Characterization and distribution of the *Flavobacterium psychrophilum* phage-host system – from local to global scales

AQUAPHAGE
WP5

Mathias Middelboe
Marine Biological Section
University of Copenhagen

Scandinavian Culture Collection of Algae & Protozoa

IOC Science and Communication Centre on Harmful Algae

ØRESUNDSAKVARIET

Outreach

- Public exhibitions
- Special arrangements
- High Schools
- Primary schools
- Kindergardens

Education

- Biology students
- DTU students
- PhD courses
- International IOC courses
- High school students

Research

- Marine microbiology
- Ecology and physiology of marine organisms

Support

- Labs
- Ships
- Aquaria
- Administration
- Sea water system

Marine Biological Section

Staff

- Faculty scientists (12)
- Head of IOC center
- Head of Øresund Aquarium
- Natural interpreter
- SCCAP curator
- Technical staff (13)
- Emeriti (7)
- Post docs (4)
- Ph.D. students (~30)
- M.Sc. students (~25)
- Animal keepers/-students (8)

A total of ~100 people

The Phage Ecology Group at MBS

PI: Mathias Middelboe

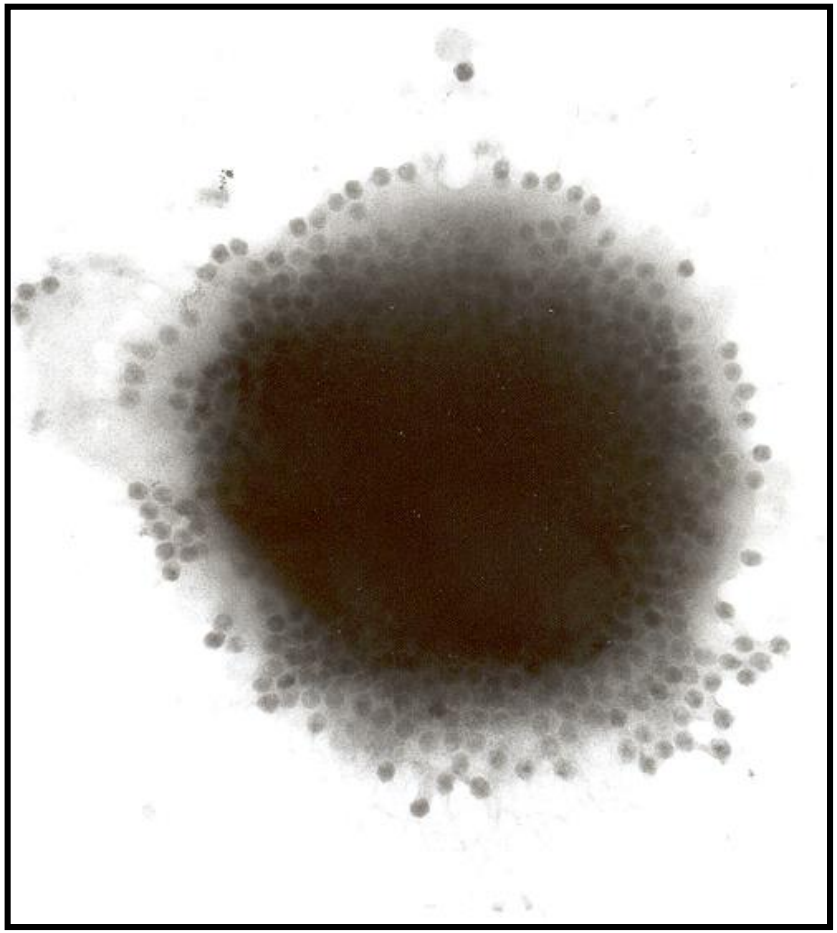
1 Post Doc

5 PhD students

6 Master students

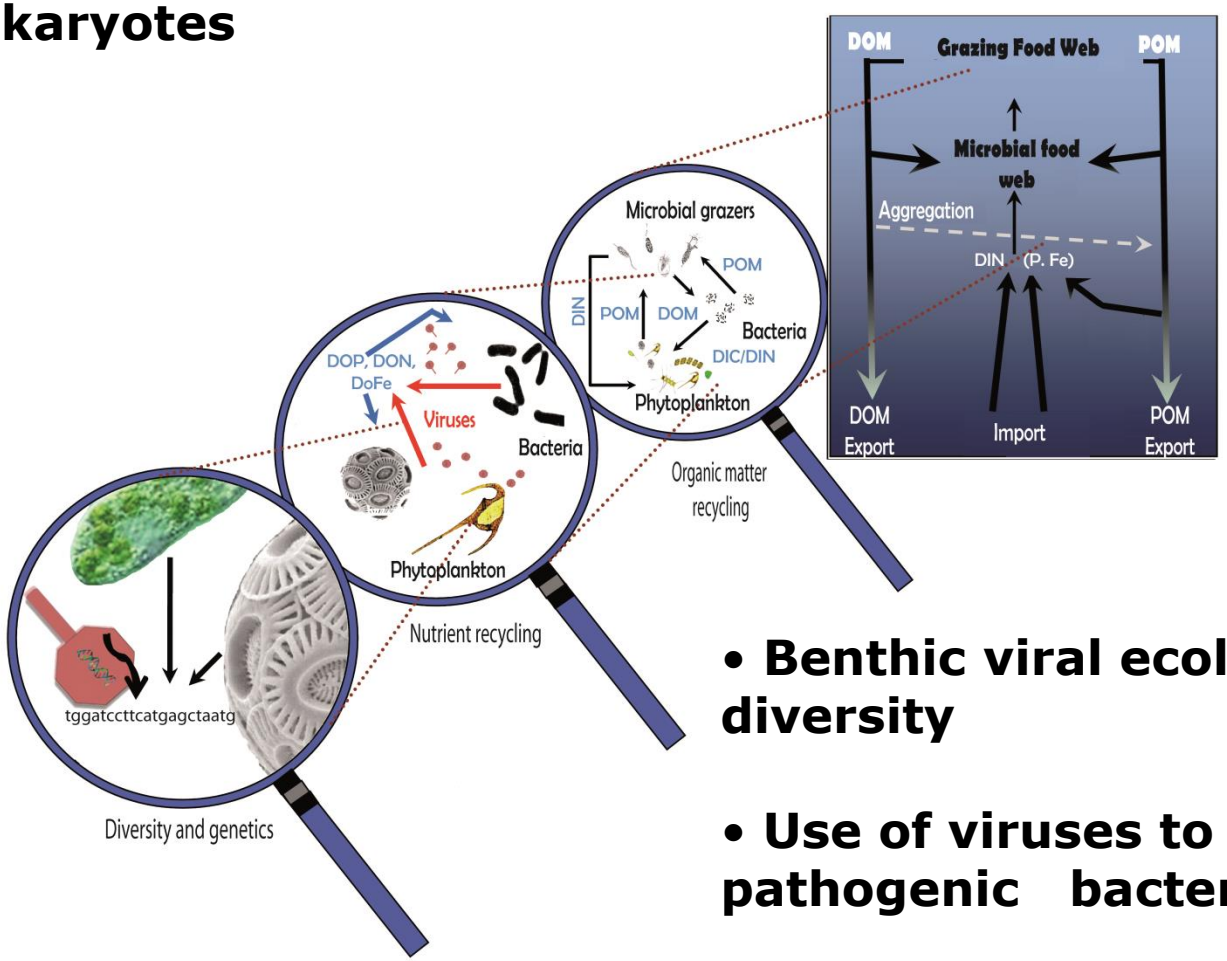
3 Bachelor students

1 Technician



Current research topics in the group

- **The role of viruses in pelagic carbon and nutrient cycling**
- **Viruses as a driving force for genetic and functional diversity of prokaryotes**



- **Benthic viral ecology and diversity**
- **Use of viruses to control pathogenic bacteria**

Specific research projects on bacteriophage control of bacteria

1) "Use of bacteriophages to control *Flavobacterium psychrophilum* in aquaculture" (2010-2014). (The Directorate for Food, Fisheries and Agri Business)

2) "AQUAPHAGE - Network for the development of phage therapy in aquaculture" (2011-2015). EU 7th Frame Programme.

3) "Role of bacteriophages in *Pseudomonas aeruginosa* biofilms in Cystic Fibrosis patients" (2012-2014) University of Copenhagen

4) "ProAqua: Reducing antibiotic use in marine larviculture by a novel combinatory probiotic strategy". (2013-2016) The Danish Strategic Research Council.

<u>AQUAPHAGE team:</u>

PhD student Rói Christiansen,
Currently 1 Master student and 1 Bachelor student are working on the project.

WP5: Evolution and distribution of broad-host range phages specific to *Flavobacterium psychrophilum* – from local to global scales.

