Genotyping of *Flavobacterium psychrophilum* isolates from wild and farmed salmonids in Norway and Chile

P. Apablaza[1], A. Dyrkorn Løland[1], Ø. J. Brevik[1], P. Ilardi[2], J. Battaglia[2] & A. Nylund[1]

[1] *Fish Diseases Group, Institute of Biology, University of Bergen, Post box 7803, 5020 Bergen, Norway*

[2] *VETERQUIMICA SA, Camino a Lonquén 10.387, Maipú, Santiago, Chile*

Correspondence: Patricia.Apablaza@bio.uib.no

Abstract

Flavobacterium psychrophilum, a member of the family Flavobacteriaceae, has from the first descriptions in North America, been distributed to many countries worldwide causing serious losses to the aquaculture industry. The Norwegian and Chilean aquacultures are also affected by outbreaks due to *F. psychrophilum*. The bacterium has been one of the most common causes of mortality in fresh water aquaculture in Chile since the 1980's and outbreaks have occurred more frequently during the last years [1]. Of the farmed fish species, most susceptible are rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and coho salmon (*O. kisutch*). *F. psychrophilum* is also present in farmed and wild trout (*S. trutta*) and Atlantic salmon in Norway. The biggest losses occur among juvenile fish where mortality can reach up to 90% [2]. The bacterium has also been found in several non-salmonid species, such as carp (*Cyprinus carpio*), ayu (*Plecoglossus altivelis*), tench (*Tinca tinca*) and eel (*Anguilla anguilla*). The importance of wild fish as reservoirs is still not very well known. *F. psychrophilum* can be both horizontally and vertically transmitted and has been isolated from salmonid ovarian fluid and gonads even after routine standard iodophor disinfection. Hence, it is suggested that the transport of embryos from infected broodfish may be important in long distance transmission.

To better understand the epizootiological factors associated with the bacterium, host specificity, pathogenicity, virulence and geographical spreading, it is critical to perform a genetic characterization of *F. psychrophilum*. In the literature there are many studies pointing out this, but a robust and fast method that can be used a standard in strain differentiation is still missing. The aim of this study was to define the genetic variation among *F. psychrophilum* isolates from farmed and wild fish species in Norway and among farmed fish in Chile in order to find markers that permit the differentiation of the isolates of both countries. This study was done by the phylogenetic analysis of the 16S rRNA and seven housekeeping genes (HK), *atp*A, *dna*K, *mur*G, *tuf*, *gyr*B, *fum*C, and *trp*B [3]. Based on the sequence types (STs) of these HK a multilocus sequence typing (MLST) was performed with the 44 isolates from Norway and Chile in this study, and with 50 isolates from many different countries of Europe, America, Australia and Asia.

The results showed that analysis of individual phylogenetic relationship based on the 16S rRNA gene was relatively poor in resolution for differentiation of the isolates giving only two supported clades among the 94 isolates. The clade, 16S rRNA-I, had seven isolates that included two separate sequences of the type strain (NCIMB1947) isolated from Coho salmon in North America. Of the remaining five isolates, four had been collected from wild Atlantic

salmon and trout in Norway, and the fifth had been isolated from Atlantic salmon in Chile. Clade 16S rRNA-II consisted of the remaining isolates of *F. psychrophilum*.

The analysis of HK *trp*B, *fum*C, *tuf*, *mur*G and *dna*K (Fig. 1) showed two and *atp*A three groups (Fig. 2). The groups did not represent a geographical distribution. On the other side, the phylogeny of *gyr*B presented five supported clades (figure not shown). The *gyr*B-I and *gyr*B-II groups included only Norwegian wild and farmed Atlantic salmon isolates.

The study of HK in the MLST system presented 53 STs among the 94 isolates, combining the allele types for the seven loci. Based on MLST results there was not a clear separation between systemic isolates and isolates from the host surfaces considering the different tissues from which they originated.

Even though some isolates from wild and farmed Atlantic salmon in Norway were grouped together in the phylogeny of *gyr*B, the analyses of 16S rRNA and phylogeny considering the seven loci did not conclude hosts specificity, geographical distribution, or differences in the virulence among the isolates. MLST had a better resolution showing that the Chilean isolates were more closely related to isolates from North America and Europe compared to Norwegian ones. Still, it is necessary to perform a further molecular approach to define critical aspects of the epidemiology of *F. psychrophilum* in the Norwegian and Chilean aquaculture.

