discriminatory marker for pathogenicity with inconclusive results (Bertolini *et al.* 1994, Faruk 2000, Madetoja *et al.* 2002). Previous studies with experimentally injected rainbow trout have suggested that the production of elastinase and gelatinase (Madsen & Dalsgaard 1998, Ostland *et al.* 2000) may be important for

the pathogenicity of *F. psychrophilum*. The results from this thesis showed that epidemic and sporadic STs could not be distinguished from each other by their ability to hydrolyze gelatin or elastin (**paper II**). All isolates were gelatin-producing and some of the elastinase-negative *F. psychrophilum* isolates represented STs (CC-ST125 and ST92) previously associated with high virulence in rainbow trout (**paper I**). Thus, the results from this thesis strongly indicate that the ability of *F. psychrophilum* to hydrolyze elastin or gelatin *in vitro* does not correlate with propensity to cause BCWD.

The comparative analysis of 32 distinct STs showed that CC-ST2 genotypes were distinguished from others by the presence of IS256 in the collagenase encoding gene (**paper II**). While it has been suggested that IS256 can contribute to virulence by influencing phase variation, antimicrobial resistance and niche adaptation of bacterial pathogens (Conlon *et al.* 2004, Kozitskaya *et al.* 2004, Gu *et al.* 2005), the consequences of its integration in the collagenase encoding gene and/or other parts of the genome of *F. psychrophilum* are not yet understood. However, the results from this thesis imply that IS256 could serve as a molecular marker for rapid differentiation of epidemiologically important CC-ST2 genotypes.

5.2 Within-population heterogeneity

Genetic and phenotypic diversification may be significant in *F. psychrophilum* due to its highly recombinogenic nature. Since routine diagnostics of bacterial pathogens from infected fish often involves identification and characterization of only a few isolates, population heterogeneity within an outbreak might be left unrecognized.

The work done in this thesis showed that within a BCWD outbreak, *F. psychrophilum* strains with different colony morphology, plasmid content, antimicrobial susceptibility and PFGE pattern could be isolated (**paper III**), suggesting that *F. psychrophilum* populations infecting rainbow trout in farm environments can be diverse and thus complicate the control of BCWD. These findings suggest that *F. psychrophilum* populations within BCWD outbreaks, as opposed to being clonal, display heterogeneity which might provide for their persistence in fish farm environments. In addition to improving the chances of survival of the bacterial population under varying environmental conditions, diversity within an infective bacterial population might have a significant impact on its global gene expression, on host interactions, and thus, on the severity of a disease outbreak.

Although BCWD outbreaks in rainbow trout were caused by genetically homogeneous *F. psychrophilum* strains (Fig. 3), individual outbreaks often involved multiple genotypes (**paper III**). The diversity of *F. psychrophilum* within disease outbreaks and the ubiquity of the pathogen in fish farms create an opportunity for coinfection and subsequent recombination in the environment and possibly within the host. *F. psychrophilum* infections with multiple genotypes may not only have important consequences on disease control, because theory predicts coinfections to drive pathogen evolution towards higher virulence (May & Nowak 1995).

The majority of the outbreak strains carried plasmids (**paper III**), but the function of these extra chromosomal pieces of DNA in *F. psychrophilum* remains unknown. No direct correlation between plasmid content and antimicrobial resistance (Izumi & Aranishi 2004a, Del Cerro *et al.* 2010, Henríquez-Núñez *et al.* 2012) or virulence (Madsen & Dalsgaard 2000, Kim *et al.* 2010) in *F. psychrophilum* has been found in previous studies. Strains lacking plasmids have been isolated from BCWD outbreaks in different geographical regions (Chakroun *et al.* 1998, **paper III**) and shown to be pathogenic in experimentally infected fish (Pérez-Pascual *et al.* 2015), indicating that plasmids are not required for pathogenicity of *F. psychrophilum*.

Both R and S morphotypes were present in the majority of disease outbreaks and although phase variation in *F. psychrophilum* was shown to occur *in vivo*, its biological role remains to be elucidated in future research. It has been suggested that phase variation is involved in biofilm formation of *F. psychrophilum* due to the different adhesion abilities of the R and S colony phenotypes (Högfors-Rönnholm 2014). Biofilm formation on the other hand was shown to increase the tolerance of *F. psychrophilum* against two different antimicrobials *in vitro* (**paper IV**). The results also indicated that when grown in a biofilm, *F. psychrophilum* is highly tolerant to antimicrobial agents and may rapidly develop resistance if exposed to subinhibitory antimicrobial concentrations. Thus, the ability to form biofilms might explain in part why treatment with antimicrobial agents sometimes gives a poor response in *F. psychrophilum* infected fish under farming conditions although isolates from the same fish population have shown clinical susceptibility *in vitro*. In addition, since oxolinic acid-resistant and sensitive *F. psychrophilum* strains were concurrently isolated both from eggs and infected fish (**paper III**), it is possible that relapses of BCWD after antimicrobial treatments are a consequence of undetected mixed infections with susceptible and resistant *F. psychrophilum* genotypes. The results from this work also indicate that

resistant genotypes could further be disseminated through contaminated eggs, particularly since *F. psychrophilum* is able to survive currently used egg disinfection procedures used in hatcheries (Cipriano *et al.* 1995, Brown *et al.* 1997, Kumagai *et al.* 1998, **paper II**).

Due to the diversity of *F. psychrophilum* and the epidemic population structure it displays, the work done in this thesis shows that for the purpose of detecting and monitoring emergent genotypes (**paper I**), it is important to examine more than a single colony from a clinical sample. The high observed prevalence of antimicrobial resistance in *F. psychrophilum* isolated from infected fish (**paper II & III**) underpins the importance of antimicrobial susceptibility testing of clinical isolates in order to predict the *in vivo* success or failure of antimicrobial therapy and to avoid unnecessary administration of drugs exerting a selective pressure on the target bacterial population.

6 CONCLUSIONS

The gradual shift from conventional phenotypic microbiology methods towards molecular genetic analyses has increased our knowledge on the diversity of bacterial pathogens. Ultimately, genetic diversity can explain most of the observed phenotypic variability in pathogenic bacteria, including geographic distribution, host specificity and pathogenicity. Together with data from other countries, this work showed that BCWD outbreaks in rainbow trout in European aquaculture has primarily been attributed to a geographically widely distributed *F. psychrophilum* lineage, CC-ST2, containing specific STs associated with high fish mortalities. As the population structure of *F. psychrophilum* seems to be epidemic, control should be directed towards identification and prevention of spread of epidemic CC-ST2 genotypes in particular to countries with a growing aquaculture production.

Rapid identification of virulent bacterial variants is crucial in preventing the spread of epidemic clones. This work showed that MLST provides a high discriminatory power applicable for both acute outbreak investigations, and for global epidemiological studies and surveillance of specific *F. psychrophilum* genotypes. Even though MLST does not possess high enough resolution to automatically discriminate between virulent and non-virulent *F. psychrophilum* strains, future typing results certainly has some predictive value for the pathogenicity of specific genotypes in particular fish species even though the correlation between ST and virulence is not consistent for all strains (unpublished results). Still, MLST is laborious and expensive and requires multiple PCRs and sequence analyses before generating a typing result. To aid in rapid discrimination of CC-ST2 genotypes, IS256 could serve as a rapid molecular marker and its potential should be further evaluated.

