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# Assessment of genetic variability and relatedness among atypical *Aeromonas salmonicida* from marine fishes, using AFLP-fingerprinting

**Vera Lund[1,*], Liss M. Jenssen[1], Mette S. Wesmajervi[2]**

[1]Fiskeriforskning, Norwegian Institute of Fisheries and Aquaculture Ltd, 9291 Tromsø, Norway
[2] DNA-Sequencing Laboratory, University Hospital of Tromsø, 9038 Tromsø, Norway

ABSTRACT: Atypical strains of *Aeromonas salmonicida* are the causal agent of atypical furunculosis or ulcer disease in various fish species, including spotted wolffish *Anarhichas minor*, which is a promising species in the Norwegian fish-farming industry. Isolates of atypical *A. salmonicida* comprise a very heterogenous group showing large variety in biochemical, molecular and virulence characteristics. The genetic variability among atypical isolates from wolffish was characterised using amplified fragment length polymorphism analysis: AFLP-fingerprinting. Additional isolates from halibut *Hippoglossus hippoglossus*, turbot *Scophthalmus maximus*, cod *Gadus morhua* and several salmonid fishes were included for assessment of variability and relatedness among a total of 56 atypical isolates of *A. salmonicida*. They were compared to reference strains of *A. salmonicida* subspecies and to other *Aeromonas* species pathogenic in fishes. AFLP-fingerprints subjected to similarity analysis yielded a grouping of the isolates into several clusters, revealing genetic heterogeneity among the isolates. There seems to be a correlation between genetic similarity among isolates and the fish host. The Icelandic isolates, mainly from cod, formed a very homogeneous subcluster, which was closely related to the wolffish isolates. All atypical isolates from spotted and common wolffish grouped together in a large cluster and appear to be very homogeneous, even though they had been isolated over a period of 8 yr at different locations in Norway. On the other hand, most of the isolates from turbot and halibut grouped together into 2 different clusters, while the 9 atypical isolates from salmonids appeared in 4 different clusters. Thus, the atypical isolates of *A. salmonicida* from halibut, turbot and salmonid fishes seem to be more genetically diverse than those from wolffish and cod.

KEY WORDS: Atypical *Aeromonas salmonicida* · Fish · Pathogen · AFLP · DNA-fingerprinting



## INTRODUCTION

*Aeromonas salmonicida* subspecies *salmonicida* is the causative agent of furunculosis, a disease mostly restricted to salmonid fishes. This subspecies comprises a homogeneous group and is referred to as typical *A. salmonicida*. Other subspecies of *A. salmonicida* i.e. the subspecies *achromogenes*, *masoucida*, *smithia* and *pectinolytica* (Smith 1963, Kimura 1969, Austin et al. 1989, Pavan et al. 2000) along with an increasing number of isolates reported from various fish species and geographical areas that are not included in any of the described subspecies of *A. salmonicida*, are referred to as atypical strains. The atypical strains are heterogeneous in terms of molecular and phenotypic characteristics. They cause various conditions of ulcer diseases or atypical furunculosis and are of increasing economic concern in aquaculture (reviewed by Wiklund & Dalsgaard 1998).

DNA-fingerprinting methods in studies of genome polymorphism are useful for taxonomic and epizootio-

*E-mail: vera.lund@fiskforsk.norut.no

logical studies of bacterial pathogens as well as for vaccine development. Methods applied to typical *Aeromonas salmonicida* such as DNA:DNA reassociation (Belland & Trust 1988), plasmid profiling (Nielsen et al. 1993, Sørum et al. 2000), ribotyping (Nielsen et al. 1994), DNA probes (Gustafson et al. 1992), restriction endonuclease analysis (McCormick et al. 1990), random amplified polymorphic DNA analysis (RAPD) (Hänninen et al. 1995, Miyata et al. 1995) and restriction fragment length polymorphism analysis by pulsed field gel-electrophoresis (PFGE) (Livesley et al. 1999), all demonstrate the typical isolates to be extremely homogeneous. Similar studies on atypical strains using RAPD analysis (Kwon et al. 1997, O'hIci et al. 2000), ribotyping (Hänninen & Hirvelä-Koski 1997), PCR-typing (Høie et al. 1999), plasmid profiling (Sørum et al. 2000) and restriction fragment analysis by PFGE (Umelo & Trust 1998, O'hIci et al. 2000), have shown them to be more genetically diverse compared to the typical strains. Phenotypic and molecular variations are not only observed between strains from different fish species, but also between strains from one and the same species. However, regarding the atypical strains, the different molecular and phenotypic methods are not congruent, as was shown when 52 atypical strains from 26 fish species were genetically characterised by various methods (Austin et al. 1998).