Aim:

- To obtain a collection of *F. psychrophilum* phages from Danish and Chilean fish farms

- To examine the diversity, distribution sensitivity/infectivity of *Flavobacterium psychrophilum* and their phages across large spatial scales

- To determine the mechanisms of phage susceptibility and resistance in *F. psychrophilum*

WP5: Evolution and distribution of broad-host range phages specific to *Flavobacterium psychrophilum* – from local to global scales.

<u>Secondments:</u>

Daniel Castillo, UDC, working at University of Copenhagen for 3 months in 2011 and 8 months in 2013.

Rói Christiansen, UC, working at UDC for 2.5 months in 2013

Mathias Middelboe, UC, working at UDC for 3 weeks in 2013

Publications:

1. *Castillo, D, Higuera, G., Villa, M, Middelboe, M. Dalsgaard I, Madsen, L, Espejo, R*. (2012). Diversity of *Flavobacterium psychrophilum* and the potential use of its phages for protection against bacterial cold water disease in salmonids. J. Fish Dis. 35:193-201

2. *Madsen, L., Dalsgaard, I. and Middelboe, M.* (2013). Dispersal and survival of *Flavobacterium psychrophilum* phages in vivo in rainbow trout and in vitro under laboratory conditions: Implications for their use in phage therapy. Appl. Environ. Microbiol. (in revision)

3. *Castillo, D., Espejo, R. and Middelboe,* M. Characterization and genomic structure of the temperate bacteriophage 6H, and its distribution as prophage in *Flavobacterium psychrophilum* strains. Submitted to Appl. Environ. Microbiol

4. *Christiansen et al.* (in prep): Effect of bacteriophages on the growth of *Flavobacterium psychrophilum* and development of phage-resistant strains

5. *Castillo et al* (in prep): Host range and geographical distribution of *Flavobacterium psychrophilum* phages: Dynamics in susceptibility

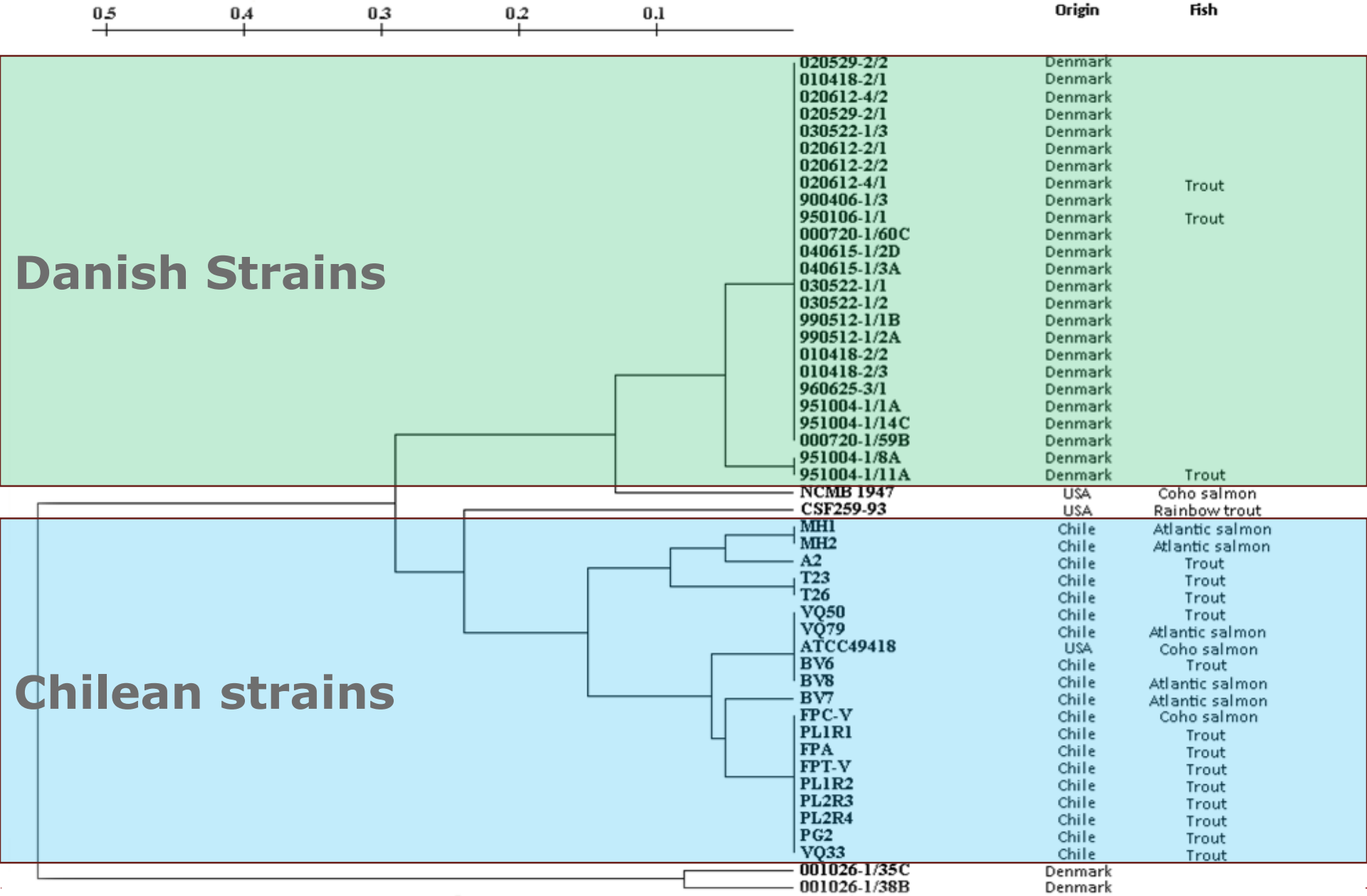
6. *Castillo et al* (in prep): Genomic characterization of bacteriophage resistance mechanisms in *Flavobacterium psychrophilum*

7. *Laigaard* et al (in prep): Dynamics and stability of phage resistance mutations in *Flavobacterium psychrophilum*

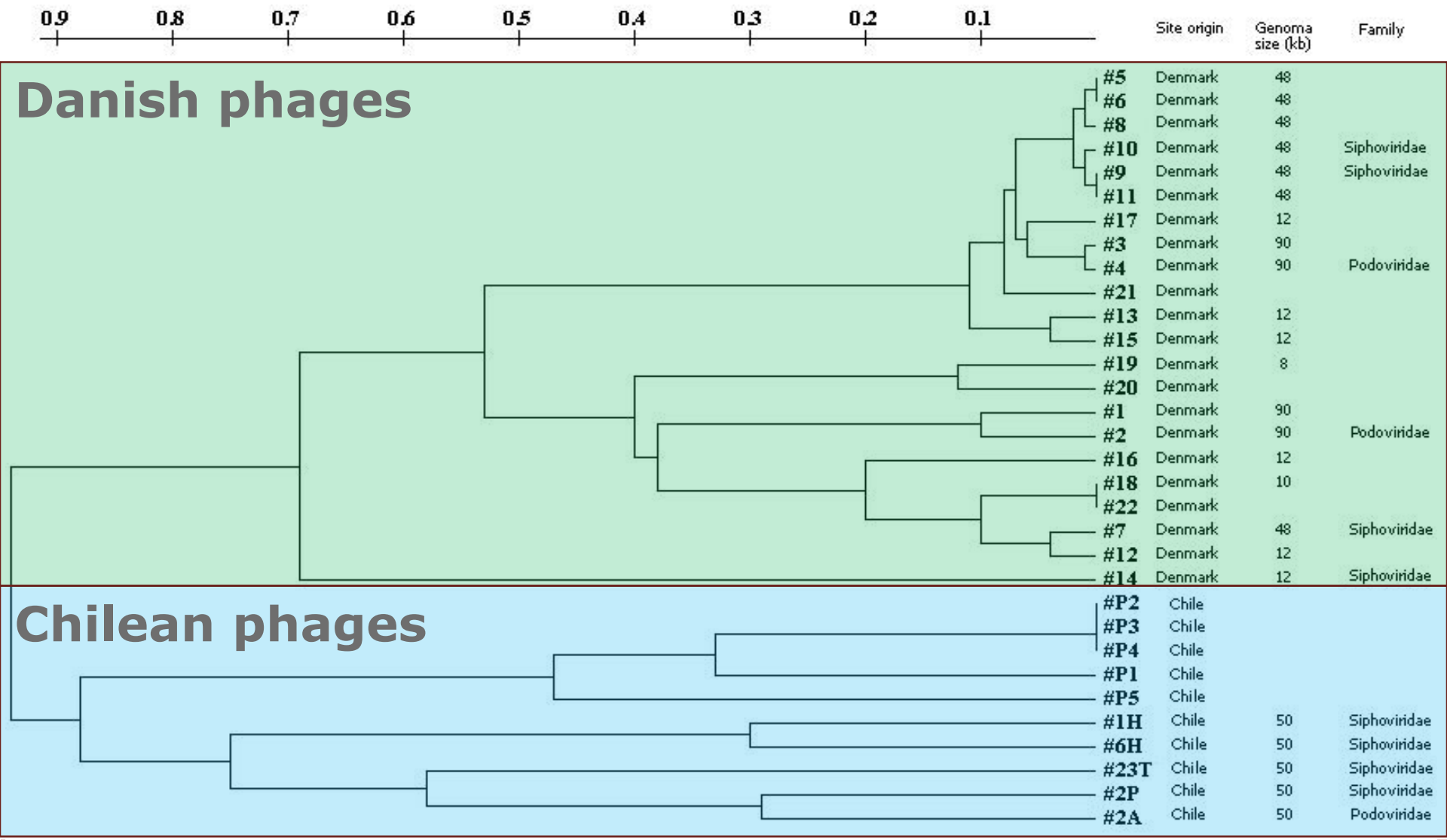
Presentations:

1. *Christiansen et al*: Meeting in the Danish Marine Research Society 2011 (Oral)
2. *Christiansen et al*: Aquatic Virus Workshop, Holland, 2011 (Poster)
3. *Christiansen et al*: Meeting in the Danish Microbiological Society, 2011. (Poster)
4. *Christiansen et al*: DMS møde 2011 (Oral)
5. *Middelboe*: AQUAPHAGE workshop, Crete, 2011 (Oral)
6. *Middelboe et al*: ISME Meeting; København, 2012, (Oral)
7. *Christiansen et al*: KU, Section seminar, 2012, (Oral)
8. *Laigaard et al*: KU, Section seminar, 2012 (Oral)
9. *Middelboe et al*.: Seminar at Chr. Hansen A/S, 2012 (Oral)
10. *Laigaard et al*: Meeting in the Danish Marine Research Society 2013 (Oral)
11. *Christiansen et al*: Dafinet workshop, 2013 (Oral)
12. *Christiansen et al*: EAFP Conference, Tampere Finland 2013 (Oral)

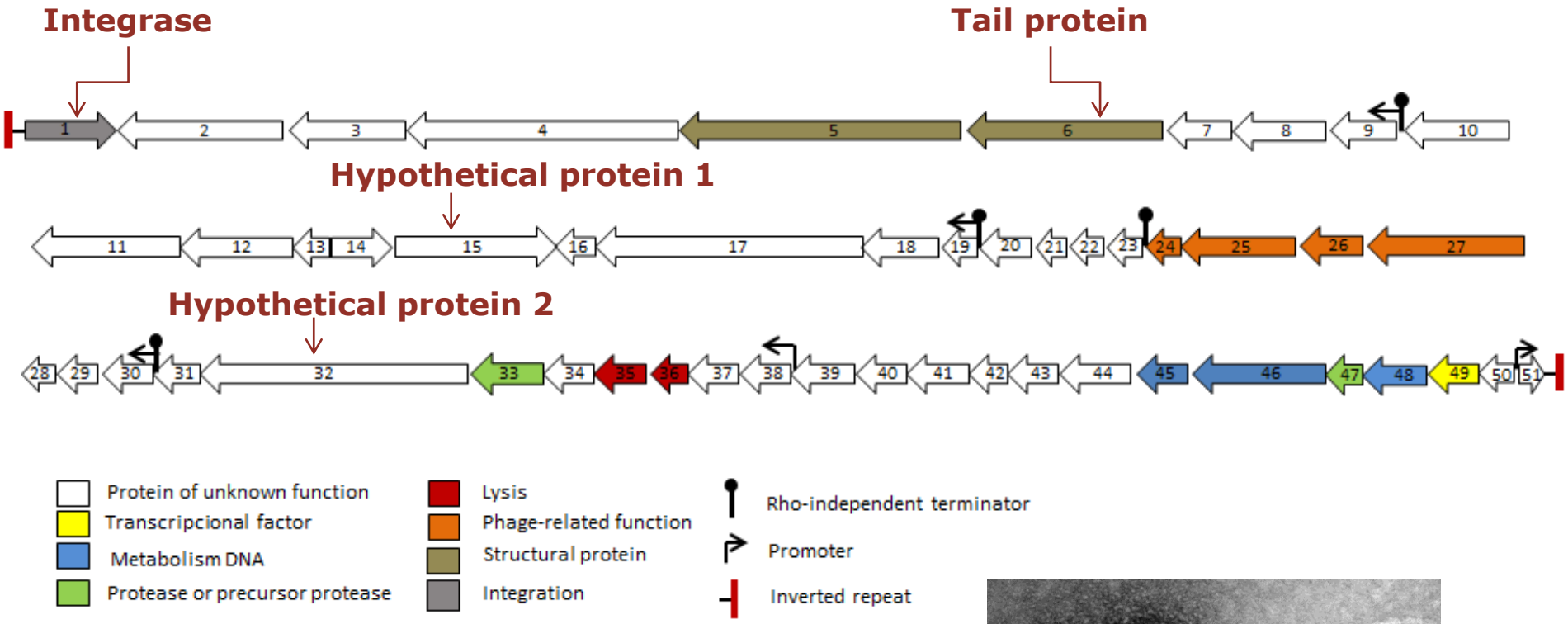
DGREA analysis of *F. psychrophilum*

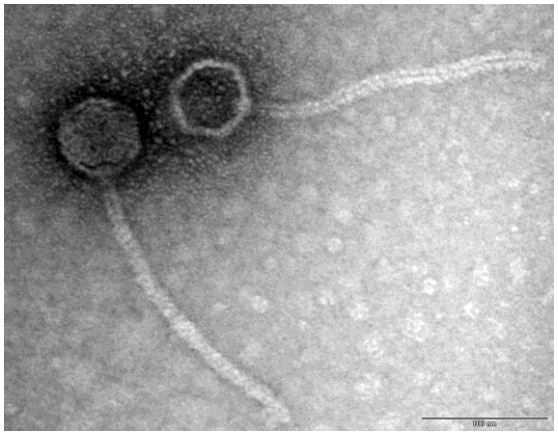


Clustering of 32 *F. psychrophilum* phage isolates based on their host ranges of infectivity against 89 bacterial isolates.