The dominance of CC-ST2 over a large geographical area indicates that this lineage has a selective advantage over other more sporadically isolated genotypes. Without a selective advantage, it is unlikely that a widespread clone like CC-ST2 could persist over several decades without being broken down by recombination. Antimicrobial resistance, particularly to quinolones, was strongly associated with *F. psychrophilum* genotypes causing disease outbreaks and may therefore constitute one of the main explanations for the persistence and success of epidemic genotypes in aquaculture environments. In general, the evolution and spread of resistance have occurred mainly during the past 50 years, i.e. since the

first use of antimicrobial agents. Outbreaks of BCWD in the Nordic countries Denmark, Finland, Norway and Sweden have been occurring over the last 40 years which is relatively recent in relation to the original import of rainbow trout from North America over 100 years ago. Based on this knowledge, it can only be speculated that the intense nature of fish farming has selected for highly virulent and quinolone resistant *F. psychrophilum* genotypes, which have been spread to aquaculture settings around the world through international trade.

Although *F. psychrophilum* strains involved in disease outbreaks in rainbow trout seem genetically highly homogeneous both on a global and on a local scale, analysis of individual BCWD outbreaks showed that population heterogeneity was a common feature. Multiple genotypes and strains with different colony morphology, plasmid content and antimicrobial susceptibility were concurrently isolated from individual outbreaks and infected fish. Whether heterogeneity is a universal trait in infective *F. psychrophilum* populations in fish farm environments needs further investigation as it may have important functional consequences on the pathogenesis and control of BCWD. At least till now, population heterogeneity resulting from of coinfections with antimicrobial-resistant and sensitive strains and from biofilm forming cells might provide and explanation to why antimicrobial treatment of *F. psychrophilum* infections in fish farms sometimes fail.

As aquaculture production continues to grow and expand, more efficient pathogen control strategies are required to ensure animal health and to decrease the use of antimicrobial agents in fish farming. Infections with *F. psychrophilum* pose a significant threat to salmonid farming around the world and preventative measures are urgently needed. Future prevention of BCWD outbreaks is challenged by the presence of genetically divergent *F. psychrophilum* clones with a pathogenic potential. For a universal vaccine to be effective a combination of antigens might be required, and in such cases, the pathogen population structure must be taken into account. Fortunately, the increased knowledge on the genetic diversity and population structure of *F. psychrophilum* from this and related studies enable a rational selection of representative genotypes for discovering ubiquitous virulence genes as potential targets for a reverse vaccinology approach.

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APPENDIX

Supplementary Table 1. Origin (year of isolation, country, source, tissue), allelic profile (*trp*B, *gyr*B, *dna*K, *fum*C, *mur*G, *tuf*, *atp*A) and sequence type (ST) of 560 *F. psychrophilum* isolates analyzed by multilocus sequence typing (**paper I**). Abbreviations of sources: ACh (*Salvelinus alpinus*), AtS (*Salmo salar*), BkT (*Salvelinus fontinalis*), BrT (*Salmo trutta*), ChH (*Salvelinus fontinalis x Salvelinus namaycush)*, CoS (*Oncorhynchus kisutch*), Flo (*Platichthys flesus*), Per (*Perca fluviatilis*), RbT (*Oncorhynchus mykiss*), StB (*Gasterosteus aculeatus*), S-to-R (smooth-to-rough converted laboratory mutant strains)

Origin						
Year	**Country**	**Source**	**Tissue**	**Isolate**	**Allelic profile**	**ST**
1983	Finland	RbT	Unknown	FI016	10,48,12,10,10,47,14	ST122
1986	France	RbT	Kidney	JIP02/86	8, 8, 2, 2, 2, 2, 2	ST20
1988	Denmark	RbT	Kidney	DK119	2, 8, 8, 2, 2, 2, 2	ST79
1988	Denmark	RbT	Eye	DK120	2, 8, 8, 2, 2, 2, 2	ST79
1988	Sweden	RbT	Tail fin	SE006	2, 8, 2, 2, 2, 7, 2	ST12
1988	Sweden	RbT	Kidney	SE009	2, 8, 2, 2, 2, 7, 2	ST12
1988	Sweden	RbT	Skin	SE010	2, 8, 2, 2, 2, 7, 2	ST12
1989	Denmark	RbT	Spleen	DK121	2, 8, 8, 2, 2, 2, 2	ST79
1989	Denmark	RbT	Spleen	DK122	2, 8, 8, 2, 2, 2, 2	ST79
1989	Denmark	RbT	Spleen	DK123	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK02	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK124	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK125	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Spleen	DK126	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK127	2, 2, 2, 2, 2, 2, 2	ST2
1990	Denmark	RbT	Spleen	DK128	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Spleen	DK129	2, 2, 2, 2, 2, 2, 2	ST2
1990	Denmark	RbT	Kidney	DK130	2, 8, 2, 2, 2, 2, 2	ST10
1990	Denmark	RbT	Intestine	DK131	2, 8, 2, 2, 2, 2, 2	ST10
1990	Denmark	RbT	Kidney	DK132	1,13,15,14, 1, 7, 1	ST70
1990	Denmark	RbT	Intestine	DK133	2, 8, 2, 2, 2, 2, 2	ST10
1990	Denmark	RbT	Spleen	DK134	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK135	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK136	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Kidney	DK137	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Spleen	DK138	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Spleen	DK139	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Spleen	DK140	2, 8, 8, 2, 2, 2, 2	ST79
1990	Denmark	RbT	Spleen	DK141	2, 8, 8, 2, 2, 2, 2	ST79
1990	Sweden	RbT	Kidney	SE018	2, 8, 2, 2, 2, 7, 2	ST12
1990	Sweden	RbT	Kidney	SE019	2, 8, 2, 2, 2,48, 2	ST128
1990	Sweden	RbT	Kidney	SE022	2, 8, 2, 2, 2, 7, 2	ST12
1990	USA	CoS	Kidney	THC02/90	4, 7, 6, 5, 6, 5, 4	ST9
1991	Denmark	RbT	Kidney	BN205	2, 8, 8, 2, 2, 2, 2	ST79
1991	Denmark	RbT	Spleen	DK12	31,46, 9,10,35,50,14	ST120
1991	Denmark	RbT	Kidney	DK161	2, 8, 8, 2, 2, 2, 2	ST79
1991	Denmark	RbT	Kidney	DK162	2, 8, 8, 2, 2, 2, 2	ST79
1991	Denmark	RbT	Kidney	DK163	2, 8, 8, 2, 2, 2, 2	ST79
1991	Denmark	RbT	Kidney	DK164	2, 8, 8, 2, 2, 2, 2	ST79
1991	Denmark	RbT	Spleen	DK165	2, 2, 2, 2, 2, 2, 2	ST2
1991	Sweden	RbT	Kidney	SE021	2, 8, 2, 2, 2, 7, 2	ST12
1991	Sweden	RbT	Kidney	SE024	2, 8, 8, 2, 2, 2, 2	ST79
1991	Sweden	RbT	Kidney	SE025	39,53, 8, 7,30,54, 2	ST233
1991	Sweden	RbT	Skin	SE026	2, 8, 8, 2, 2, 2, 2	ST79
1991	Sweden	RbT	Kidney	SE027	8, 8,22, 3, 3, 2, 3	ST234
1991	Sweden	RbT	Kidney	SE028	2, 8, 8, 2, 2, 2, 2	ST79
1991	Sweden	RbT	Kidney	SE029	2, 8, 2, 2, 2, 7, 2	ST12
1992	Denmark	RbT	Kidney	BN208	2, 2, 2, 2, 2, 2, 2	ST2
1992	Denmark	RbT	Kidney	BN209	2, 8, 2, 2, 2, 7, 2	ST12
1992	Denmark	RbT	Spleen	BN210	2, 8, 2, 2, 2, 7, 2	ST12
1992	Sweden	RbT	Kidney	SE051	2, 8, 8, 2, 2, 3, 2	ST235