In our work with vaccine development for atypical furunculosis in marine farmed fishes (Ingilæ et al. 2000, Lund et al. 2001), amplified fragment length polymorphism analysis, AFLP-fingerprinting, was used for the assessment of genetic variability and relatedness among atypical isolates from species of marine and salmonid fishes. The AFLP analysis is based on selective amplification of restriction fragments of whole chromosomal DNA and is reported to discriminate between bacterial strains below the species level (Vos et al. 1995, Janssen et al. 1996). For characterisation and epidemiological purposes, AFLP analysis has been used to differentiate strains of bacteria including *Aeromonas* spp. (Huys et al. 1996) and *Vibrio viscosus* and *V. wodanis* associated with winter ulcer in salmonid fishes (Benediktsdottir et al. 2000). Here we report the results of AFLP analysis of a total of 56 atypical *A. salmonicida* isolates from Atlantic salmon *Salmo salar*, rainbow trout *Oncorhynchus mykiss*, Arctic char *Salvelinus alpinus*, grayling *Thymallus thymallus*, spotted wolffish *Anarhichas minor*, common wolffish *A. lupus*, halibut *Hippoglossus hippoglossus*, turbot *Scophthalmus maximus* and cod *Gadus morhua*. For comparison, 3 strains of typical *Aeromonas salmonicida*, 3 subspecies reference strains of *A. salmonicida*, and 3 other *Aeromonas* species pathogenic in fishes were included.

## MATERIALS AND METHODS

**Strains and culture conditions.** A total of 56 atypical isolates of *Aeromonas salmonicida* from 10 species of salmonid and marine fishes were compared in this study. Also included were strains of typical *A. salmonicida*, reference strains of *A. salmonicida* subspecies *salmonicida* (*Ass*-4010), *masoucida* (*Asm*-4035) and *achromogenes* (*Asa*-403), along with strains of other *Aeromonas* sp. pathogenic in fishes. (See Table 1 for strain designations, host data and location of isolation in addition to the results from some of the reactions of the standardised biochemical test API 20E [bioMérieux sa]). For the purpose of the present study the Icelandic isolates from Atlantic cod were kindly supplied by Bjarnheidur Gudmundsdottir (Institute of Experimental Pathology, University of Iceland), while a total of 22 isolates from various fish species were from the strain collection at The Norwegian Veterinary Institute. The remaining strains were from the strain collection in our laboratory, including strains previously obtained for use in other studies. Most of the isolates from wolffish were from wild-caught fish from the coast of northern Norway or the Barents Sea, used as breeding stocks at 2 locations in northern Norway. The bacteria were isolated from diseased fish in late summer when water temperature increases, or during the spawning season.

Prior to the study, isolates were confirmed by their phenotypic characteristics such as Gram-negative rods, lack of mobility and production of a brown pigment in most of the isolates. All marine atypical isolates were grown in brain heart infusion medium (BHI, Difco) supplemented with 2% NaCl at 12°C for 2 to 3 d, depending on the growth rate. The remaining isolates were grown in BHI at 12°C.

**AFLP — amplified fragment length polymorphism.** AFLP™ microbial fingerprinting (PE Applied Biosystems) is based on the selective amplification of restriction fragments (Vos et al. 1995, Janssen et al. 1996). In short, the technique consists of 5 steps: (1) digestion of total cellular DNA with 2 restriction endonucleases and ligation of double-stranded restriction half site-specific adaptors to all restriction fragments; (2) low-level preselective amplification of the restriction fragments with preselective primers; (3) selective amplification of a specific subset of these fragments with primers containing the same sequence as their corresponding adaptors and an additional base; (4) analysis of the polymerase chain reaction (PCR) products on a regular polyacrylamide sequencing gel; (5) similarity analysis of the AFLP fingerprints, resulting in dendrograms.