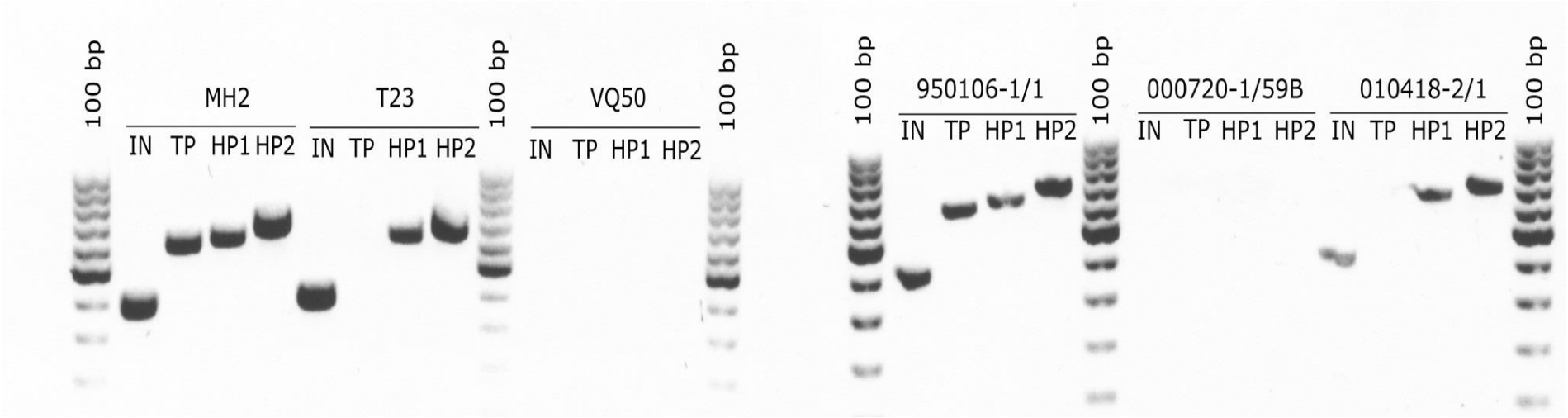


Phage 6H genome organization

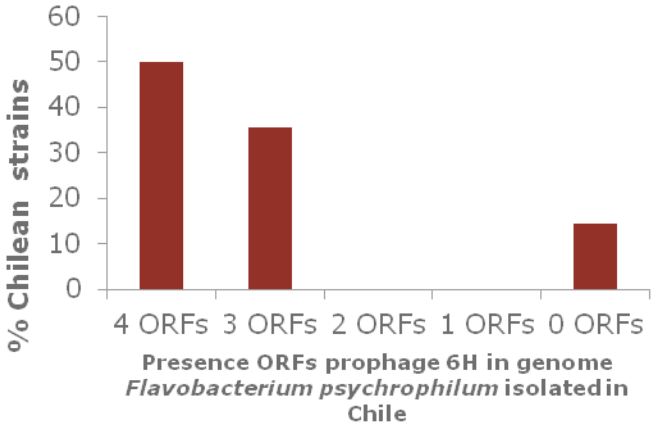




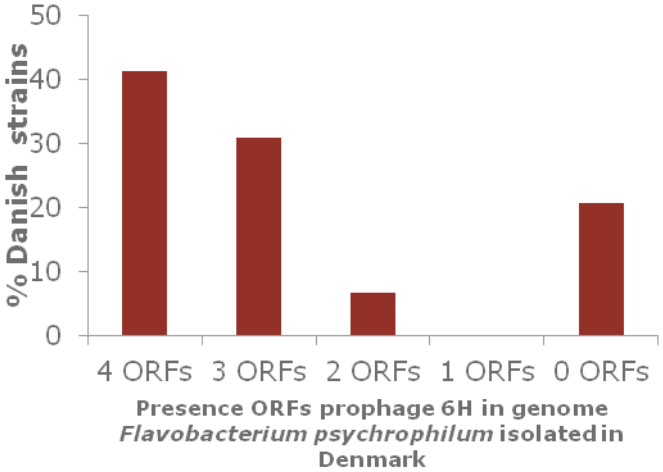
Presence of four ORFs from genome 6H in *F. psychrophilum* strains isolated in Chile and Denmark



21 strains isolated in Chile



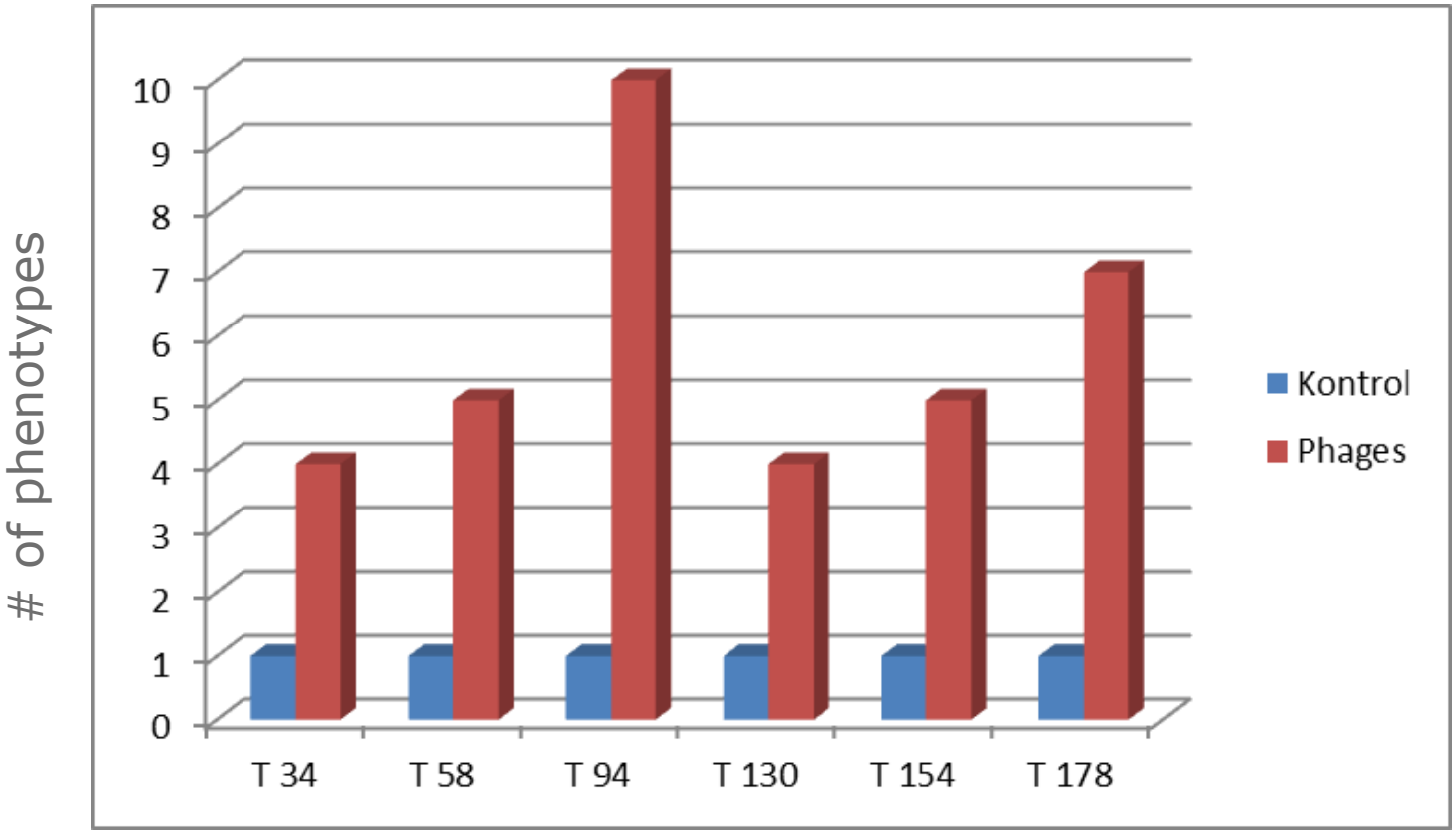
28 strains isolated in Denmark



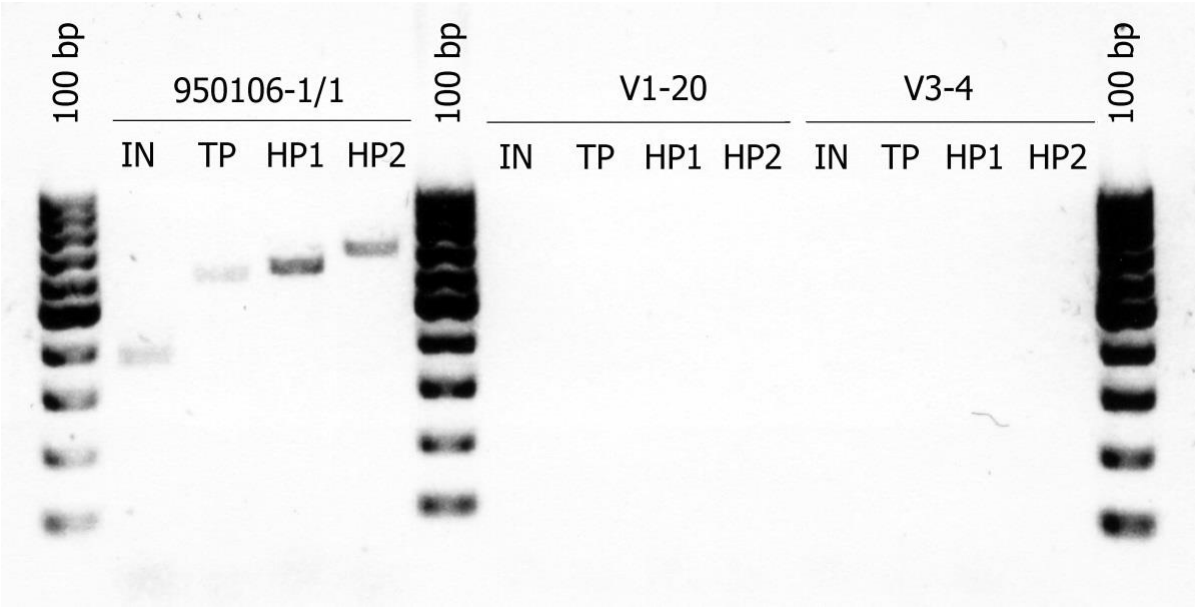
Phage driven development of resistance in *F. psychrophilum*

