

Genome characterization of flatfish alphavirus and its risk to aquaculture

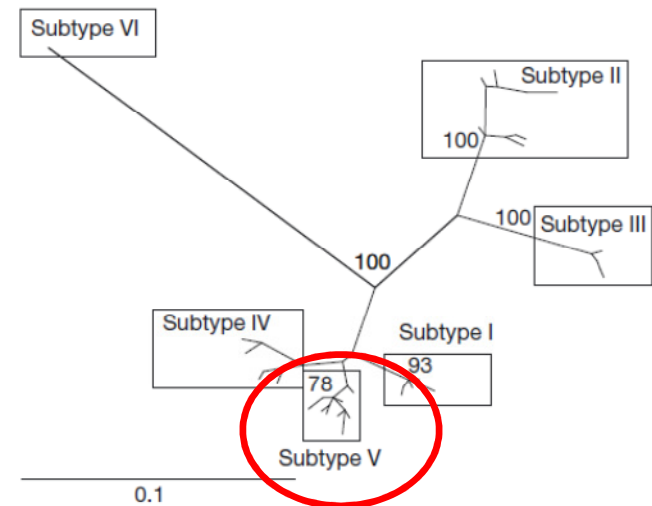


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marine scotland
science

INTRODUCTION – SAV genotypes

- SAV can be divided into six subtypes based on the partial nsP3 and E2 genes (Fringuelli et al., 2008)
- SAV RNA was detected in tissue of variety of flatfish (common dab, long rough dab, plaice (Snow et al., 2010)



- partial E2 sequence alignment showed 0.3 % variability among Atlantic salmon and common dab SAV sequences and phylogeny confirmed flatfish SAV as type IV-like (Snow et al., 2010)
- replication of dab-derived SAV confirmed in a salmonid-derived cell culture may suggest a wild reservoir of SAV with a direct risk to aquaculture

AIMS OF THE STUDY

1. INVESTIGATE COMPLETE GENOME SEQUENCE OF A COMMON DAB-DERIVED ALPHAVIRUS
2. DISCUSS A POTENTIAL RISK FROM THIS VIRUS TO SALMONID AQUACULTURE

TOOLS:

- **complete non-structural and structural polyprotein sequence comparisons of:**
 - a/ SAV type V from common dab**
 - b/ SAV type V from Atlantic salmon**
 - c/ SAV type I from Atlantic salmon**

MATERIALS & METHODS

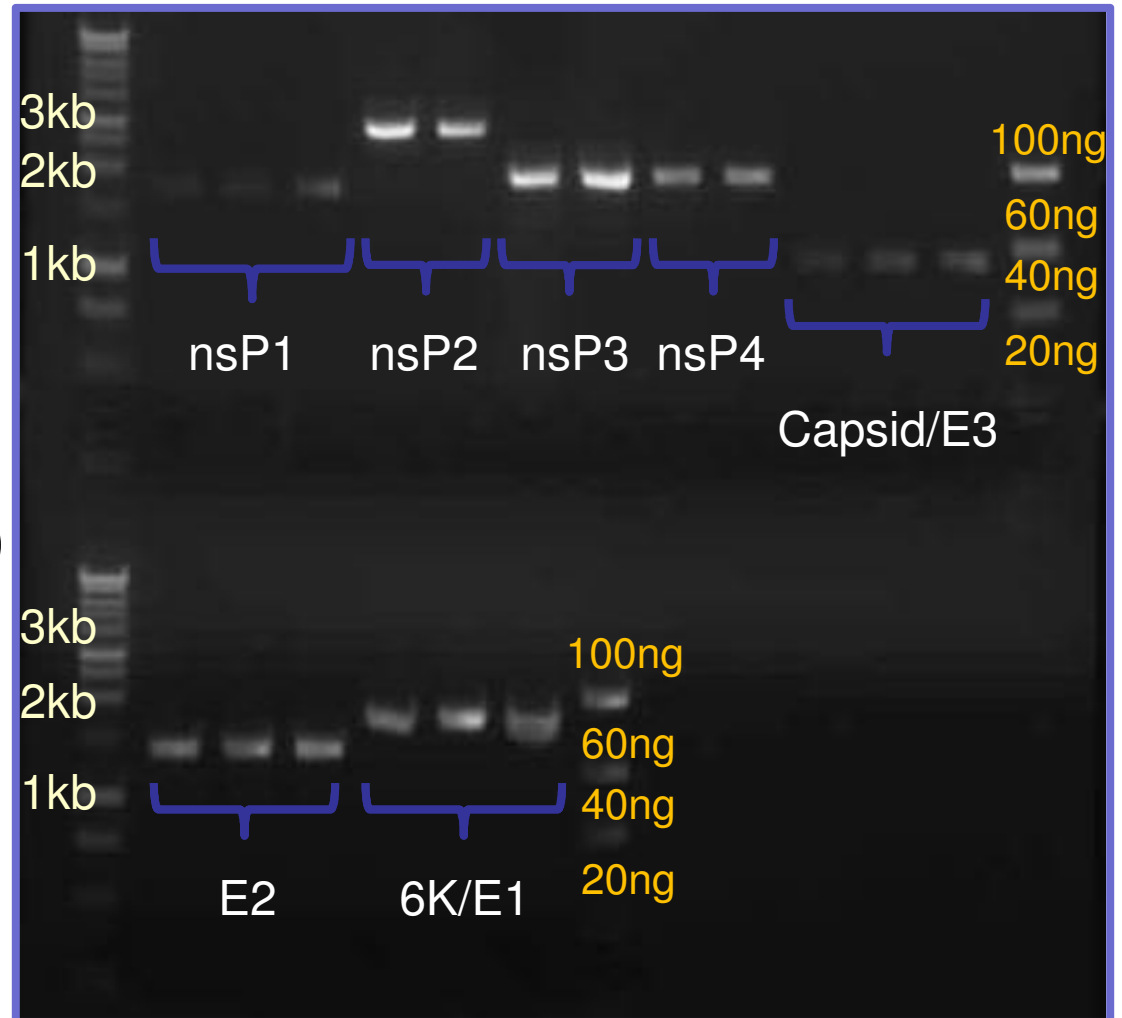
- Total RNA extraction from heart tissue (RNeasy Minikit, Qiagen), cDNA synthesis (Applied Biosystems)
- seven overlapping PCR (proof-reading polymerase) (KOD Hot Start DNA polymerase, Merck) in triplicate reaction
- PCR product purification (MinElute gel extraction kit, Qiagen)
- 60 ng purified product sequenced in both directions (GenomeLab DTCS Quick Start Kit, CEQ8800 DNA Sequencer, Beckman Coulter)
- sequences analysed in Sequencher software (Gene Codes) and aligned using ClustalW implemented in BioEdit (Hall, 1999)