***Genomic DNA extraction:*** Cells were harvested from 20 ml culture by centrifugation of 1 ml at 14000 × *g* for 3 min followed by extraction of genomic DNA

using GenomicPrep™ (Amersham Pharmacia Biotech). High-quality unsheared genomic DNA was confirmed on a 1% agarose gel. The DNA concentration was determined by measuring $A_{260}$ on a spectrophotometer (1 $OD_{260}$ unit = 50 µg DNA $ml^{-1}$), before dilution in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to 10 µg $ml^{-1}$ for use in the AFLP™ reactions.

***AFLP™ reagents:*** Oligonucleotide adaptors, primers and amplification mix were provided by the producer (PE Applied Biosystems), and the manufacturer's protocol was followed.

***Selection of selective primer pair:*** Initially, 24 different *Eco*RI/*Mse*I primer pairs provided by the manufacturer were screened using the typical strain *Aeromonas salmonicida* 4017 in order to choose 1 pair producing 25 to 130 fragments evenly dispersed between 50 and 500 bases. The *Eco*RI-A/*Mse*I-T primer pair, which resulted in 38 amplified fragments, was selected for analysis of genome polymorphism among the *A. salmonicida* strains. This primer pair was also the one recommended by the manufacturer for use with *Aeromonas* sp. No other primer pairs were used in the analysis of this strain collection.

***Template preparation:*** Genomic DNA was digested with restriction enzymes *Eco*RI and *Mse*I (New England BioLabs). Subsequently, double-stranded restriction half site-specific adaptors were ligated to the restriction fragments by T4 DNA ligase (Promega).

All isolates were subjected to the AFLP™ reactions at least twice to determine the reproducibility of the method. Also, the AFLP fingerprint of *Aeromonas salmonicida* subspecies *salmonicida* Strain 4017 was used as an internal reference in each electrophoresis run.

***AFLP™ reaction:*** The *Eco*RI-A/*Mse*I-T primer pair was used for amplification of the PCR products, which were labelled during amplification by the 5′ fluorescent dye-label on the *Eco*RI-A primer for use with ABI Prism™ detection technology.

***Gel analysis:*** Prior to gel-loading, GeneScan-500 was added to each sample as an internal lane size standard to size all amplification fragments accurately. The PCR products were separated on a regular polyacrylamide sequencing gel on an ABI PRISM 377 (PE Applied Biosystems). The results were analysed with ABI Prism™ GeneScan® Analysis and Genotyper® 2.0 software (PE Applied Biosystems). The strains produced 104 amplification fragments in total, evenly dispersed from 50 to 495 bases. Each strain was visually coded for the presence (coded 1) and absence (coded 0) of each DNA fragment. The data obtained for all the *Aeromonas* isolates in the study were combined into 1 data matrix, and AFLP-based similarity dendrograms were derived from unweighted average-pair group-cluster analysis using Pearson similarity coefficient (SYSTAT 1999).

## RESULTS

The strain collection used in the study, including 56 atypical *Aeromonas salmonicida* isolates from 9 different species of marine and salmonid fish, is shown in Table 1. The results from some of the biochemical reactions of the standardised API 20E test, showing variation in arginine dihydrolase (ADH), lysine decarboxylase (LDC), fermentation of sugars, indole and acetoin production and gelatin degradation, are also listed in Table 1. However, the API 20E patterns cannot be related to the fish host or geographical location.

The results of the genome characterisation using AFLP analysis are presented as similarity dendrograms. The different *Aeromonas* species and the 3 reference strains of *A. salmonicida* subspecies *Ass*-4010, *Asm*-4035 and *Asa*-4036 are compared in the similarity dendrogram in Fig. 1. *A. hydrophila*, *A. caviae* and *A. sobria*, isolated from roach, eel and carp, respectively, were shown to be genetically very different from each other and from the *A. salmonicida* strains, which all were more closely related. A cluster delineation of 70% similarity resulted in grouping of the reference strains of subspecies *salmonicida Ass*-4010 and *achromogenes Asa*-4036 with the *A. salmonicida* subspecies *salmonicida* isolates from Norway (4017), Scotland (4012) and Canada (4004). The reference strain *Asm* 4035 was more distantly related with an AFLP-based similarity of less than 70% to the reference strain *Ass* 4010.