Phage driven development of resistance in *F. psychrophilum*

Many resistant phenotypes appear after exposure to phages



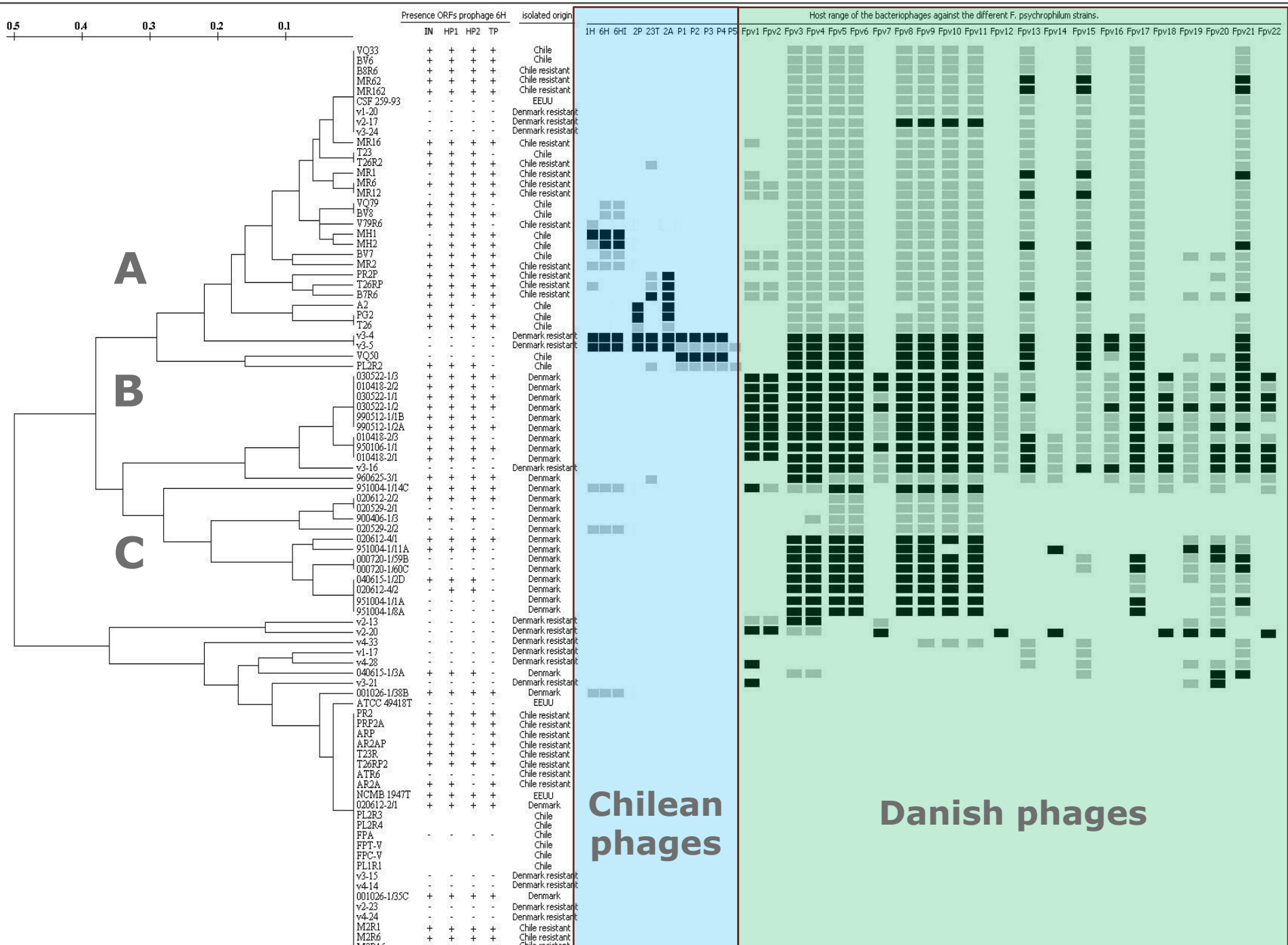
Presence of four ORFs from genome 6H in *F. psychrophilum* resistant strains



V1-17
v1-20
v2-13
v2-17
v2-20
v2-23
v3-4
v3-5
v3-15
v3-16
v3-21
v3-24
v4-14
v4-24
v4-28
v4-33

All Danish phage-resistant strains had lost the 6H prophage

Complete host range analysis including all Chilean and Danish *F. psychrophilum* strains and *p*hages