1992	Sweden	BrT	Fin	SE052	8,50,22, 3, 3, 2, 3	ST236
1992	Sweden	RbT	Kidney	SE053	8,50,22, 3, 3, 2, 3	ST236
1993	Denmark	RbT	Kidney	BN206	2, 2, 2, 2, 2, 2, 2	ST2
1993	Denmark	RbT	Kidney	BN207	2, 2, 2, 2, 2, 2, 2	ST2
1993	Denmark	RbT	Kidney	BN211	2, 2, 2, 2, 2, 2, 2	ST2
1993	Finland	RbT	Lesion	FI017	2, 8, 8, 2, 2, 2, 2	ST79
1993	Finland	RbT	Lesion	FI018	2, 8, 8, 2, 2, 2, 2	ST79
1993	Finland	BrT	Jaw lesion	FI182	32,23, 8,10,10,22,42	ST121
1993	Sweden	BrT	Tail fin	SE055	11,49, 2,11,44, 2, 3	ST237
1993	Sweden	RbT	Spleen	SE056	2, 8, 8, 2, 2, 3, 2	ST235
1994	Finland	BrT	Kidney	3558/1/94	13,22, 8,10, 6,20,20	ST36
1994	Denmark	RbT	Kidney	DK03	2, 8, 8, 2, 2, 2, 2	ST79
1994	Denmark	RbT	Brain	DK07	2, 8, 2, 2, 2, 2, 2	ST10
1994	Denmark	RbT	Kidney	DK08	2, 2, 2, 2, 2, 2, 2	ST2
1994	Denmark	RbT	Brain	DK145	2, 2, 2, 2, 2, 2, 2	ST2
1994	Denmark	RbT	Eye	DK154	2, 8, 2, 2, 2, 7, 2	ST12
1994	Denmark	RbT	Kidney	DK155	2, 2, 2, 2, 2, 2, 2	ST2
1994	Finland	RbT	Kidney	FI181	13,22, 8,10, 6,20,20	ST36
1994	Sweden	RbT	Kidney	SE076	2, 8, 2, 2, 2, 2, 2	ST10
1994	Sweden	RbT	Kidney	SE080	2, 8, 8, 2, 2, 3, 2	ST235
1994	Sweden	RbT	Kidney	SE091	2, 8, 8, 2, 2,49, 2	ST238
1994	Sweden	ChH	Spleen	SE100	8,63,31, 3, 2, 3, 3	ST239
1995	Denmark	RbT	Kidney	DK009	11,29, 8, 3,32,41,31	ST138
1995	Denmark	RbT	Spleen	DK04	2, 2, 2, 2, 2, 2, 2	ST2
1995	Denmark	RbT	Spleen	DK06	2, 8, 2, 2, 2, 2, 2	ST10
1995	Denmark	RbT	Brain	DK115	2, 2, 2, 2, 2, 2, 2	ST2
1995	Denmark	RbT	Kidney	DK116	2, 2, 2, 2, 2, 2, 2	ST2
1995	Denmark	RbT	Ulcer	DK117	2, 2, 2, 2, 2, 2, 2	ST2
1995	Denmark	RbT	Ulcer	DK118	2, 2, 2, 2, 2, 2, 2	ST2
1995	Denmark	RbT	Brain	DK146	11,29, 8, 3,32,41,31	ST138
1995	Denmark	RbT	Brain	DK147	2, 8, 2, 2, 2, 7, 2	ST12
1995	Denmark	RbT	Spleen	DK148	11,29, 8, 3,32,41,31	ST138
1995	Denmark	RbT	Brain	DK149	11,29, 8, 3,32,41,31	ST138
1995	Denmark	RbT	Kidney	DK150	11,29, 8, 3,32,41,31	ST138
1995	Denmark	RbT	Kidney	DK151	2, 8, 8, 2, 2, 2, 2	ST79
1995	Denmark	RbT	Brain	DK152	38,21,28, 2,18,20,53	ST231
1995	Denmark	RbT	Spleen	DK153	2, 2, 2, 2, 2, 2, 2	ST2
1995	Denmark	RbT	Brain	DK156	4, 3,22, 3,20, 3, 3	ST192
1995	Denmark	RbT	Brain	DK157	3,19,13, 9,12,16,15	ST31
1995	Denmark	RbT	Brain	DK158	2, 8, 2, 2, 2, 2, 2	ST10
1995	Denmark	RbT	Brain	DK159	4, 3,22, 3,20, 3, 3	ST192
1995	Denmark	RbT	Brain	DK160	4, 3,22, 3,20, 3, 3	ST192
1995	Finland	RbT	Jaw lesion	FI176	8,50,22, 3, 3, 3, 3	ST139
1995	Sweden	RbT	Kidney	SE117	2, 8, 8, 2, 2, 7, 2	ST240
1996	Finland	BrT	Spleen	10424/1/96	9,13,11, 7, 9,13,12	ST23
1996	Finland	Ach	Skin	12714/1/96	9,13,11, 7, 9,13,12	ST23
1996	Finland	BrT	Brain	P14-5/96	12,21, 8, 7,17,19,19	ST35
1996	Finland	BrT	Brain	P14-6/96	4, 7, 6, 5, 6, 8, 4	ST13
1996	Denmark	RbT	Spleen	DK114	2, 2, 2, 2, 2,48, 2	ST98
1996	Finland	RbT	Inner organs	FI055	2, 8, 2, 2, 2, 7, 2	ST12
1996	Finland	RbT	Inner organs	FI056	2, 8, 8, 2, 2, 2, 2	ST79
1996	Finland	RbT	Brain	FI155	8,13,15,14, 1, 7, 1	ST73
1996	Finland	RbT	Liver	FI156	2, 8, 2, 2, 2, 7, 2	ST12
1996	Finland	RbT	Brain	FI157	2, 8, 2, 2, 2, 7, 2	ST12
1996	Finland	RbT	Inner organs	FI160	2, 8, 8, 2, 2, 2, 2	ST79
1996	Finland	BkT	Spleen	FI183	13,21,16, 7,17,21,21	ST37
1996	Finland	RbT	Inner organs	FI189	2, 8, 2, 2, 2, 7, 2	ST12
1996	Finland	RbT	Brain	FI190	2, 8, 8, 2, 2, 2, 2	ST79
1996	Finland	RbT	Spleen	FI191	2, 8, 8, 2, 2, 2, 2	ST79
1996	Finland	RbT	Kidney	FI192	2, 8, 8, 2, 2, 2, 2	ST79
1996	Finland	RbT	Liver	FI193	2, 8, 8, 2, 2, 2, 2	ST79
1996	Norway	AtS	Fin	NO019	4,49,22,11, 6,50, 3	ST184
1996	Sweden	RbT	Kidney	SE139	2, 8, 8, 2, 2, 7, 2	ST240
1996	Sweden	RbT	Kidney	SE164	2, 8, 8, 2, 2, 7, 2	ST240
1997	Finland	RbT	Inner organs	FI177	1,13,15, 2, 2, 7, 2	ST125
1997	Finland	RbT	Inner organs	FI178	2, 8, 8, 2, 2, 2, 2	ST79
1997	Finland	RbT	Inner organs	FI179	2, 8, 2, 2, 2, 7, 2	ST12
1997	Finland	RbT	Inner organs	FI180	2, 8, 8, 2, 2, 2, 2	ST79
1997	Finland	RbT	Inner organs	FI184	2, 8, 2, 2, 2, 7, 2	ST12
1997	Finland	RbT	Inner organs	FI185	2, 8, 2, 2, 2, 7, 2	ST12
1997	Finland	RbT	Inner organs	FI186	4, 3,22, 3, 3, 7, 3	ST140
1997	Finland	RbT	Spleen	FI187	2, 8, 2, 2, 2, 7, 2	ST12
1997	Finland	RbT	Spleen	FI188	2, 8, 8, 2, 2, 2, 2	ST79

