

# Characterization of *Vibrio anguillarum* O2 isolated from Atlantic cod, *Gadus morhua* L.

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Fiskeriforskning

## Introduction

Vibriosis is a major bacterial disease in farmed cod in Norway and due to the lack of effective vaccines the use of antibiotics is increasing. The disease is caused by *Vibrio anguillarum* which is a heterogenic group of bacteria. Atlantic cod become well protected after vaccination against classical vibriosis and today commercial vaccines are available, containing bacterins of three serotypes of *V. anguillarum*. However, *V. anguillarum* serotype O2 is still related to outbreaks of disease in farmed Atlantic cod and biochemical characterization of these isolates show that they deviate from other *V. anguillarum* isolates in some important characteristics.

## Materials and Methods

### Characterization of *V. anguillarum* isolates from diseased cod:

- API 20 E kit
- Polyclonal antibodies against *V. anguillarum* serotype O2a and O2b (pAb  $\alpha$ Va-O2a and pAb  $\alpha$ Va-O2b)
- Monoclonal antibodies (mAb) made against outer membrane components of *V. anguillarum* O2b
  - **mAb 6E10**: locate epitopes in the O-antigen side chain
  - **mAb 2H3**: locate epitopes in the core region of LPS
- Amplified fragment length polymorphisms (AFLP) analysis

### Vaccine efficacy

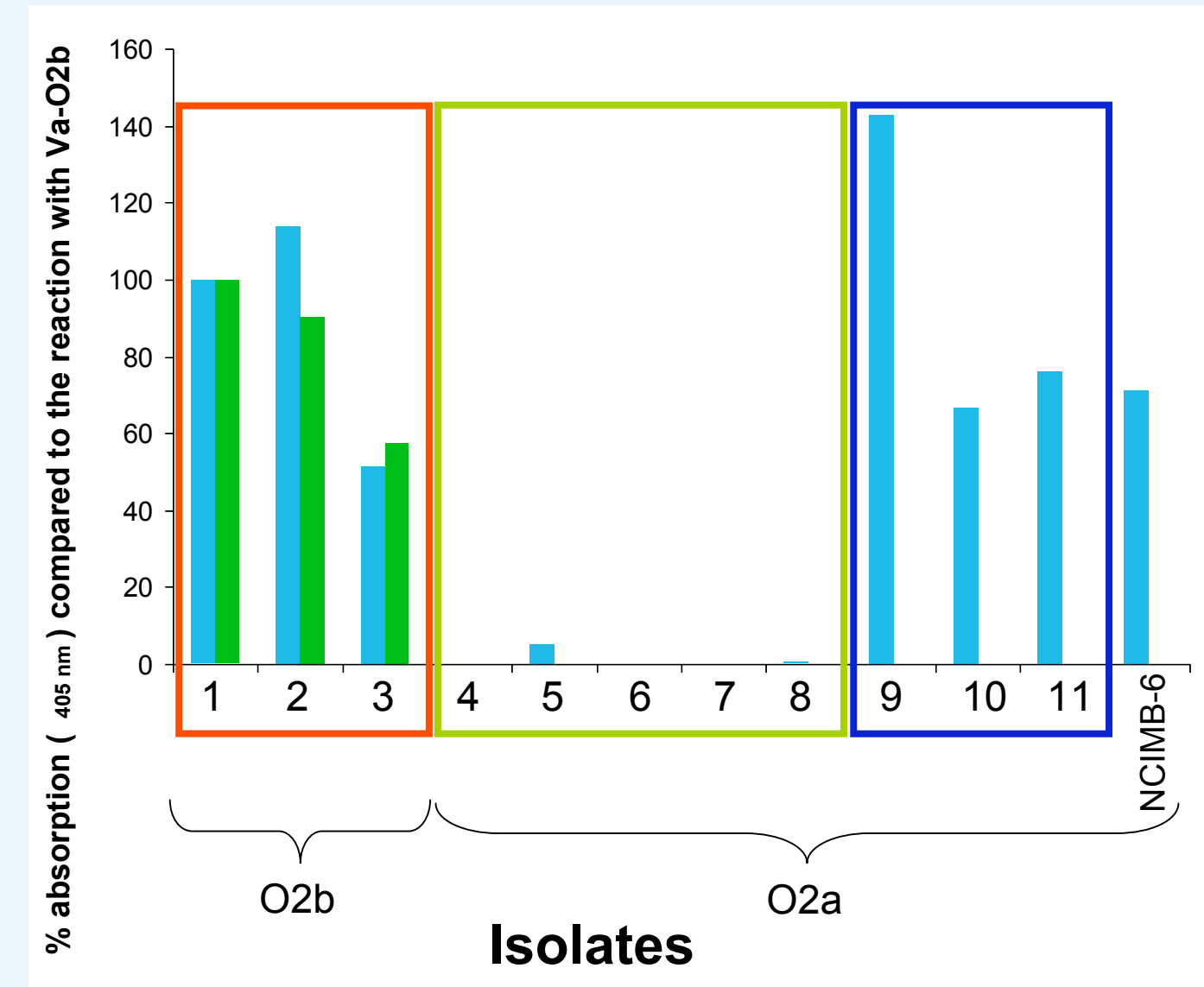
- Cod (2.5 g) were dip vaccinated with the commercial vaccine ALPHA MARINE™ Vibrio (PHARMAQ AS). Thirteen weeks post vaccination the fish were bath challenged in parallel tanks with three isolates of *V. anguillarum* O2a and one isolate of *V. anguillarum* O2b.