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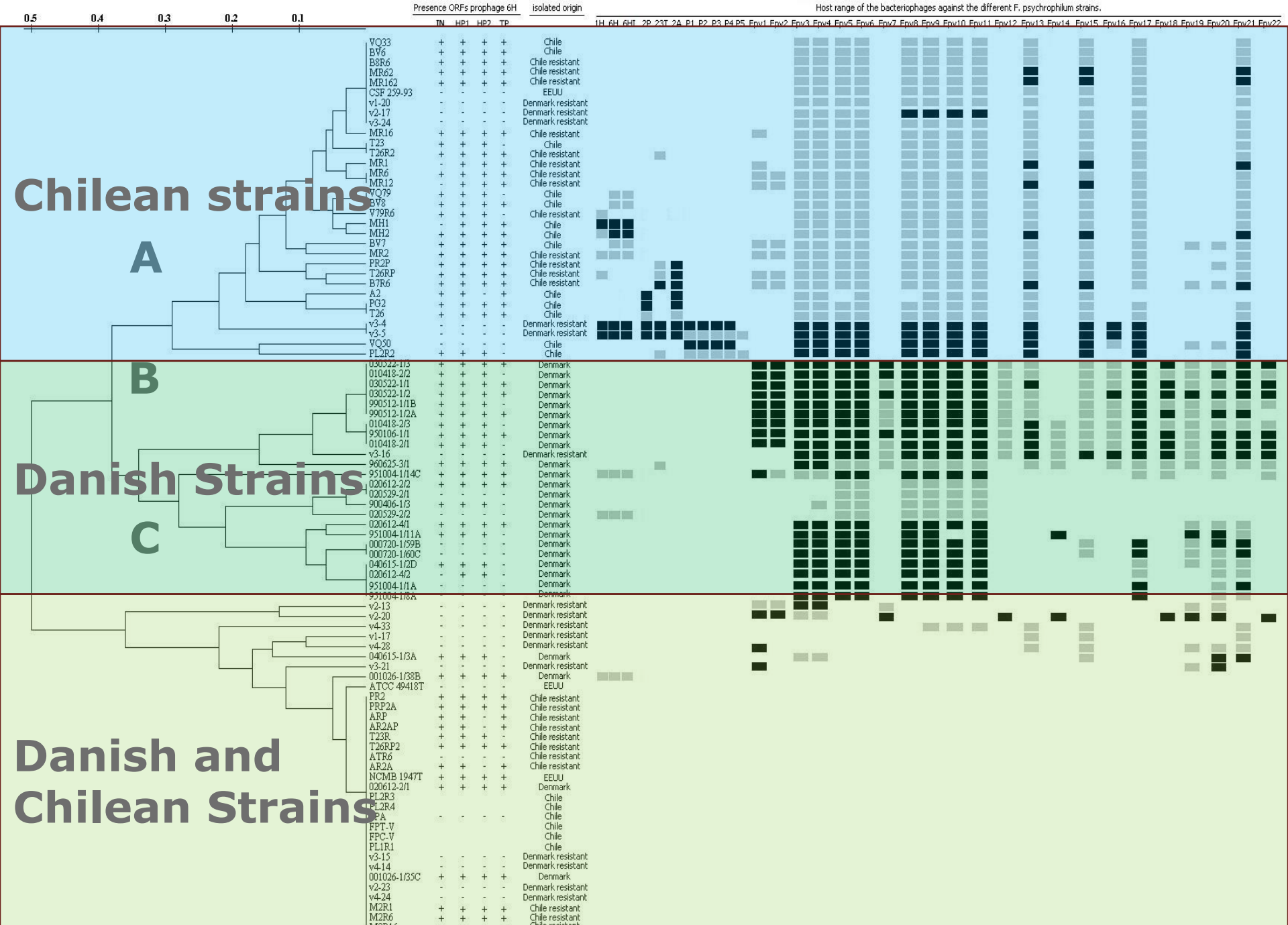
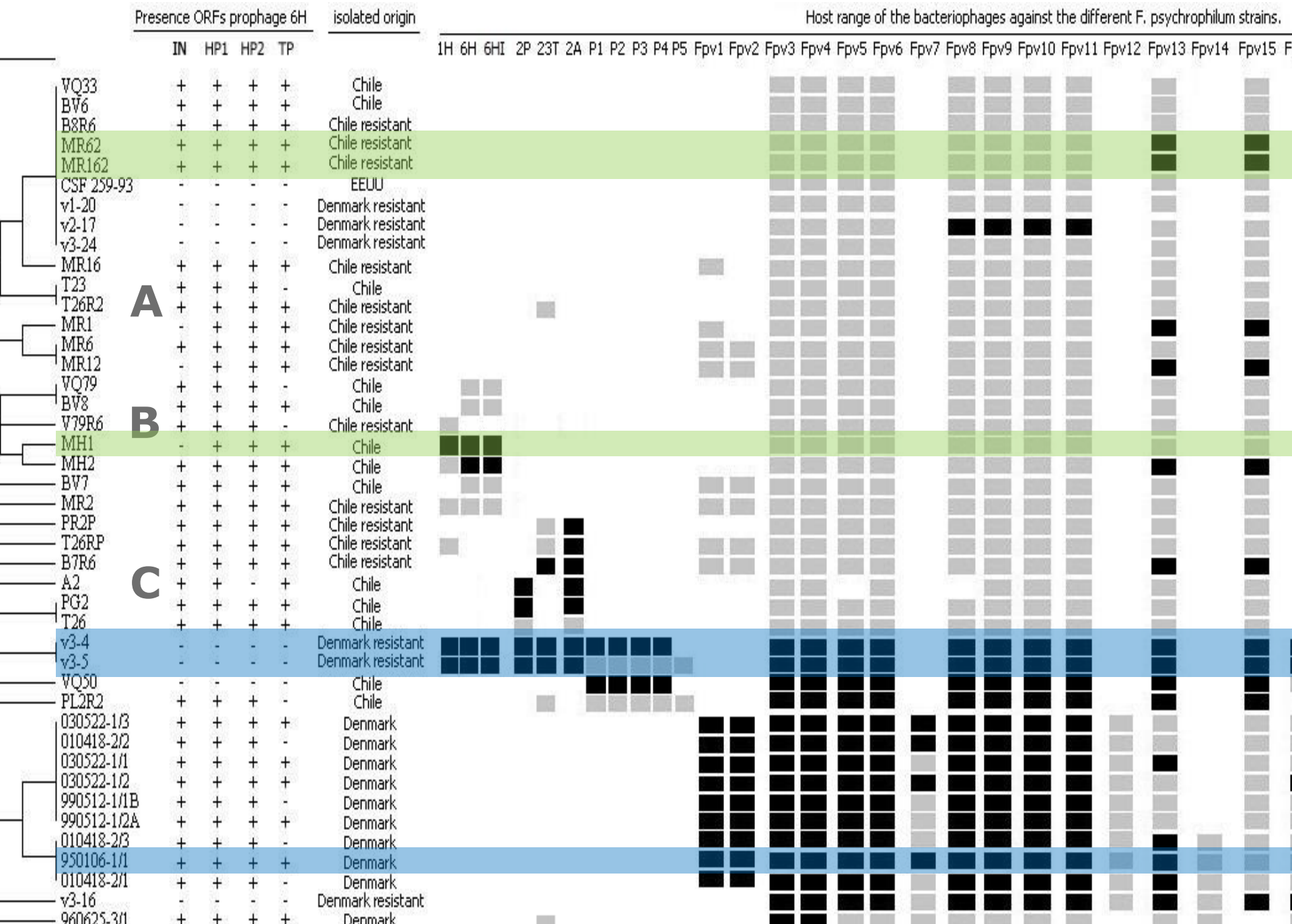
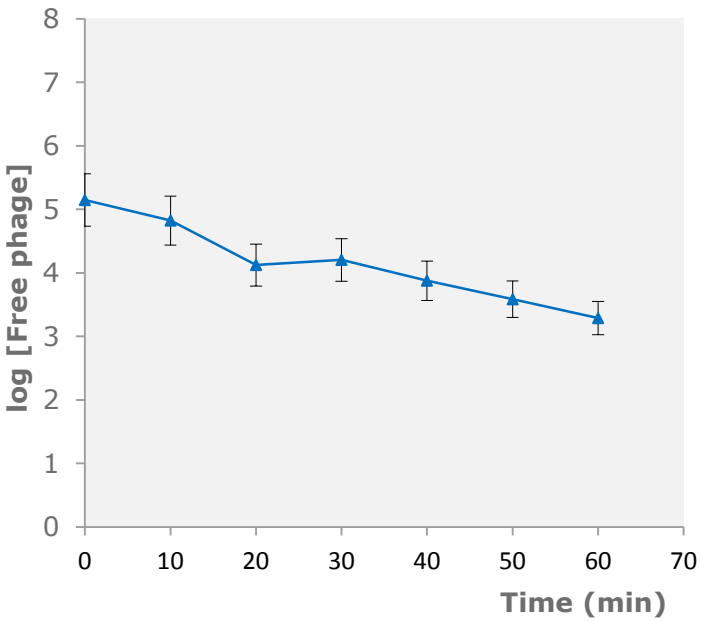
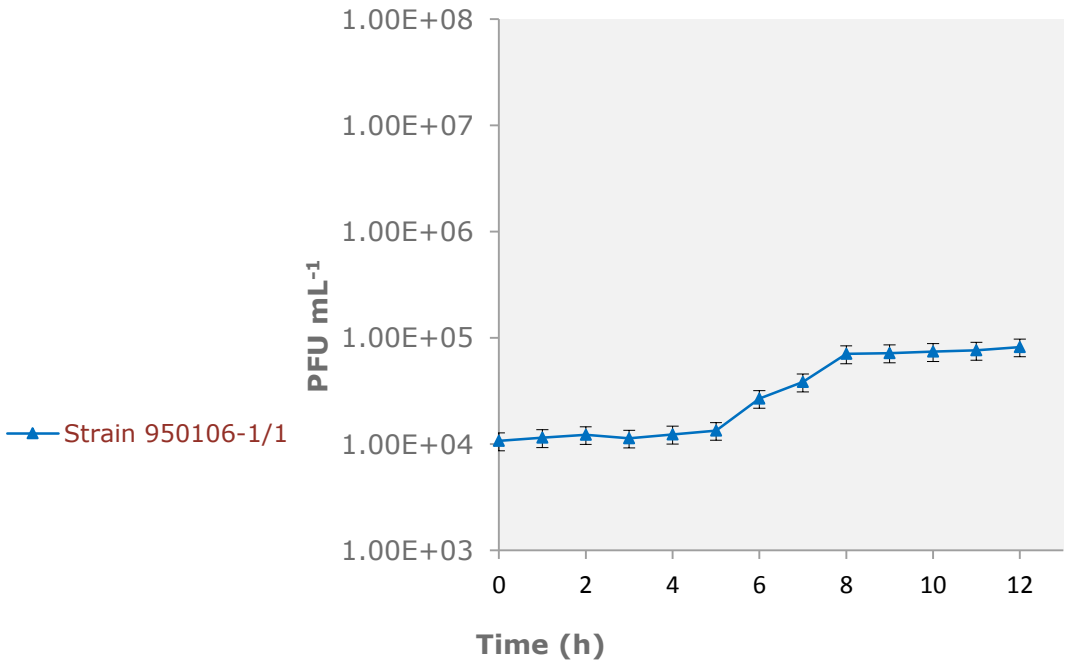


Resistance against some phages provides increased sensitivity to others



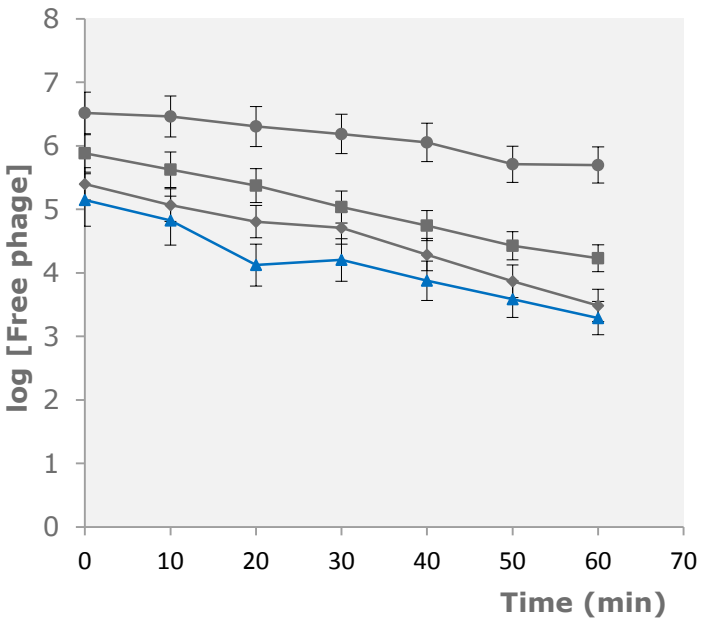
Resistance against some phages provides increased sensitivity to others
E.g.: FpV-15