The dendrogram in Fig. 2 shows the AFLP-based similarity between atypical isolates of *A. salmonicida* from different species of salmonid and marine fishes. When the isolates were compared with a cluster delineation of 70% similarity, they grouped into 6 clusters (A to F) while 1 isolate was unclustered. The 2 reference strains of subspecies *salmonicida Ass*-4010 and *achromogenes Asa*-4036 formed a cluster (B) of their own, while the *masoucida* reference strain *Asm*-4035

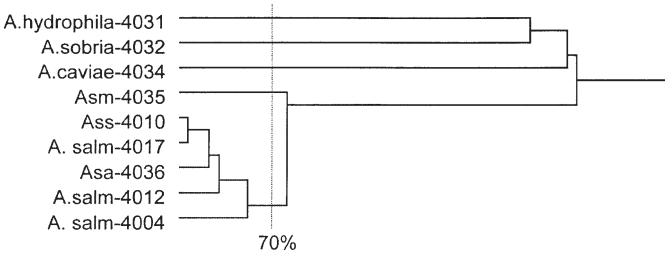


Fig. 1. *Aeromonas* spp. Dendrogram derived from a cluster analysis of the AFLP patterns of fish pathogenic *Aeromonas* species, including reference strains of *A. salmonicida* subspecies *salmonicida Ass*-4010, *achromogenes Asa*-4036 and *masoucida Asm*-4035. Vertical line denoting a hypothetical node of 70% has been added for comparison purposes

Table 1. *Aeromonas* spp. Strain designations, host data, isolation locations and biochemical test reactions. ADH: arginine dihydrolase; LDC: lysine decarboxylase; nd: no data

| Strain | Designation | *Aeromonas* species/subspecies | Host | Country of origin | API 20E tests | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ADH | LDC | Indole | Acetoin | Glucose | Mannitol | Sucrose | Gelatinase |
| 4000 | 172/87 | *achromogenes* | Atlantic salmon | Iceland | + | – | + | + | + | + | + | + |
| 4001 | 265/87 | *achromogenes* | Atlantic salmon | Iceland | + | – | + | + | + | + | + | – |
| 4002 | D1-03/89 | Atypical | Char | Norway | + | – | + | + | + | + | + | – |
| 4004 | 429-R CAE-144 | *salmonicida* | Atlantic salmon | Canada | – | – | – | – | + | + | – | + |
| 4010 | NCMB 1102 | *salmonicida* | Atlantic salmon | England | – | – | – | – | + | + | – | + |
| 4012 | MT 028 | *salmonicida* | Atlantic salmon | Scotland | + | – | – | – | + | + | – | – |
| 4017 | 3329/89 | *salmonicida* | Atlantic salmon | Norway | – | – | – | – | + | + | – | ++ |
| 4021 | 937/80 | Atypical | Atlantic salmon | Norway | + | – | + | + | + | + | + | + |
| 4031 | v1 Gat NH | *A. hydrophila* | Roach | nd | + | + | + | + | + | + | + | – |
| 4032 | 87025/Da | *A. sobria* | Carp | nd | + | + | + | + | + | + | + | – |
| 4034 | 851122/1HB | *A. caviae* | Eel | nd | + | – | + | – | + | + | + | – |
| 4035 | ATCC 27017 | *masoucida* | nd | nd | + | + | + | + | + | + | + | + |
| 4036 | ATCC 33659 | *achromogenes* | nd | nd | – | – | + | + | + | + | + | – |
| 4042 | 113-92 | *achromogenes* | Grayling | Finland | – | – | + | – | + | + | + | – |
| 4043 | 117-92 | *achromogenes* | Char | Finland | – | – | + | – | + | + | + | – |
| 4047 | 56.2192 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4048 | 99/92 | Atypical | Spotted wolffish | Norway | + | – | + | + | + | – | + | + |
| 4050 | AL2939 | Atypical | Halibut | Norway | – | – | – | – | + | – | + | – |
| 4051 | 22/93 | Atypical | Common wolffish | Norway | – | – | + | + | + | + | + | – |
| 4052 | 72/92 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4053 | 99/92 | Atypical | Common wolffish | Norway | – | nd | + | + | + | + | + | + |
| 4054 | K-06/96 | Atypical | Common wolffish | Norway | – | – | + | + | + | + | + | + |
| 4056 | K-08/96 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | – |
| 4058 | K-108/97 | Atypical | Spotted wolffish | Norway | – | – | + | – | + | + | – | – |
| 4059 | K-208/97 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4060 | K-110/97 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4061 | K-09/97 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | – |
| 4062 | K-210/97 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4063 | K-494/97 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | – |
| 4064 | K-30/98 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | – |
| 4065 | K-06/98 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | – | + | – |
| 4066 | K-08/98 | Atypical | Common wolffish | Norway | + | – | + | + | + | + | + | + |
| 4067 | K-9/98 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4068 | R-57/98 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | + |
| 4069 | R-565/98 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | – |
| 4070 | R-55/98 | Atypical | Spotted wolffish | Norway | – | – | + | + | + | + | + | – |
| 4072 | R-552/99 | Atypical | Spotted wolffish | Norway | – | – | + | – | + | + | – | – |
| 4073 | R-576/99 | Atypical | Spotted wolffish | Norway | + | – | – | – | – | – | – | – |
| 4075 | th233/91 | *achromogenes* | Cod | Iceland | – | – | + | – | + | + | + | – |
| 4076 | th7/94 | *achromogenes* | Cod | Iceland | + | – | + | + | + | + | + | – |
| 4077 | th62/94 | *achromogenes* | Cod | Iceland | + | – | + | + | + | + | + | – |
| 4078 | th63/94 | *achromogenes* | Cod | Iceland | + | – | + | + | + | + | + | – |
| 4078 | th82/94 | *achromogenes* | Cod | Iceland | + | – | + | + | + | + | + | – |