## Biochemical characterization

API-20E								
Isolate	Serotype	LDC	CIT	IND	GEL	SOR	AMY	ARA
1	O2b	-	-	-	+	+	-	-
2	O2b	nt	nt	nt	nt	nt	nt	nt
3	O2b	-	-	-	+	+	-	-
4	O2a	+	-	+	+	-	-	+
5	O2a	-	-	+	-	-	-	+
6	O2a	-	-	+	+	-	-	+
7	O2a	-	-	+	-	-	-	+
8	O2a	+	-	+	+	-	-	+
9	O2a	-	-	+	+	+	-	-
10	O2a	-	-	+	+	+	-	-
11	O2a	-	-	+	+	+	-	-
NCIMB 6	O2a	-	+	+	+	+	+	nt

nt: not tested  
All strains tested were positive for the traits ONPG, ADH, VP, MAN, SAC, GLU and negative for the traits ODC, H<sub>2</sub>S, URE, TDA, INO, RHA, MEL.

## Serological characterization

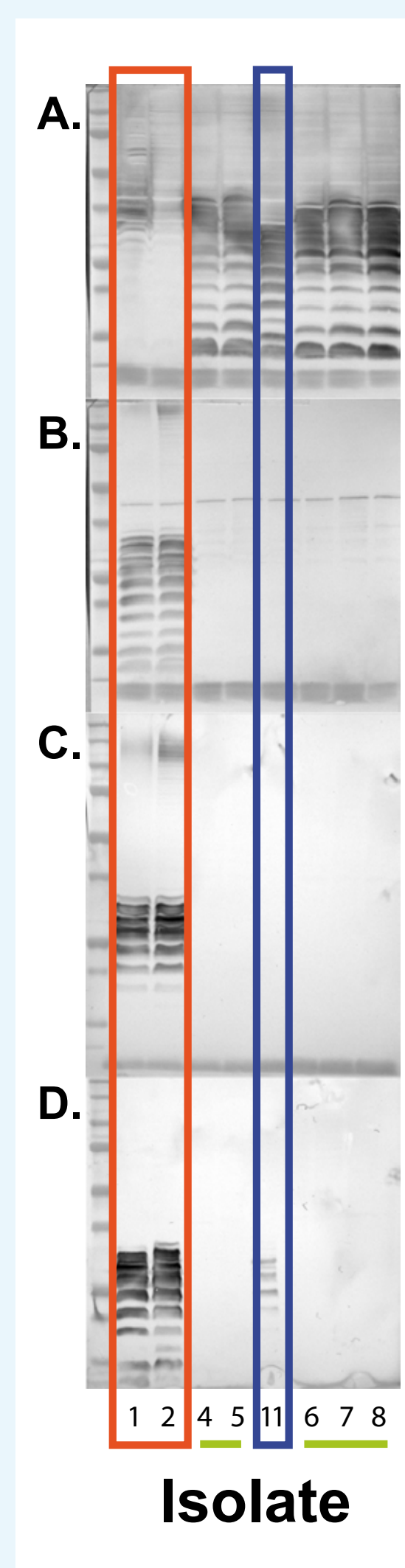


Serotyping of *V. anguillarum* strains by the use of two monoclonal antibodies in ELISA.

- mAb 6E10, detecting Va-O2a and Va-O2b isolates
- mAb 2H3, specific for Va-O2b isolates

Western blot of lysates of different *V. anguillarum* isolates immunostained by the use of polyclonal and monoclonal antibodies against *V. anguillarum*.