1997	Norway	BrT	Fin	NO020	4,49,15,11, 6, 3, 3	ST185
1997	Norway	BrT	Fin	NO021	13,22, 8,10,17,22,42	ST186
1997	Norway	BrT	Fin	NO022	9,13,11, 7, 9,13,12	ST23
1997	Norway	BrT	Fin	NO023	9,13,11, 7, 9,13,12	ST23
1997	Norway	BrT	Fin	NO024	13,21,16, 7,17,21,21	ST37
1997	Norway	BrT	Fin	NO025	13,22, 8,10,17,22,42	ST186
1997	Norway	BrT	Fin	NO026	34,19,26, 5,39,24,20	ST188
1997	Norway	AtS	Fin	NO044	4,49,22,11, 6, 3, 3	ST123
1998	Norway	BrT	Fin	NO003	9,13,11, 7, 9,13,12	ST23
1998	Norway	BrT	Fin	NO004	32,23, 8,10,10,22,42	ST121
1998	Norway	BrT	Fin	NO028	7,51, 8,17,33,17,43	ST171
1998	Norway	BrT	Fin	NO029	9,13,11, 7, 9,13,12	ST23
1999	Denmark	Rbt	Milt	DK037	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	Water	Water	DK050	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Milt	DK078	4,29,16, 5, 2,49,20	ST177
1999	Denmark	RbT	Brain	DK112	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK113	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK166	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK167	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK168	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK169	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK170	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK171	2, 2, 2, 2, 2,48, 2	ST98
1999	Denmark	RbT	Kidney	DK172	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK173	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Spleen	DK174	2, 2, 2, 2, 2,48, 2	ST98
1999	Denmark	RbT	Brain	DK175	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK176	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK177	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Spleen	DK178	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK179	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK180	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Spleen	DK181	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK182	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK183	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Spleen	DK184	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK185	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Brain	DK186	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Spleen	DK187	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Gills	DK26	4,43, 2, 3,22, 3, 3	ST175
1999	Denmark	RbT	Ovarian fluid	DK27	2,15, 2, 2, 2, 2, 2	ST26
1999	Denmark	RbT	Liver	DK28	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Spleen	DK29	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Ovarian fluid	DK30	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Milt	DK31	4, 3,22, 2, 3, 3, 3	ST176
1999	Denmark	RbT	Milt	DK32	2, 2, 2, 2, 2,48, 2	ST98
1999	Denmark	RbT	Milt	DK33	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Milt	DK34	4, 3,22, 2, 3, 3, 3	ST176
1999	Denmark	RbT	Milt	DK35	4, 3,22, 2, 3, 3, 3	ST176
1999	Denmark	RbT	Ovarian fluid	DK36	2, 2, 2, 2, 2,48, 2	ST98
1999	Denmark	RbT	Ovarian fluid	DK38	2,15, 2, 2, 2, 2, 2	ST26
1999	Denmark	RbT	Milt	DK39	4, 3,22, 2, 3, 3, 3	ST176
1999	Denmark	RbT	Milt	DK40	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Ovarian fluid	DK41	2, 2, 2, 2, 2,48, 2	ST98
1999	Denmark	RbT	Ovarian fluid	DK42	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	Water	Water	DK43	2, 2, 2, 2, 2,48, 2	ST98
1999	Denmark	RbT	Milt	DK44	4,29,16, 5, 2,49,20	ST177
1999	Denmark	RbT	Milt	DK45	15,29, 8, 3,32,41,31	ST178
1999	Denmark	RbT	Milt	DK46	11,29, 8, 3,32,53,31	ST179
1999	Denmark	RbT	Milt	DK47	15,29, 8, 3,32,41,31	ST178
1999	Denmark	RbT	Ovarian fluid	DK49	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Gills	DK51	4, 3,22, 2, 3, 3, 3	ST176
1999	Denmark	RbT	Perit. cavity	DK52	4, 3,22, 2, 3, 3, 3	ST176
1999	Denmark	RbT farm	Water	DK53	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT farm	Water	DK54	2, 2, 2, 2, 2, 2, 2	ST2
1999	Denmark	RbT	Kidney	DK57	2, 2, 2, 2, 2, 2, 2	ST2
1999	Finland	Water	Water	FI152	2, 8, 2, 2, 2, 2, 2	ST10
1999	Finland	Water	Water	FI153	1,13,15, 2, 2, 7, 2	ST125
1999	Finland	Water	Water	FI154	9,13,11, 7, 9,13,12	ST23
1999	Finland	AtS	Unknown	FI195	1,13,15,14, 1, 7, 1	ST70
1999	Finland	AtS	Unknown	FI197	2,28, 8,10,17,19,42	ST198
1999	Finland	AtS	Unknown	FI198	21,23, 8, 7,38,51,42	ST141
1999	Norway	BrT	Fin	NO005	9,13,11, 7, 9,13,12	ST23