Table 1 (continued)

| Strain | Designation | *Aeromonas* species/subspecies | Host | Country of origin | API 20E tests | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ADH | LDC | Indole | Acetoin | Glucose | Mannitol | Sucrose | Gelatinase |
| 4080 | th85/94 | *achromogenes* | Cod | Iceland | + | – | + | + | + | + | + | – |
| 4081 | 89/09/2403 | Atypical | Atlantic salmon | Norway | + | – | + | + | + | + | + | + |
| 4082 | 88/09/3377 | Atypical | Atlantic salmon | Norway | + | – | + | – | – | – | + | – |
| 4083 | 88/09/222 | Atypical | Char | Norway | – | – | + | – | + | + | + | – |
| 4084 | 86/092120 | Atypical | Rainbow trout | Norway | – | – | + | + | + | + | + | – |
| 4085 | 97/09/1025 | Atypical | Spotted wolffish | Norway | + | – | – | + | + | – | + | – |
| 4086 | 95/09/0404 | Atypical | Common wolffish | Norway | + | – | + | + | + | + | + | – |
| 4087 | 93/09/1676 | Atypical | Common wolffish | Norway | + | – | – | + | + | – | + | – |
| 4088 | 92/09/1777 | Atypical | Spotted wolffish | Norway | + | – | – | + | + | + | + | – |
| 4089 | 98/09/0632 | Atypical | Turbot | Norway | – | – | – | – | + | + | – | + |
| 4090 | 98/09/0225 | Atypical | Turbot | Norway | + | – | – | – | + | + | – | + |
| 4091 | 90/09/2717 | Atypical | Turbot | Norway | – | – | – | + | – | – | – | + |
| 4092 | 88/09/02778 | Atypical | Turbot | Norway | + | – | + | – | + | + | + | – |
| 4093 | 96/09/1517 | Atypical | Halibut | Norway | + | – | + | – | + | + | + | – |
| 4094 | 96/09/0844 | Atypical | Halibut | Norway | – | – | – | – | + | + | – | + |
| 4095 | 95/09/0104 | Atypical | Halibut | Norway | – | – | – | – | + | + | – | + |
| 4096 | 94/09/0273 | Atypical | Halibut | Norway | – | – | + | + | + | + | + | – |
| 4097 | | Atypical | Halibut | Norway | – | – | + | + | + | + | + | – |
| 4099 | 93/09/914 | Atypical | Cod | Norway | + | – | + | + | + | + | + | – |
| 4100 | th98/94 | *achromogenes* | Cod | Iceland | + | – | + | + | + | + | + | – |
| 4101 | th19/99 | *achromogenes* | Cod | Iceland | – | – | + | + | + | + | + | – |
| 4102 | Olivier; 81377 | *achromogenes* | Cod | Canada | – | – | + | + | + | + | + | – |