References

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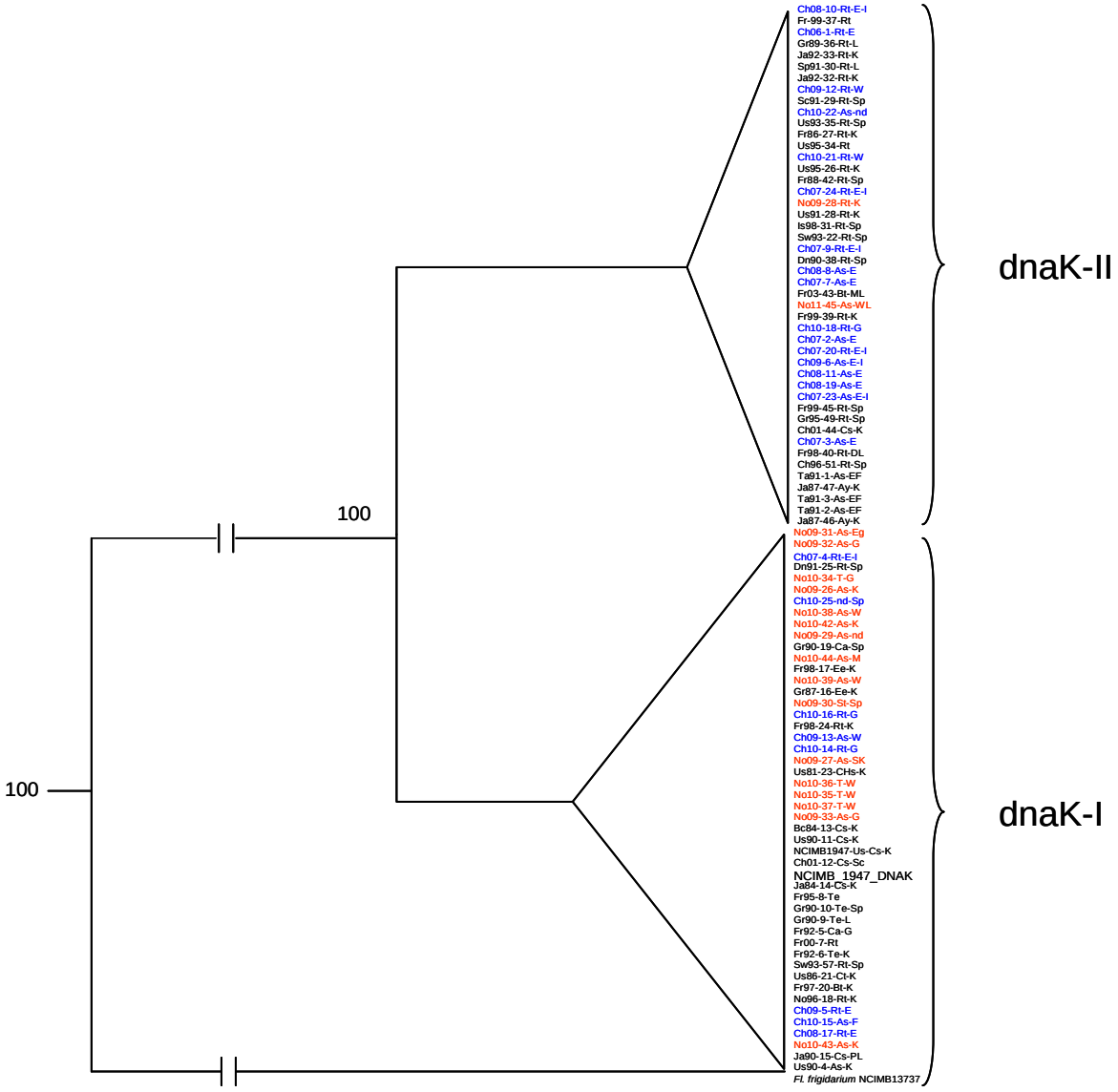

Figure 1. The phylogenetic positioning of the 44 *F. psychrophilum* isolates from the present study and sequences from 50 previously analyzed isolates [3], obtained from GeneBank. The phylogeny was constructed from an analysis on 897 nt. within the *dna*K gene. The phylogenetic tree was obtained by Bayesian method using the Beast package v1.6.1. Posterior probability values below 50% are not shown. NCIMB1947: type strain for the present study. The order in the code indicates, the country of isolation Chile (Ch), Norway (No), Japan (Ja), Tasmania (Ta), United States (Us), British Columbia (BC), France (Fr), Switzerland (Sw), Denmark (Dn), Germany (Gn), Israel (Is), Scotland (Sc), Spain (Sp); year of isolation (2006 to 2011), number of the isolate; fish species, ayu (Ay), Coho salmon (Cs), brown trout (Bt), Chinook salmon (CHs), carp (Ca), tench (Te), eel (Ee), A. salmon (As), Rainbow trout (Rt), trout (T); tissue, gill (G), wound (W), kidney (K), eggs (Eg), milt (M), spleen (Sp), skin (SK), internal (int) and internal-external (int-ext) surface of fish, eroded fin (EF), spinal cord (Sc), liver (L), peduncle lesion (PL), dorsal lesion (DL), mouth lesion (ML). Nd, no information available.