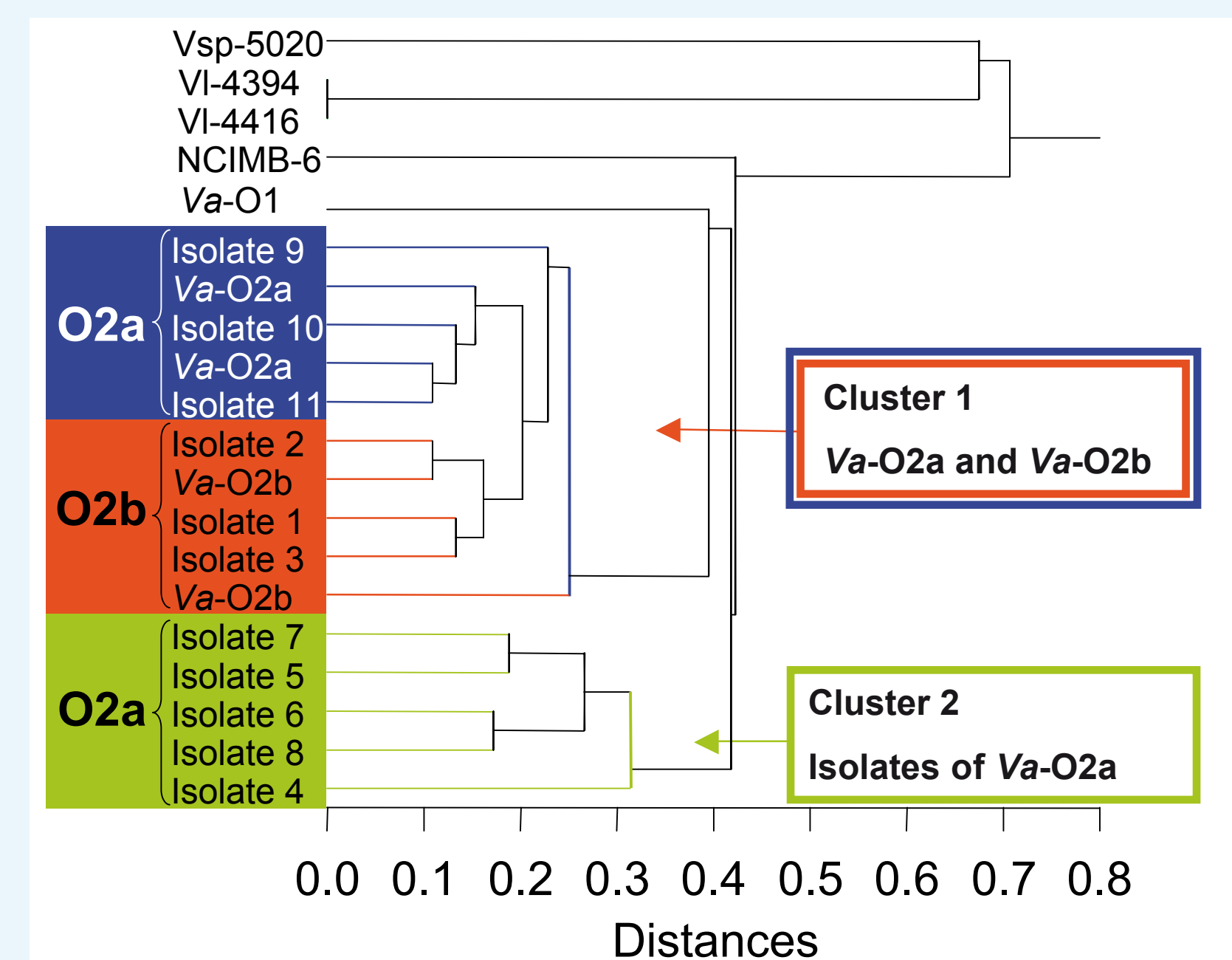
- A. pAb  $\alpha$ Va-O2a. B. pAb  $\alpha$ Va-O2b.
- C. mAb 2H3. D. mAb 6E10.



## Aim of the study

- Characterize new isolates of *Vibrio anguillarum* O2 from diseased cod.
- Evaluate the efficacy of the commercial dip vaccine ALPHA MARINE™ Vibrio (PHARMAQ AS) against newly isolated *V. anguillarum* O2 isolated from diseased cod.