grouped with atypical isolates from wolffish, cod and salmonid species in Cluster C. Thus, the isolates from cod and salmonids previously characterised as subspecies *achromogenes* (Table 1) did not cluster with the corresponding reference strain *Asa* 4036. Cluster C contained all the wolffish and cod isolates in addition to 5 isolates from salmonid fishes (4000, 4001, 4002, 4021 and 4084) and 1 from halibut (4097). The atypical isolates of *A. salmonicida* from wolffish appeared to be very homogeneous, regardless of whether they were isolated from spotted or common wolffish.

The atypical isolates from halibut and turbot grouped into 2 clusters, A and D, while the turbot isolate 4091 was unclustered. Finally, 2 Norwegian isolates from salmon and char (4081 and 4083) appeared in Cluster E, and 2 Finnish isolates from grayling and char (4042 and 4043) in another cluster, F.

## DISCUSSION

Genome heterogeneity among atypical isolates of *Aeromonas salmonicida* from a wide range of fish species has been demonstrated by various methods exploring DNA polymorphism (Hänninen & Hirvelä-Koski 1997, Kwon et al. 1997, Høie et al. 1999, O'hIci et al. 2000). In the present study, genetic variability and relatedness among 56 atypical strains of *A. salmonicida* from various species of marine and salmonid fishes mostly from Norway and Iceland, were assessed by AFLP analysis. For comparison, reference strains of 3 *A. salmonicida* subspecies and 3 other *Aeromonas* species also pathogenic in fishes, were included. From each *A. salmonicida* strain, the chosen selective primer pair *Eco*RI-A/*Mse*I-T produced 30 to 64 PCR products evenly dispersed between 50 and 500 bases. All the strains were run at least twice to determine the reproducibility of the method. The number of fragments obtained for 1 isolate in the different runs varied by ~3 fragments. However, the isolates always grouped in identical clusters when using a cluster delineation of 90% similarity for comparison of the different runs.

The AFLP analysis clearly discriminated between *Aeromonas* species, as previously reported (Huys et al. 1996), and the different strains of *A. salmonicida*, including the reference strains of subspecies *salmonicida Ass*-4010, *masoucida Asm*-4035 and *achromo-*