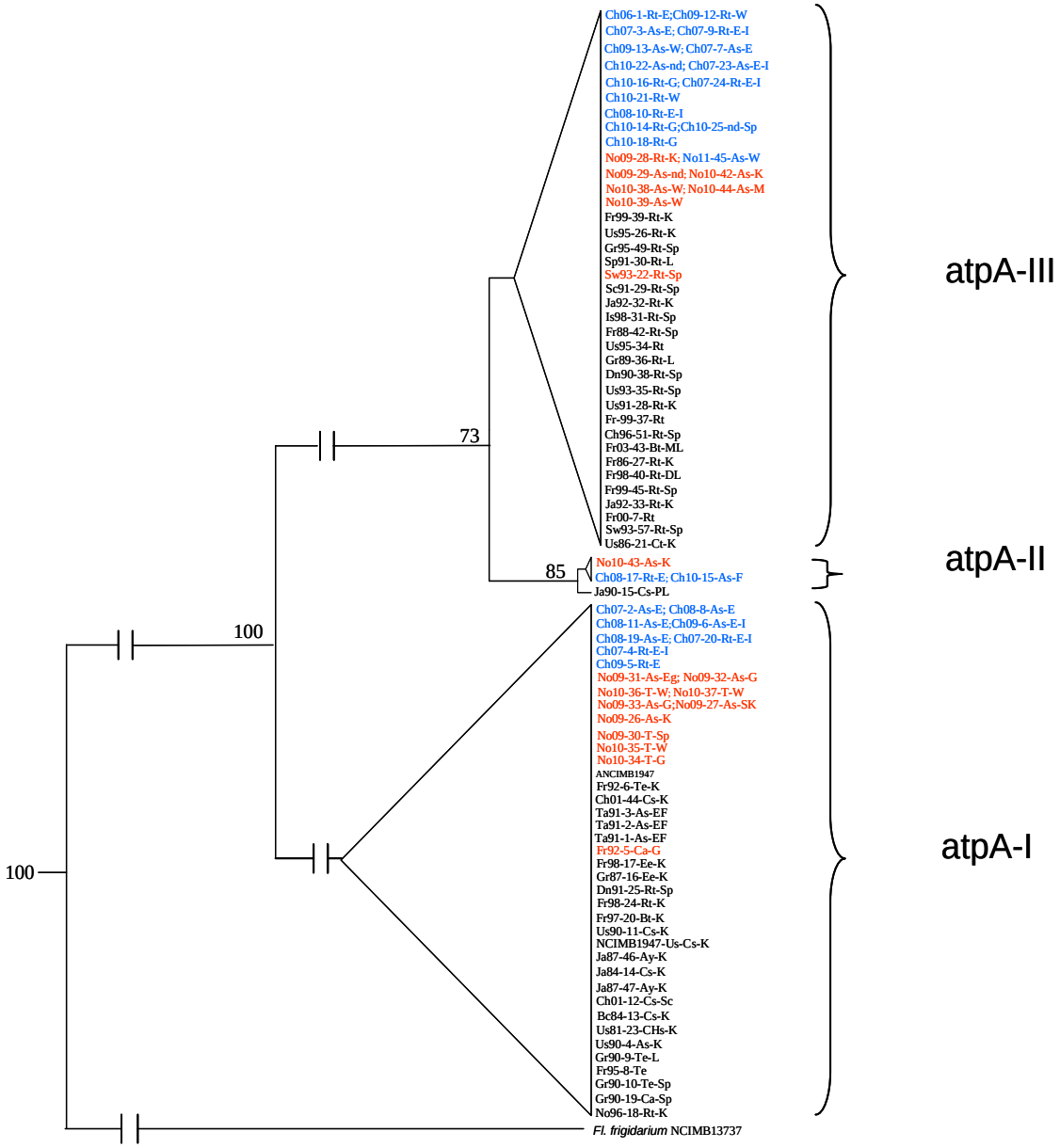

Figure 2. The phylogenetic positioning of the 44 isolates *F. psychrophilum* isolates from the present study and sequences from 50 previously analyzed isolates [3], obtained from GeneBank. The phylogeny was constructed from an analysis on 979 nt. within the *atpA* gene. The phylogenetic tree was obtained by Bayesian method using the Beast package v1.6.1. Posterior probability values below 50% are not shown. NCIMB1947: type strain for the present study. The order in the code indicates, the country of isolation Chile (Ch), Norway (No), Japan (Ja), Tasmania (Ta), United States (Us), British Columbia (BC), France (Fr), Switzerland (Sw), Denmark (Dn), Germany (Gn), Israel (Is), Scotland (Sc), Spain (Sp); year of isolation (2006 to 2011), number of the isolate; fish species, ayu (Ay), Coho salmon (Cs), brown trout (Bt), Chinook salmon (CHs), carp (Ca), tench (Te), eel (Ee), A. salmon (As), Rainbow trout (Rt), trout (T); tissue, gill (G), wound (W), kidney (K), eggs (Eg), milt (M), spleen (Sp), skin (SK), internal (int) and internal-external (int-ext) surface of fish, eroded fin (EF), spinal cord (Sc), liver (L), peduncle lesion (PL), dorsal lesion (DL), mouth lesion (ML). Nd, no information available.