1999	Norway	AtS	Fin	NO030	10,48,12,10,10,47,14	ST122
1999	Norway	AtS	Fin	NO031	10,48,12,10,10,47,14	ST122
1999	Norway	BrT	Fin	NO032	32,23, 8,10,10,22,42	ST121
2000	Denmark	RbT	Brain	DK080	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	RbT	Brain	DK081	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	RbT	Kidney	DK082	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	RbT	Brain	DK083	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	RbT	Spleen	DK084	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	RbT	Spleen	DK085	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	BrT	Gills	DK086	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	BrT	Gills	DK088	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	BrT	Gills	DK089	4,49,22,17, 2, 3,47	ST194
2000	Denmark	BrT	Gills	DK090	2, 2, 2, 2, 2, 2, 2	ST2
2000	Denmark	BrT	Skin	DK091	4, 3,22, 3, 3, 3, 3	ST191
2000	Denmark	BrT	Gills	DK092	4, 3,22, 3,20, 3, 3	ST192
2000	Denmark	RbT	Gills	DK093	4, 3,22, 3,20, 3, 3	ST192
2000	Denmark	RbT	Gills	DK094	4,18,22,17, 2, 3,47	ST193
2000	Denmark	StB	Skin	DK095	4,18,22,17, 2, 3,47	ST193
2000	Denmark	StB	Abd. cavity	DK096	4,18,22,17, 2, 3,47	ST193
2000	Denmark	StB	Gills	DK097	4,49,22,17, 2, 3,47	ST194
2000	Denmark	Flo	Gills	DK098	18,54,22, 3,40, 3, 3	ST195
2000	Finland	RbT	Skin lesion	FI131	1, 1,15, 2, 2, 7, 2	ST131
2000	Finland	RbT	Skin mucus	FI132	1, 1,15, 2, 2, 7, 2	ST131
2000	Finland	RbT	Skin lesion	FI133	1, 1,15, 2, 2, 7, 2	ST131
2000	Finland	RbT	Skin mucus	FI134	1, 1,15, 2, 2, 7, 2	ST131
2000	Finland	RbT	Skin lesion	FI135	1, 1,15, 2, 2, 7, 2	ST131
2000	Finland	RbT	Skin mucus	FI136	1, 1,15, 2, 2, 7, 2	ST131
2000	Finland	RbT	Spleen	FI137	2, 8, 2, 2, 2, 7, 2	ST12
2000	Finland	RbT	Spleen	FI138	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Spleen	FI139	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Kidney	FI140	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Skin lesion	FI141	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Kidney	FI142	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Skin lesion	FI143	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Kidney	FI144	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	RbT	Skin lesion	FI145	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	Water	Water	FI146	11,49, 2,11, 3, 3, 3	ST132
2000	Finland	Water	Water	FI147	9,13,11, 7, 9,13,12	ST23
2000	Finland	Water	Water	FI148	9,13,11, 7, 9,13,12	ST23
2000	Finland	Water	Water	FI149	33,37, 8, 5, 2,26, 2	ST133
2000	Finland	Per	Skin mucus	FI150	11,49, 2,11, 3, 3, 3	ST132
2000	Finland	Per	Skin mucus	FI151	34,19, 8, 5,37,24,20	ST134
2000	Finland	RbT	Skin lesion	FI158	2, 8, 8, 2, 2, 2, 2	ST79
2000	Finland	Per	Skin mucus	FI159	11,49, 2,11, 3, 3, 3	ST132
2000	Finland	RbT	Ovarian fluid	FI161	1,13,15, 2, 2, 3, 2	ST135
2000	Finland	RbT	Ovarian fluid	FI162	1,13,15, 2, 2, 7, 2	ST125
2000	Finland	RbT	Ovarian fluid	FI164	2, 8, 2, 2, 2, 7, 2	ST12
2000	Norway	AtS	Unknown	NO006	9,13,11, 7, 9,13,12	ST23
2000	Norway	AtS	Fin	NO033	9,13,11, 7, 9,13,12	ST23
2000	Norway	AtS	Unknown	NO045	10,48,12,10,10,47,14	ST122
2001	Denmark	RbT	Spleen	DK109	2, 8, 2, 2, 2, 2, 2	ST10
2001	Denmark	RbT	Spleen	DK110	2, 2, 2, 2, 2, 2, 2	ST2
2001	Denmark	RbT	Spleen	DK111	2, 2, 2, 2, 2, 2, 2	ST2
2001	Denmark	RbT	Ulcer	DK142	4, 3,22, 3, 3, 3, 2	ST181
2001	Denmark	RbT	Kidney	DK60	2, 2, 2, 2, 2, 2, 2	ST2
2001	Sweden	RbT	Kidney	SE328	2,13,15,14, 1, 2, 2	ST241
2002	Denmark	RbT	Spleen	DK103	2, 2, 2, 2, 2, 2, 2	ST2
2002	Denmark	RbT	Spleen	DK104	2, 2, 2, 2, 2, 2, 2	ST2
2002	Denmark	RbT	Spleen	DK105	2, 2, 2, 2, 2, 2, 2	ST2
2002	Denmark	RbT	Spleen	DK106	2, 2, 2, 2, 2, 2, 2	ST2
2002	Denmark	RbT	Spleen	DK107	2, 8, 2, 2, 2, 2, 2	ST10
2002	Denmark	RbT	Spleen	DK108	2, 2, 2, 2, 2, 2, 2	ST2
2002	Denmark	RbT	Spleen	DK59	2, 2, 2, 2, 2, 2, 2	ST2
2002	Finland	AtS	Unknown	FI196	9,13,11, 7, 9,13,12	ST23
2002	Sweden	RbT	Kidney	SE374	2, 2, 2, 2, 2, 2, 2	ST2
2003	Denmark	RbT	Spleen	DK100	2, 2, 2, 2, 2, 2, 2	ST2
2003	Denmark	RbT	Spleen	DK101	2, 2, 2, 2, 2, 2, 2	ST2
2003	Denmark	RbT	Spleen	DK102	2, 2, 2, 2, 2, 2, 2	ST2
2003	Finland	RbT	Spleen	FI095	2, 8, 2, 2, 2,48, 2	ST128
2003	Finland	RbT	Spleen	FI096	2, 8, 2, 2, 2,48, 2	ST128
2003	Finland	RbT	Spleen	FI097	2, 8, 2, 2, 2,48, 2	ST128
2003	Finland	RbT	Inner organs	FI109	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI110	2, 8, 8, 2, 2, 2, 2	ST79