RESULTS

Amplification of SAV V viral genes:

- Common dab SAV V
– isolates 2010/0684 (heart)

- Atlantic salmon SAV V
– isolate 4638 (passage no. 5)



RESULTS

NON-STRUCTURAL GENES – nucleic acid comparison:

- 7800 bp, resembles identical genome organization as F93-125 (accession number AJ316244, Weston et al., 2002)

Dab SAV vs. Salmon SAV type V:

Gene	% similarity	No. differences
nsP1:	99.5 %	9 bp (3 bp deletion)
nsP2:	99.2 %	21 bp
nsP3:	99.0 %	16 bp
nsP4:	99.4 %	11 bp

Dab SAV vs. Salmon SAV type I:

Gene	% similarity	No. differences
nsP1:	96.6 %	58 bp
nsP2:	96.2 %	98 bp
nsP3:	93.9 %	105 bp (3 bp deletion)
nsP4:	95.9 %	75 bp

RESULTS

NON-STRUCTURAL GENES – amino acid comparison:

- 2600 amino acids

Dab SAV vs. Salmon SAV type V:

Gene	% similarity	No. differences
		conserv/semi-con/non-con

nsP1: 99.5 % 2/0/1

nsP2: 99.3 % 5/1/0

nsP3: 99.6 % 0/2/0

nsP4: 99.5 % 1/2/0

Dab SAV vs. Salmon SAV type I:

Gene	% similarity	No. differences
		conserv/semi-con/non-con

nsP1: 97.3 % 8/4/3 (glutamic acid del)

nsP2: 98.7 % 11/3/2

nsP3: 94.2 % 9/13/11 (arginine ins)

nsP4: 98.5 % 2/5/2

RESULTS

STRUCTURAL GENES – nucleic acid comparison:

- 3963 bp, resembles identical genome organization as F93-125 (accession number AJ316244, Weston et al., 2002)

Dab SAV vs. Salmon SAV type V:

Gene	% similarity	No. differences
capsid:	99.2 %	7 bp
E3:	98.6 %	3 bp
E2:	99.1 %	12 bp
6K:	99.0 %	2 bp
E1:	99.6 %	6 bp

Dab SAV vs. Salmon SAV type I:

Gene	% similarity	No. differences
capsid:	96.2 %	32 bp
E3:	95.8 %	9 bp
E2:	94.6 %	68 bp
6K:	98.5 %	3 bp
E1:	97.7 %	32 bp

RESULTS

STRUCTURAL GENES – amino acid comparison:

- 1321 amino acids

Dab SAV vs. Salmon SAV type V:

Gene	% similarity	No. differences		
		conserv	semi-con	non-con
capsid:	100 %			
E3:	100 %			
E2:	99.8 %	0	0	1
6K:	98.5 %	0	1	0
E1:	99.3 %	2	0	1

Dab SAV vs. Salmon SAV type I:

Gene	% similarity	No. differences		
		conserv	semi-con	non-con
capsid:	97.9 %	1	3	2
E3:	97.2 %	1	1	0
E2:	97.2 %	5	5	3
6K:	100 %			
E1:	99.7 %	2	1	1

SUMMARY AND DISCUSSION

BOTH POLYPROTEINS COMPARISON:

- overall similarity between dab-derived SAV and salmon SAV type V

99.3 % nucleic acid similarity **99.5 % amino acid similarity**

- overall similarity between dab-derived SAV and salmon SAV type I

95.9 % nucleic acid similarity **97.5 % amino acid similarity**



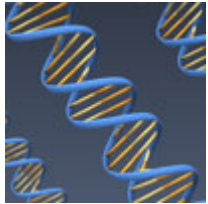
SUMMARY AND DISCUSSION

- dab-derived SAV poses a high risk to Atlantic salmon aquaculture due to high level of similarities in the complete polyprotein sequences – SAV type V

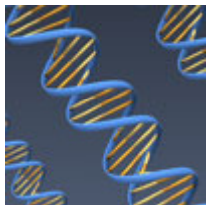
- NON-STRUCTURAL GENES: main differences in nsP3, function poorly understood but can have effect on RNA synthesis and virus replication

- STRUCTURAL GENES: main differences in E2, might effect binding to the host receptors (viral entry), or due to differences in antibody recognition epitope there might be differences in which host system recognizes SAV type I and SAV type V

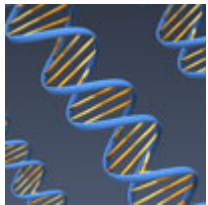
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