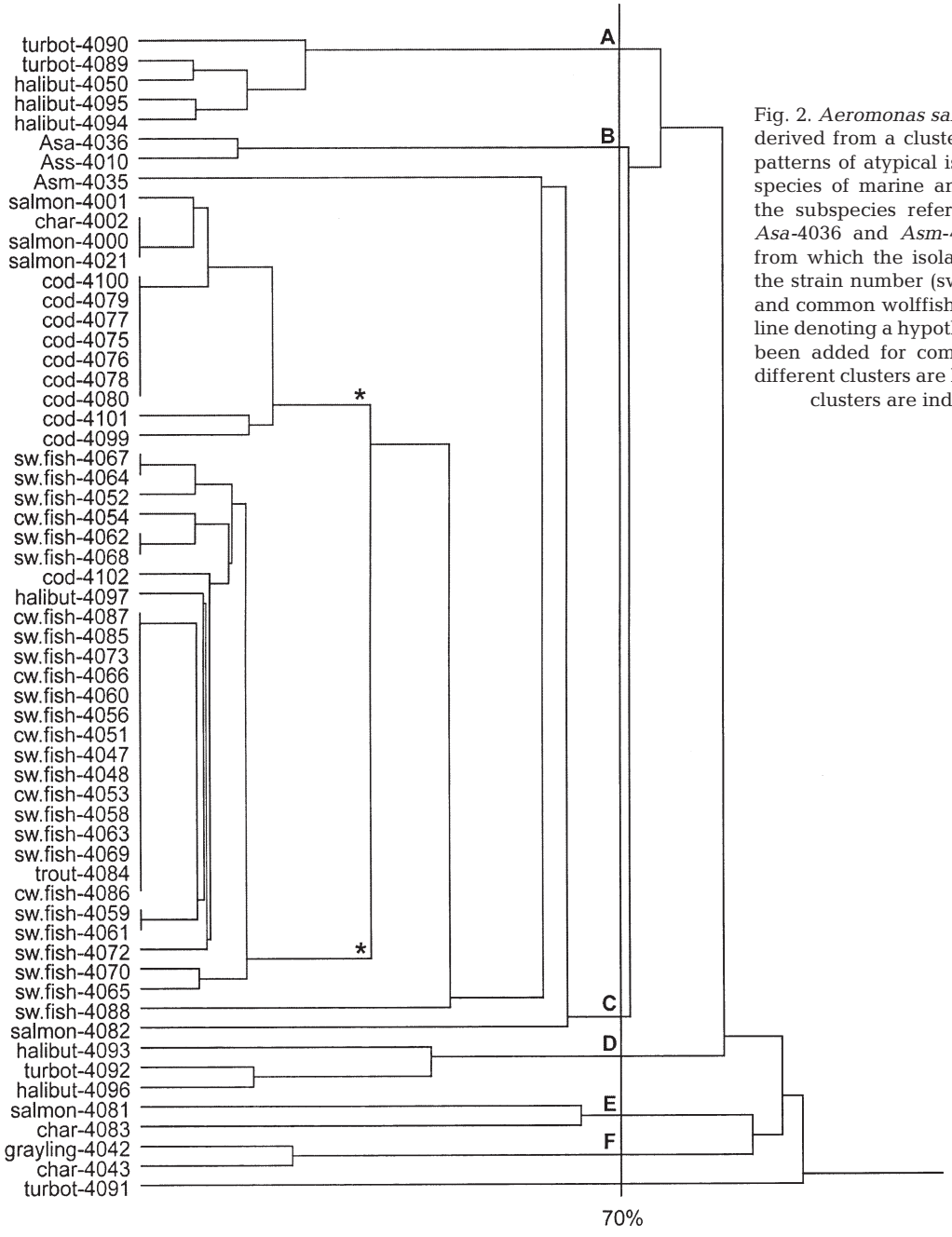


Fig. 2. *Aeromonas salmonicida*. Dendrogram derived from a cluster analysis of the AFLP patterns of atypical isolates from 9 different species of marine and salmonid fishes and the subspecies reference strains *Ass*-4010, *Asa*-4036 and *Asm*-4035. The fish species from which the isolates originated precede the strain number (sw.fish, cw.fish = spotted and common wolffish, respectively). Vertical line denoting a hypothetical node of 70% has been added for comparison purposes. The different clusters are labelled A to F and subclusters are indicated by asterisk

*genes Asa*-4036, showed an AFLP-based similarity of a little less than 70% (Fig. 1). Although different restriction enzymes and primers were used in the study of Huys et al., the corresponding subspecies reference strains of *A. salmonicida* in their study also showed approximately 70% similarity. In the present study, the subspecies *salmonicida* reference strain *Ass*-4010 was more closely related to the *achromogenes* reference strain *Asa*-4036 than to the *masoucida* reference strain *Asm*-4035. The 3 typical *A. salmonicida* isolates from Norway, Scotland and Canada grouped with both the subspecies reference strains *Ass*-4010 and *Asa*-4036, with a similarity of approximately 80%. Unfortunately, too few typical *A. salmonicida* isolates were included to assess the AFLP-based similarity or diversity among typical isolates. However, when 17 typical strains were subjected to similar RAPD analysis, they formed 2 clusters of 70% similarity correlated with geographical location (North America and North Europe), while restriction fragment analysis by PFGE showed the same strains to have >90% similarity to each other (O'hIci et al. 2000).

Our strain collection of atypical *Aeromonas salmonicida* isolates together with the subspecies reference strains grouped into 6 clusters (A to F) when using a cluster delineation of 70% similarity. Thus, genetic heterogeneity is demonstrated among the isolates from the various fish species even when they originated from a restricted geographical area (Fig. 2). None of the atypical isolates clustered with Subspecies Type Strains *Ass*-4010 or *Asa*-4036, but the wolffish, cod and some of the salmonid isolates clustered with the *masoucida* Type Strain *Asm*-4035. The AFLP analysis suggests the cod and wolffish isolates to be most related to the *masoucida* Type Strain *Asm*-4035, and does not support the subspecies classification of the Icelandic *achromogenes* isolates (Table 1). Similarly, in the work of O'hIci et al. (2000) using PFGE analysis, no atypical strain showed a 70% similarity to the subspecies *salmonicida* or *achromogenes* Type Strains, while 3 atypical strains from Finland showed a 75% PFGE-based similarity to a subspecies *masoucida* type strain.