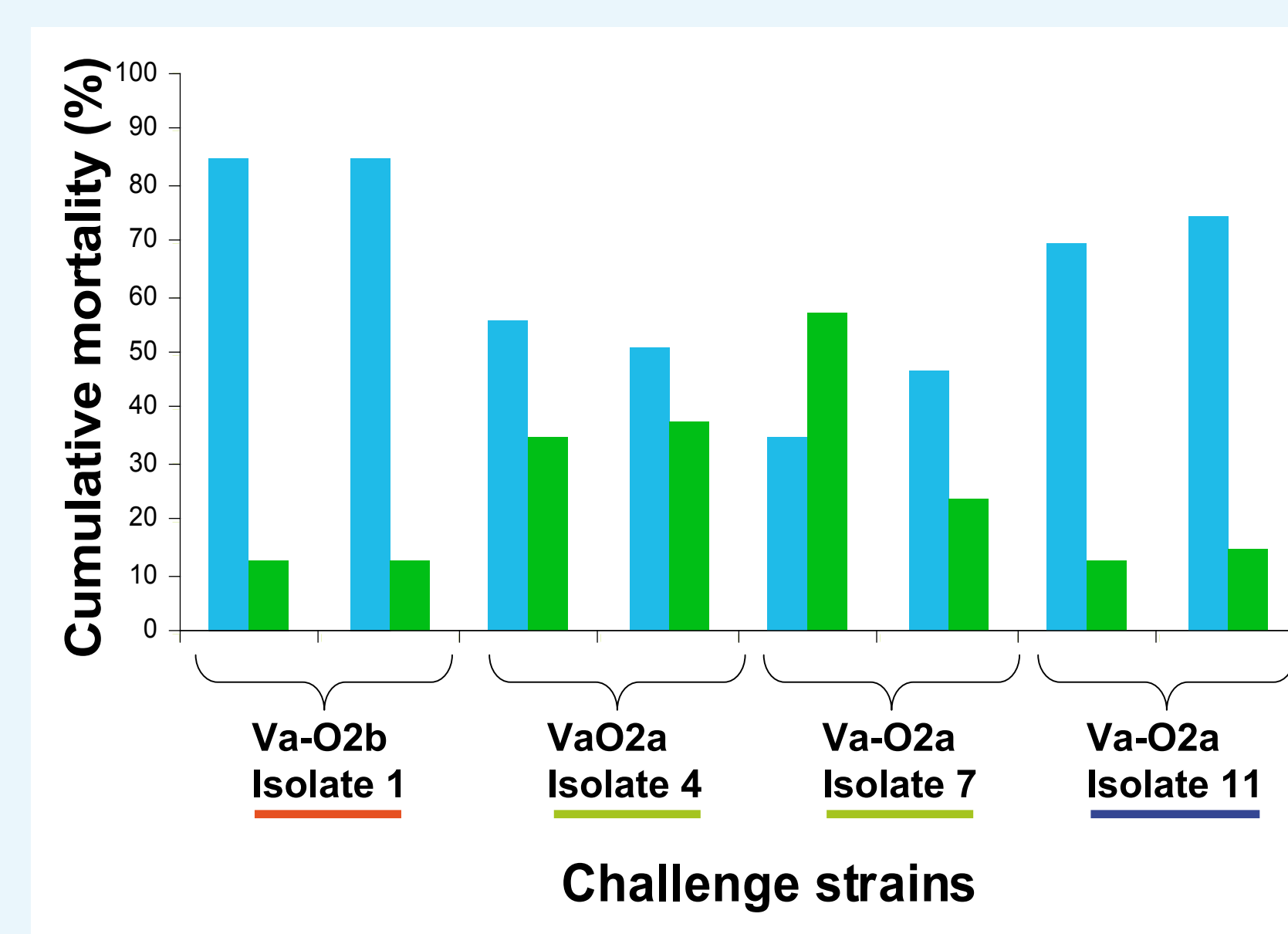
## Genetic comparison



Dendrogram derived from cluster analysis of the AFLP patterns of *V. anguillarum* serotype O2a and O2b isolates from Atlantic cod.

The following strains were also included in the analysis: *V. anguillarum* serotype O2a (NCIMB 6), *Vibrio* sp. (VI-5020) and two *Vibrio lorgei* like isolates (VI-4394 and VI-4416).

## Vaccine efficacy



Cumulative mortality (%) in parallel tanks of cod juveniles vaccinated with ALPHA MARINE™ Vibrio and bath challenged 13 weeks post vaccination with:  
Va-O2b Isolate 1 ( $1.2 \times 10^4$  cfu/ml),  
Va-O2a Isolate 4 ( $6 \times 10^5$  cfu/ml),  
Va-O2a Isolate 7 ( $5 \times 10^6$  cfu/ml) and  
Va-O2a Isolate 11 ( $8 \times 10^3$  cfu/ml).

## Summary

- Some isolates of *V. anguillarum* found in diseased cod differed genetical, serological and in some biochemical reactions compared to earlier isolates.
- ALPHA MARINE™ Vibrio protected very well against *V. anguillarum* isolates biochemically and serologically similar to the strains used in the vaccine, whereas no or only limited protection was obtained against two deviating strains:  
Va-O2b Isolate 1 and Va-O2a Isolate 11: Good protection (RPS: 85 and 81)  
Va-O2a Isolate 4: Limited protection (RPS: 31)  
Va-O2a Isolate 7: No protection

PHARMAQ

The project is financially supported by the Norwegian Research Council, Innovation Norway and PHARMAQ AS.

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Design: Rudi Caeyers, NFH - UIT