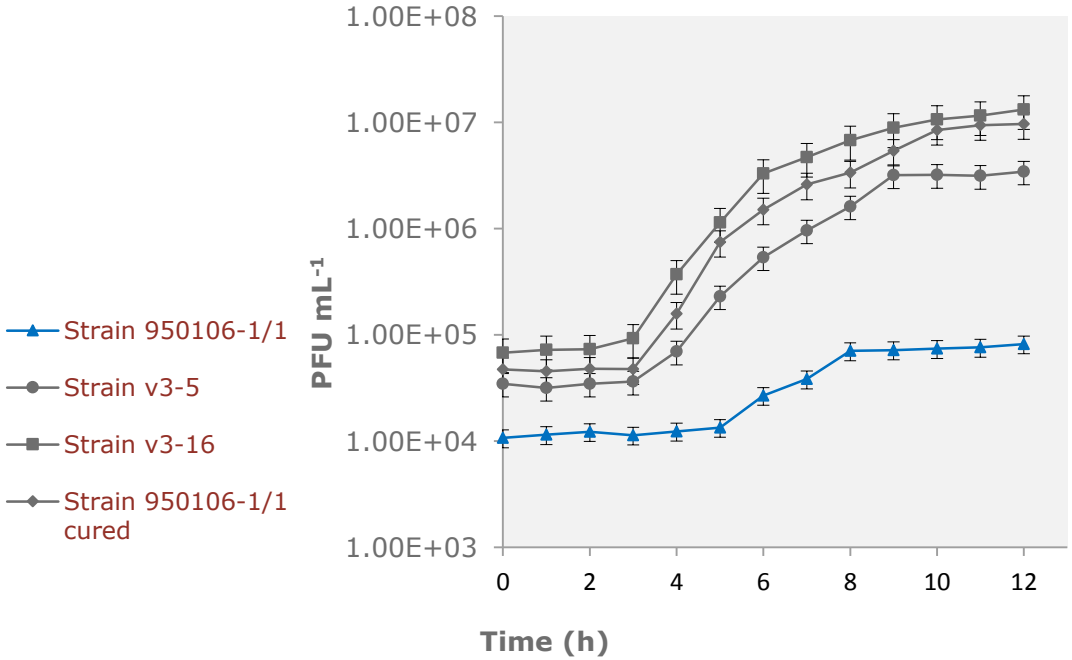




Strain	Burst size	latency time (hr)	Sensitivity
950106-1/1: **WT**	9 ± 1	5	**$3x10^6$**

Resistance against some phages provides increased sensitivity to others E.g.: FpV-15





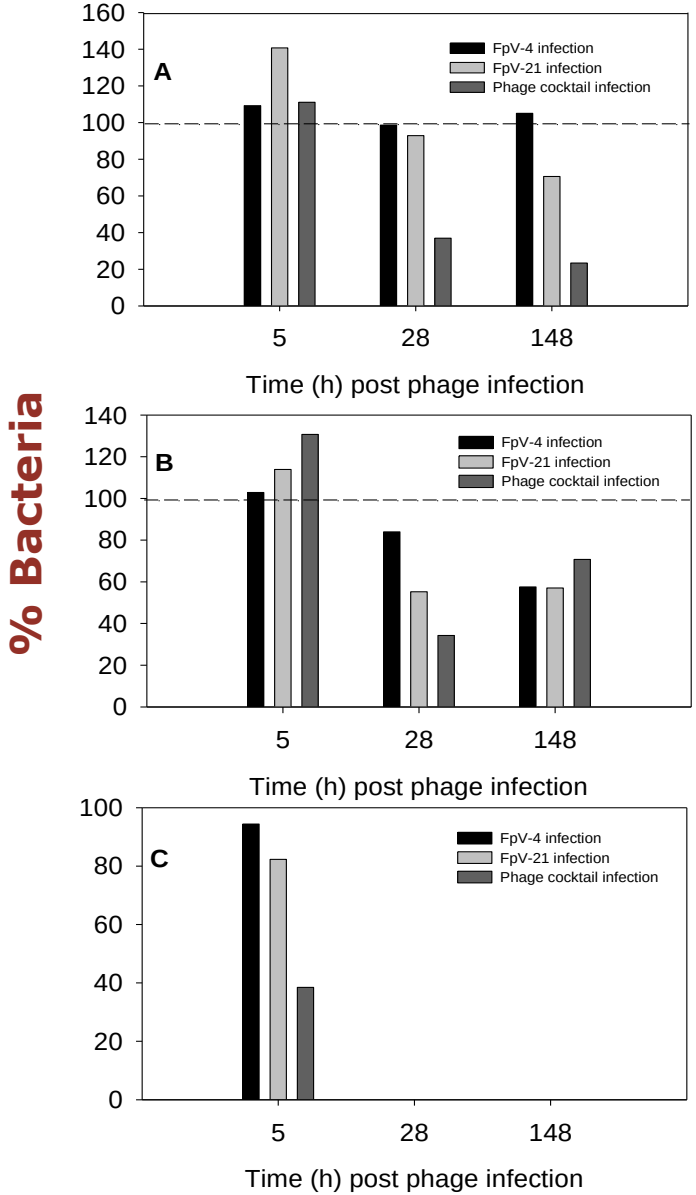
Strain	Burst size	latency time (hr)	Sensitivity
950106-1/1: **WT**	9 ± 1	5	**$3x10^6$**
950106-1/1c: **Cured**	195 ± 10	3	ND
v3-5: **Resistant**	125 ± 20	3	**$>10^{10}$**
v3-16: **Resistant**	183 ± 9	3	**$5x10^9$**

Multiple costs of resistance:

- Enhanced infection by other phages
- Reduced growth rates
- Loss of ability to utilize specific substrates

Metabolic and enhanced infection costs of resistance may be key drivers of host strain diversity and the co-existence of phages and hosts on local and global scales