The cod and wolffish isolates were all closely related to each other and grouped together in Cluster C. The atypical isolates from halibut, turbot and salmonid fishes were shown to be more genetically diverse by appearing in several clusters (A, C, D, E and F). In addition, 1 turbot isolate (4091) did not cluster with any other isolate, and was also the only isolate displaying urease activity in the API 20E test (reaction not included in Table 1).

Although all the atypical *Aeromonas salmonicida* isolates from wolffish were isolated over a period of 8 yr (1992 to 1999) at different locations along the coast of Norway and in the Barents Sea, all but 4088 appeared to be very homogeneous. Several of the isolates from both common and spotted wolffish even showed 100% AFLP-based similarity. At least 17 of the wolffish isolates were from wild-caught fish used as breeding stocks at 2 locations in northern Norway. They were isolated from diseased fish during a period of 4 yr from 1996 to 1999. The remaining isolates were from other locations in Norway and were isolated in the period 1992 to 1997. On the other hand, the 8 Icelandic cod isolates were all from the same farm, and 6 of them were isolated the same year (1994), while Isolates 4075 and 4101 were isolated 3 yr earlier and 5 yr later, respectively. In addition, 7 of the 8 isolates showed 100% AFLP-based similarity (Fig. 2). This is probably the result of an epidemiological incident, whereby the atypical isolate has spread within the farm from a common clonal origin.

A certain correlation between AFLP-based similarity and fish host or geographical location was observed. All wolffish isolates grouped together in a sub-cluster in Cluster C, which also contained 1 Canadian isolate from cod and 2 Norwegian isolates from halibut and rainbow trout. Of the 10 cod isolates, 9 grouped together with 2 Icelandic isolates from Atlantic salmon and 2 Norwegian isolates from salmon and Arctic char in another sub-cluster in Cluster C. The 2 Finnish isolates from char and grayling (4042 and 4043) grouped together in a separate cluster. Correlation between fish host and genome similarity has previously been reported. A clear correlation between AFLP-clustering and the origin of *Vibrio viscosus* isolates from Atlantic salmon was demonstrated by Benediktsdottir et al. (2000), whereby the Norwegian and Icelandic isolates formed 2 separate clusters. RAPD analysis of 29 atypical *Aeromonas salmonicida* strains isolated from 8 fish species in Japan using 5 random primers indicated genome homogeneity among the isolates from the different fish species, while the RAPD-profiles amplified from 4 subspecies reference strains differed from one another and from those of the atypical isolates (Kwon et al. 1997). Only one of the primers resulted in correlation between RAPD-profiles and the fish host. PFGE analysis of 17 atypical *A. salmonicida* revealed close relationship between the 6 goldfish isolates from Australia, USA and Europe (Umelo & Trust 1998). In another study, PFGE analysis of atypical *A. salmonicida* revealed correlation with geographical location by clustering of 3 Canadian isolates from eel and 4 from Atlantic salmon, while RAPD analysis of the same isolates did not show any correlation with fish host or geographical location (O'hIci et al. 2000). Also, there was no correlation between the 2 methods in identifying clusters of similar atypical strains.

In summary, the AFLP-fingerprinting of atypical *Aeromonas salmonicida* isolates from various fish species demonstrated genetic heterogeneity among the isolates. Correlation between genome similarity among the atypical isolates and the fish host was observed for the cod and wolffish isolates. The atypical strains from spotted and common wolffish isolated during a period of 8 yr along the coast of Norway were shown to be very homogeneous. Atypical isolates from halibut, turbot and salmonid fishes seemed to be genetically more diverse than those from cod and wolffish. Finally, similar to other methods used for studies of genome polymorphism, the results of the AFLP analysis do not support the existing subspecies classification of atypical *A. salmonicida.*

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