2003	Finland	RbT	Inner organs	FI111	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI112	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI113	2, 8, 2, 2, 2, 7, 2	ST12
2003	Finland	RbT	Inner organs	FI114	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI115	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI116	8, 8, 8, 2, 2, 2, 2	ST130
2003	Finland	RbT	Inner organs	FI117	2, 8, 2, 2, 2, 7, 2	ST12
2003	Finland	RbT	Inner organs	FI118	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI119	2, 8, 8, 2, 2, 2, 2	ST79
2003	Finland	RbT	Inner organs	FI120	2, 8, 8, 2, 2, 2, 2	ST79
2003	Sweden	RbT	Kidney	SE409	2, 8, 2, 2, 2, 2, 2	ST10
2004	Denmark	RbT	Ulcer	DK024	2, 2, 2, 2, 2, 2, 2	ST2
2004	Denmark	RbT	Ulcer	DK099	2, 2, 2, 2, 2, 2, 2	ST2
2004	Denmark	RbT	Spleen	DK25	2, 2, 2, 2, 2, 2, 2	ST2
2004	Norway	RbT	Unknown	NO009	2, 2, 2, 2, 2, 2, 2	ST2
2004	Norway	RbT	Inner organs	NO046	2, 2, 2, 2, 2, 2, 2	ST2
2005	Denmark	RbT	Skin	DK144	4,19, 2, 3, 3, 3, 3	ST207
2005	Finland	RbT	Kidney	FI172	11,29, 8, 3,32,41,31	ST138
2005	Finland	RbT	Kidney	FI173	11,29, 8, 3,32,41,31	ST138
2005	Finland	RbT	Spleen	FI174	11,29, 8, 3,32,41,31	ST138
2005	Finland	RbT	Kidney	FI175	11,29, 8, 3,32,41,31	ST138
2006	Denmark	RbT	Skin	DK063	2,37, 8, 2,16,47,46	ST190
2006	Denmark	RbT	Brain	DK076	4, 3,22, 3, 3, 3, 2	ST181
2006	Denmark	RbT	Skin	DK077	2, 8, 2, 2, 2, 2, 2	ST10
2006	Denmark	RbT	Ulcer	DK22	2, 8, 2, 2, 2, 2, 2	ST10
2006	Denmark	RbT	Kidney	DK23	2, 8, 2, 2, 2, 2, 2	ST10
2006	Denmark	RbT	Kidney	DK48	3, 2, 2, 2, 2,41, 2	ST92
2006	Denmark	RbT	Skin	DK61	35,21, 8, 7,28,19,44	ST180
2006	Denmark	RbT	Skin	DK62	4, 3,22, 3, 3, 3, 2	ST181
2006	Denmark	RbT	Skin	DK64	2, 2, 2, 2, 2, 2, 2	ST2
2006	Denmark	RbT	Skin lesion	DK65	2, 8, 2, 2, 2, 2, 2	ST10
2006	Denmark	RbT	Skin	DK66	2, 2, 2, 2, 2, 2, 2	ST2
2006	Denmark	RbT	Gills	DK67	15,53, 8, 7,30,54,45	ST182
2006	Denmark	RbT	Gills	DK68	4, 3,22, 3,32, 3, 3	ST183
2006	Denmark	RbT	Gills	DK69	35,21, 8, 7,28,19,44	ST180
2006	Denmark	RbT	Gills	DK70	2, 8, 2, 2, 2, 2, 2	ST10
2006	Denmark	RbT	Gills	DK71	2, 2, 2, 2, 2, 2, 2	ST2
2006	Denmark	RbT	Gills	DK72	2, 2, 2, 2, 2, 2, 2	ST2
2006	Denmark	RbT	Gills	DK73	4, 3,22, 3, 3, 3, 2	ST181
2006	Denmark	RbT	Spleen	DK74	2, 2, 2, 2, 2, 2, 2	ST2
2006	Denmark	RbT	Brain	DK75	2, 8, 2, 2, 2, 2, 2	ST10
2006	Finland	Per	Mouth	FI070	1,20,15, 3,16, 6,18	ST34
2006	Finland	Per	Mouth	FI071	1,20,15, 3,16, 6,18	ST34
2006	Finland	Per	Kidney	FI072	1,20,15, 3,16, 6,18	ST34
2006	Finland	Per	Kidney	FI073	1,55,15, 3,16, 6,18	ST196
2006	Finland	Per	Mouth	FI074	8,37, 8, 2,36,47,18	ST127
2006	Finland	Per	Mouth	FI075	1,20,15, 3,16, 6,18	ST34
2006	Finland	Per	Mouth	FI076	1,20,15, 3,16, 6,18	ST34
2006	Finland	Per	Kidney	FI077	8,37, 8, 2,36,47,18	ST127
2006	Finland	RbT	Spleen	FI108	2, 8, 8, 2, 2, 2, 2	ST79
2006	Norway	AtS	Skin ulcer	NO034	4,49, 8,11, 6, 3, 3	ST168
2007	Denmark	RbT	Brain	DK58	2, 2, 2, 2, 2, 2, 2	ST2
2007	Finland	RbT	Kidney	FI049	1,13,15, 2, 2, 7, 2	ST125
2007	Finland	RbT	Spleen	FI051	1,13,15, 2, 2, 7, 2	ST125
2007	Finland	RbT	Spleen	FI052	1,13,15, 2, 2, 7, 2	ST125
2007	Finland	RbT	Kidney	FI053	1,13,15, 2, 2, 7, 2	ST125
2007	Norway	RbT	Spleen	NO010	2, 2, 2, 2, 2, 2, 2	ST2
2007	Norway	RbT	Spleen	NO047	2, 2, 2, 2, 2, 2, 2	ST2
2007	Norway	AtS	Ulcer	NO048	4,49, 8,11, 6, 3, 3	ST168
2008	Denmark	RbT	Brain	BN212	2, 2, 2, 2, 2, 2, 2	ST2
2008	Denmark	RbT	Absces	BN213	2, 2, 2, 2, 2, 2, 2	ST2
2008	Denmark	RbT	Kidney	BN214	2, 2, 2, 2, 2, 2, 2	ST2
2008	Denmark	RbT	Spleen	DK05	2, 2, 2, 2, 2, 2, 2	ST2
2008	Finland	RbT	Kidney	FI098	3, 2, 2, 2, 2,41, 2	ST92
2008	Finland	RbT	Fin	FI099	3, 2, 2, 2, 2,41, 2	ST92
2008	Finland	RbT	Spleen	FI100	3, 2, 2, 2, 2,41, 2	ST92
2008	Finland	RbT	Spleen	FI101	3, 2, 2, 2, 2,41, 2	ST92
2008	Finland	RbT	Spleen	FI102	12,21, 8, 7,17, 7,19	ST197
2008	Finland	RbT	Kidney	FI104	2,13, 2,14, 1, 7, 1	ST129
2008	Finland	RbT	Kidney	FI105	3, 2, 2, 2, 2,41, 2	ST92
2008	Finland	RbT	Kidney	FI106	3, 2, 2, 2, 2,41, 2	ST92
2008	Finland	RbT	Kidney	FI107	3, 2, 2, 2, 2,41, 2	ST92
2008	Norway	RbT	Kidney	NO007	2, 2, 2, 2, 2, 2, 2	ST2

2008	Norway	AtS	Skin lesion	NO011	10,48,12,10,10,47,14	ST122
2008	Norway	AtS	Fin	NO012	9,13,15,14, 1, 7, 1	ST187
2008	Norway	RbT	Kidney	NO013	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Spleen	NO014	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO015	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO016	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO017	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Skin ulcer	NO018	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO035	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO036	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO037	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO038	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Skin ulcer	NO040	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	AtS	Kidney	NO041	1,13,15,14, 1, 7, 1	ST70
2008	Norway	AtS	Spleen	NO042	1,13,15,14, 1, 7, 1	ST70
2008	Norway	RbT	Kidney	NO049	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	AtS	Skin ulcer	NO050	4,49, 8,11, 6, 3, 3	ST168
2008	Norway	RbT	Kidney	NO051	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Spleen	NO052	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO053	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO054	2, 2, 2, 2, 2, 2, 2	ST2
2008	Norway	RbT	Kidney	NO055	2, 2, 2, 2, 2, 2, 2	ST2
2009	Denmark	RbT	Spleen	BN188	2, 8, 2, 2, 2, 2, 2	ST10
2009	Denmark	RbT	Spleen	DK01	2, 8, 2, 2, 2, 2, 2	ST10
2009	Denmark	RbT	Kidney	DK189	2, 8, 2, 2, 2, 2, 2	ST10
2009	Denmark	RbT	Spleen	DK190	2, 8, 2, 2, 2, 2, 2	ST10
2009	Denmark	RbT	Spleen	DK191	2, 8, 2, 2, 2, 2, 2	ST10
2009	Denmark	RbT	Spleen	DK192	2, 8, 2, 2, 2, 2, 2	ST10
2009	Finland	RbT	Spleen	FI089	3, 2, 2, 2, 2,41, 2	ST92
2009	Finland	RbT	Lesion	FI090	2, 2, 2, 2, 2,41, 2	ST91
2009	Finland	RbT	Kidney	FI091	2, 2, 2, 2, 2,41, 2	ST91
2009	Finland	RbT	Lesion	FI092	2, 2, 2, 2, 2,41, 2	ST91
2009	Finland	RbT	Spleen	FI093	3, 2, 2, 2, 2,41, 2	ST92
2009	Finland	RbT	Lesion	FI094	3, 2, 2, 2, 2,41, 2	ST92
2009	Norway	AtS	Fin	NO039	1,13,15,14, 1, 7, 1	ST70
2009	Norway	AtS	Spleen	NO043	32,23, 8,10,10,22,42	ST121
2009	Norway	AtS	Kidney	NO056	1,13,15,14, 1, 7, 1	ST70
2009	Norway	AtS	Kidney	NO057	1,13,15,14, 1, 7, 1	ST70
2009	Norway	RbT	Kidney	NO058	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Kidney	NO059	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Skin ulcer	NO060	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	AtS	Kidney	NO061	8, 3,15, 1, 1, 1, 1	ST169
2009	Norway	AtS	Kidney	NO062	32,23, 8,10,10,52,42	ST170
2009	Norway	AtS	Kidney	NO063	32,23, 8,10,10,52,42	ST170
2009	Norway	AtS	Fin rot	NO066	4,49, 8,11, 6, 3, 3	ST168
2009	Norway	AtS	Fin rot	NO067	4,49, 8,11, 6, 3, 3	ST168
2009	Norway	AtS	Fin rot	NO068	1,13,15,14, 1, 7, 1	ST70
2009	Norway	AtS	Fin rot	NO069	1,13,15,14, 1, 7, 1	ST70
2009	Norway	AtS	Fin rot	NO070	7,51, 8,17,33,17,43	ST171
2009	Norway	AtS	Fin rot	NO071	7,51, 8,17,33,17,43	ST171
2009	Norway	RbT	Spleen	NO085	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Ulcer	NO086	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Spleen	NO088	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Spleen	NO089	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Kidney	NO090	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Kidney	NO091	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Kidney	NO092	2, 2, 2, 2, 2, 2, 2	ST2
2009	Norway	RbT	Kidney	NO101	4, 3,22, 3, 3, 3, 2	ST181
2009	Norway	RbT	Kidney	NO102	4, 3,22, 3, 3, 3, 2	ST181
2009	Norway	RbT	Kidney	NO103	4, 3,22, 3, 3, 3, 2	ST181
2010	Denmark	RbT	Kidney	DK143	2, 2, 2, 2, 2, 2, 2	ST2
2010	Denmark	RbT	Kidney	DK193	2, 2, 2, 2, 2, 2, 2	ST2
2010	Denmark	RbT	Kidney	DK194	2, 2, 2, 2, 2, 2, 2	ST2
2010	Denmark	RbT	Spleen	DK195	2, 2, 2, 2, 2, 2, 2	ST2
2010	Denmark	RbT	Spleen	DK196	2, 2, 2, 2, 2, 2, 2	ST2
2010	Denmark	RbT	Spleen	DK197	2, 2, 2, 2, 2, 2, 2	ST2
2010	Denmark	RbT	Spleen	DK198	2, 8, 2, 2, 2, 2, 2	ST10
2010	Denmark	RbT	Spleen	DK199	2, 8, 2, 2, 2, 2, 2	ST10
2010	Denmark	RbT	Spleen	DK200	2, 8, 2, 2, 2, 2, 2	ST10
2010	Finland	RbT	Spleen	FI001	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Lesion	FI002	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI003	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Lesion	FI004	3, 2, 2, 2, 2,41, 2	ST92

2010	Finland	RbT	Spleen	FI005	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Eye	FI006	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI007	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI008	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI009	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Eye	FI010	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI011	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI012	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Muscle	FI013	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI014	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI015	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI079	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI080	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI081	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI082	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI083	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI084	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI085	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI086	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Kidney	FI087	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI088	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Eye	FI121	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI122	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Eye	FI123	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Egg	FI168	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Egg	FI169	4,21,26, 5,14,49,20	ST137
2010	Finland	RbT	Egg	FI170	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	S-to-R converted FI170		FI171	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	S-to-R converted FI168		FI204	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	S-to-R converted FI083		FI205	3, 2, 2, 2, 2,41, 2	ST92
2010	Finland	RbT	Spleen	FI206	3, 2, 2, 2, 2,41, 2	ST92
2010	Norway	AtS	Kidney	NO072	9,49, 8,11, 6, 3, 3	ST172
2010	Norway	AtS	Kidney	NO073	4,52,22,11, 6, 3, 3	ST173
2010	Norway	AtS	Fin rot tail	NO074	7,51, 8,17,33,17,43	ST171
2010	Norway	AtS	Fin rot tail	NO075	7,51, 8,17,33,17,43	ST171
2010	Norway	AtS	Skin ulcer	NO076	1,13,15,14, 1, 7, 1	ST70
2010	Norway	AtS	Fin rot	NO077	1,13,15,14, 1, 7, 1	ST70
2010	Norway	RbT	Kidney	NO094	2, 2, 2, 2, 2, 2, 2	ST2
2011	Denmark	RbT	Spleen	DK13	3, 2, 2, 2, 2,41, 2	ST92
2011	Denmark	RbT	Spleen	DK14	3, 2, 2, 2, 2,41, 2	ST92
2011	Denmark	RbT	Spleen	DK15	3, 2, 2, 2, 2,41, 2	ST92
2011	Denmark	RbT	Spleen	DK16	3, 2, 2, 2, 2,41, 2	ST92
2011	Denmark	RbT	Spleen	DK17	3, 2, 2, 2, 2,41, 2	ST92
2011	Denmark	RbT	Spleen	DK18	3, 2, 2, 2, 2,41, 2	ST92
2011	Denmark	RbT	Spleen	DK19	2, 2, 2, 2, 2, 2, 2	ST2
2011	Denmark	RbT	Spleen	DK20	2, 2, 2, 2, 2, 2, 2	ST2
2011	Denmark	RbT	Spleen	DK201	2, 8, 2, 2, 2, 2, 2	ST10
2011	Denmark	RbT	Spleen	DK21	2, 2, 2, 2, 2, 2, 2	ST2
2011	Denmark	RbT	Spleen	DK56	2, 8, 2, 2, 2, 2, 2	ST10
2011	Finland	RbT	Kidney	FI019	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Kidney	FI020	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Kidney	FI021	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Kidney	FI022	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Lesion	FI023	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI024	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI025	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI026	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Kidney	FI027	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Kidney	FI028	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI029	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI030	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI031	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI032	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Spleen	FI033	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Spleen	FI034	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Spleen	FI035	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Spleen	FI036	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI037	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI038	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI039	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Lesion	FI040	2,13,15,14, 1, 7, 1	ST124
2011	Finland	RbT	Kidney	FI041	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Spleen	FI042	3, 2, 2, 2, 2,41, 2	ST92

2011	Finland	RbT	Spleen	FI043	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Spleen	FI044	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Lesion	FI045	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Lesion	FI046	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Lesion	FI047	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Lesion	FI048	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Spleen	FI057	2,13,15,16, 1, 7, 1	ST126
2011	Finland	RbT	Kidney	FI058	2,13,15,16, 1, 7, 1	ST126
2011	Finland	RbT	Kidney	FI059	2, 2, 2, 2, 2, 2, 2	ST2
2011	Finland	RbT	Spleen	FI060	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Spleen	FI061	2, 2, 2, 2, 2, 2, 2	ST2
2011	Finland	RbT	Kidney	FI062	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	RbT	Kidney	FI063	2, 2, 2, 2, 2, 2, 2	ST2
2011	Finland	RbT	Spleen	FI064	2,13,15,16, 1, 7, 1	ST126
2011	Finland	RbT	Spleen	FI065	2, 2, 2, 2, 2, 2, 2	ST2
2011	Finland	RbT	Kidney	FI066	2,13,15,16, 1, 7, 1	ST126
2011	Finland	RbT	Spleen	FI067	2,13,15,16, 1, 7, 1	ST126
2011	Finland	RbT	Kidney	FI068	2, 2, 2, 2, 2, 2, 2	ST2
2011	Finland	RbT	Spleen	FI069	3, 2, 2, 2, 2,41, 2	ST92
2011	Finland	AtS	Unknown	FI194	13,22, 8,10, 6,20,20	ST36
2011	Finland	RbT	Kidney	FI200	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO078	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO079	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Skin ulcer	NO080	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Skin ulcer	NO081	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO082	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO083	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO084	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO087	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO095	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO096	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	AtS	Ovarian fluid	NO097	9,13,11, 7, 9,13,12	ST23
2011	Norway	AtS	Ovarian fluid	NO098	9,13,11, 7, 9,13,12	ST23
2011	Norway	AtS	Kidney	NO099	9,13,15,14, 1, 7, 1	ST187
2011	Norway	AtS	Kidney	NO100	9,13,15,14, 1, 7, 1	ST187
2011	Norway	RbT	Ulcer	NO104	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	AtS	Fin rot	NO105	9,13,11, 7, 9,13,12	ST23
2011	Norway	RbT	Kidney	NO106	2, 2, 2, 2, 2, 2, 2	ST2
2011	Norway	RbT	Kidney	NO107	2, 2, 2, 2, 2, 2, 2	ST2
2011	Sweden	RbT	Kidney	SE474	2,13,15,16, 1, 7, 1	ST126
2011	Sweden	RbT	Kidney	SE475	3, 2, 2, 2, 2,41, 2	ST92
2011	Sweden	RbT	Kidney	SE476	2, 8, 2, 2, 2, 7, 2	ST12
2011	Sweden	RbT	Kidney	SE477	3, 2, 2, 2, 2,41, 2	ST92
2011	Sweden	RbT	Kidney	SE479	3, 2, 2, 2, 2,41, 2	ST92
2011	Sweden	RbT	Kidney	SE480	2,13,15,16, 1, 7, 1	ST126
2011	Sweden	RbT	Kidney	SE482	3, 2, 2, 2, 2,41, 2	ST92
2011	Sweden	RbT	Kidney	SE483	3, 2, 2, 2, 2,41, 2	ST92
2012	Denmark	RbT	Ulcer	BN203	2, 2, 2, 2, 2, 2, 2	ST2
2012	Denmark	RbT	Ulcer	BN204	2, 2, 2, 2, 2, 2, 2	ST2
2012	Denmark	RbT	Eye	DK202	2, 2, 2, 2, 2, 2, 2	ST2
2012	Finland	RbT	Spleen	FI201	1,13,15, 2, 2, 7, 2	ST125
2012	Finland	RbT	Spleen	FI202	1,13,15, 2, 2, 7, 2	ST125
2012	Finland	RbT	Spleen	FI203	1,13,15, 2, 2, 7, 2	ST125
2012	Norway	AtS	Ulcer	NO108	4,49, 8,11, 6, 3, 3	ST168
2012	Norway	AtS	Ulcer	NO109	4,49, 8,11, 6, 3, 3	ST168
2012	Norway	AtS	Ulcer	NO110	7,51, 8,17,33,17,43	ST171
2012	Norway	AtS	Ulcer	NO111	7,51, 8,17,33,17,43	ST171
2012	Norway	AtS	Ulcer	NO112	9,13,15,14, 1, 7, 1	ST187
2012	Norway	AtS	Ulcer	NO113	9,13,15,14, 1, 7, 1	ST187
2012	Norway	AtS	Ulcer	NO114	28,62,15, 2,25, 1,54	ST232
2012	Norway	AtS	Ulcer	NO115	9,13,11, 7, 9,13,12	ST23
2012	Norway	AtS	Kidney	NO116	9,13,11, 7, 9,13,12	ST23
2012	Norway	RbT	Kidney	NO117	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Kidney	NO118	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Kidney	NO119	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Ulcer	NO120	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Ulcer	NO121	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Ulcer	NO122	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Kidney	NO123	2, 2, 2, 2, 2, 2, 2	ST2
2012	Norway	RbT	Kidney	NO124	2, 2, 2, 2, 2, 2, 2	ST2
Unknown	USA	CoS	Kidney	NCIMB 1947^{T}	4, 7, 6, 5, 6, 8, 4	ST13

Krister Sundell

From genotype to phenotype: diversity and population structure of *Flavobacterium psychrophilum*

This thesis integrates the tools of molecular genetics and conventional phenotypic microbiology to bring increased knowledge about the diversity and population structure of the fish pathogenic bacterium *Flavobacterium psychrophilum*. This work shows that the pathogen has an epidemic structure dominated by one particular globally distributed genetic lineage containing specific genotypes associated with high mortalities in economically important salmonid fish species. The simultaneous presence of genetically divergent clones and the observed population heterogeneity within disease outbreaks, however, present a challenge for disease control. Antimicrobial resistance was strongly associated with outbreak strains and evidence indicating dissemination of harmful strains with commercial movement of fish and fish eggs is strengthened.

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