Phage influence on host abundance and isolation of phage-resistant hosts

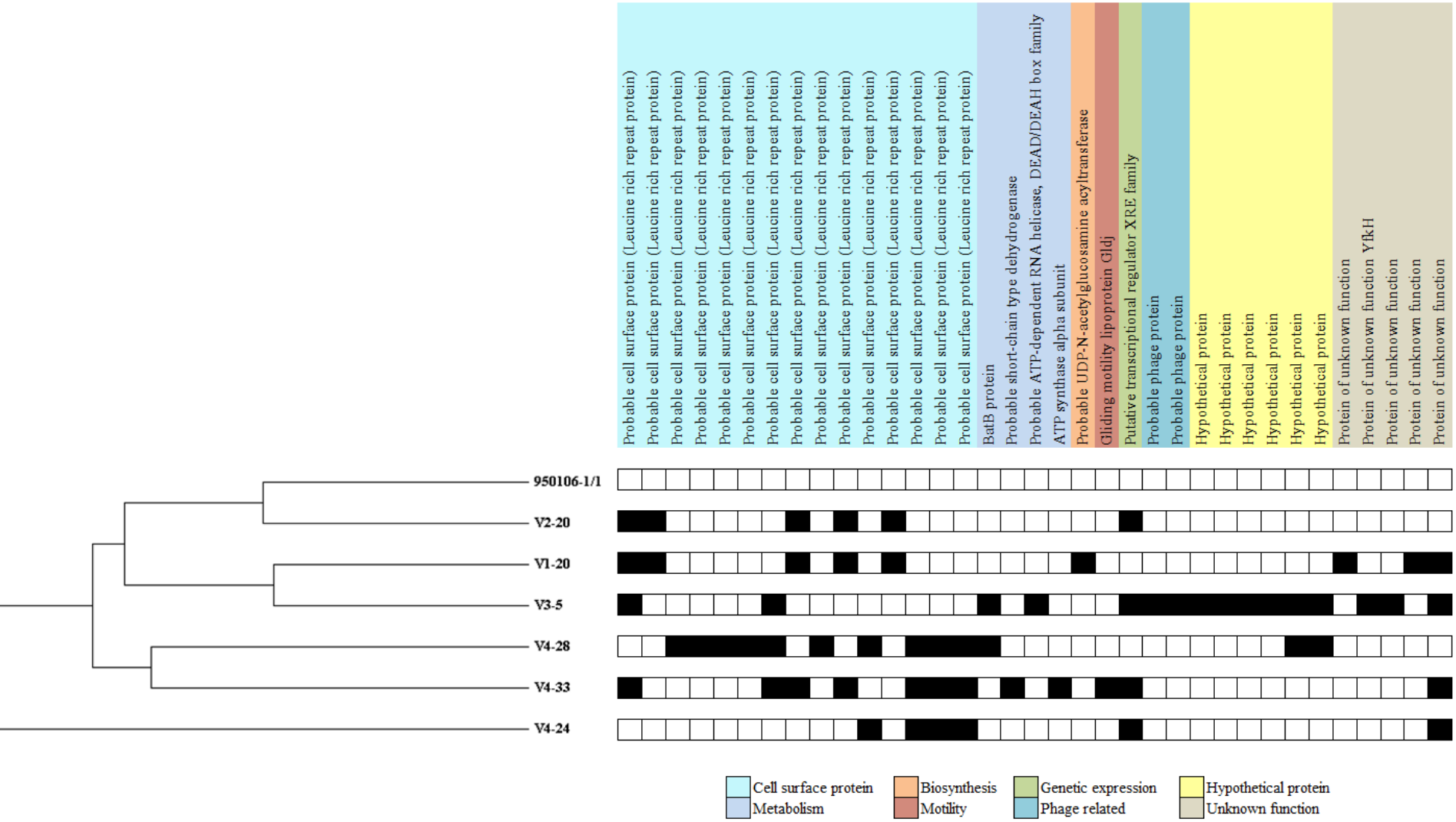


Low phage concentration. MOI:~0.01

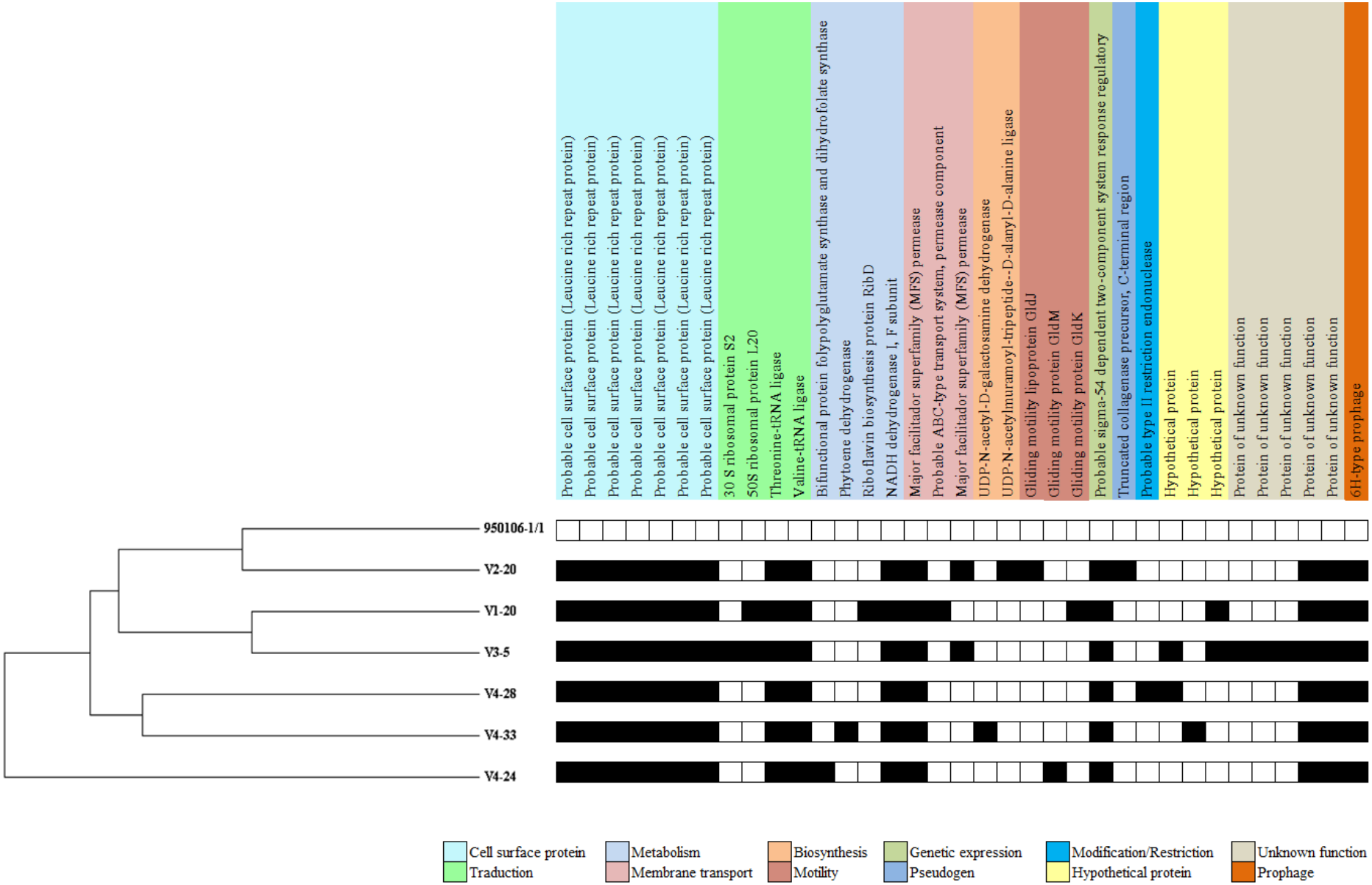
Medium phage concentration: MOI:~0.1

High phage concentration: MOI:~5

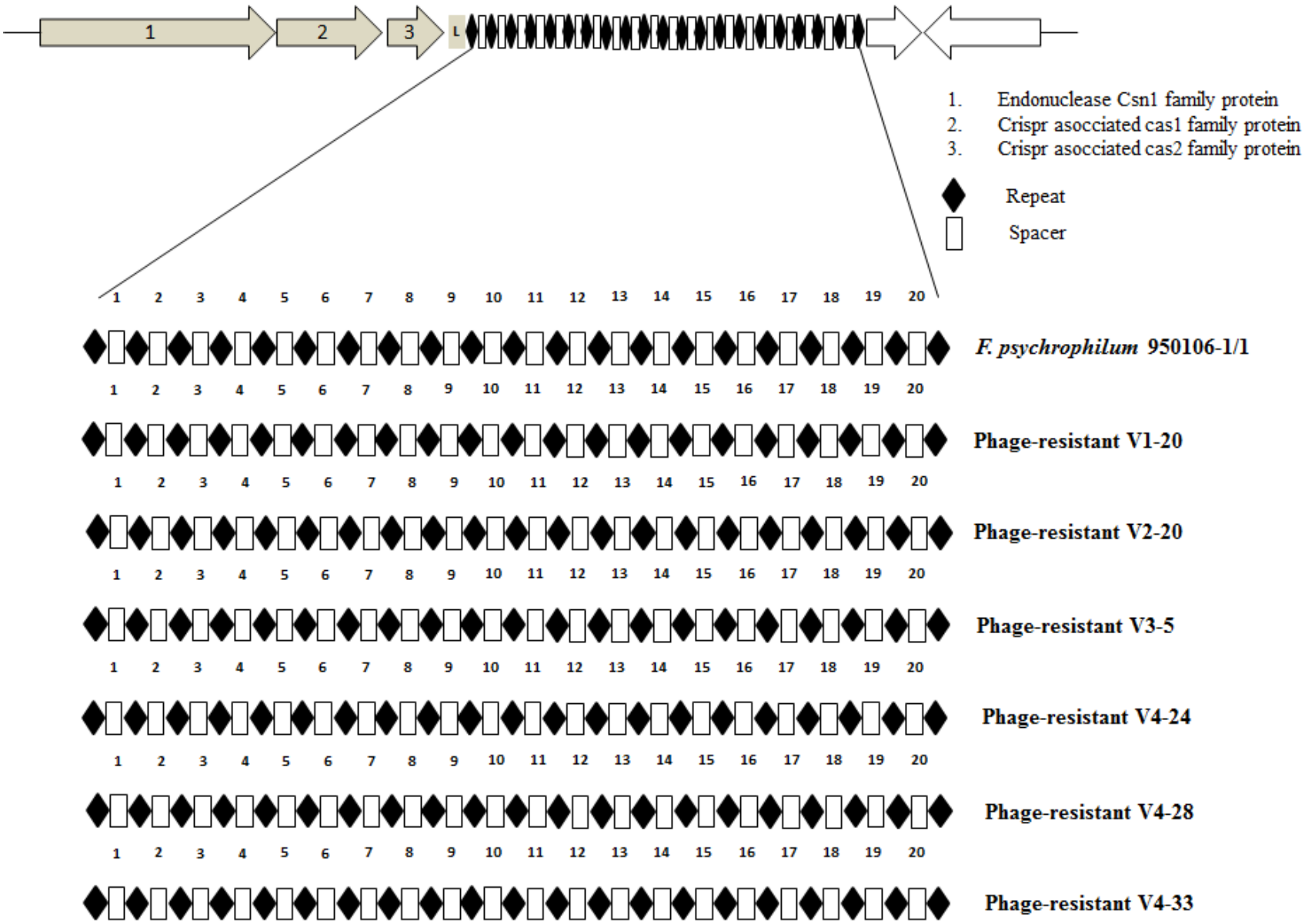
Genetic basis for resistance: Punctual mutations i resistant strains



Genetic basis for resistance: Deletions and insertions in resistant strains



CRISPR resistance in *F. psychrophilum*



Conclusions

- Phylogenetically, the Chilean and Danish strains are similar, but separated into two distinct clusters based on direct genome restriction fragment analysis (DGREA).
- Positive cross-reactions between Danish hosts and Chilean phages and vice versa.
- Presence of a specific (phage 6H) prophage in both Danish and Chilean host strains.
- Development of phage-resistance were associated with metabolic costs and/or increased susceptibility to other phages.
- Development of phage-resistance was associated with mutations in cell surface structures
- Resistance patterns and phage life cycle characterization of several phages were linked to the presence of 6H prophage in the host.

WP5: Evolution and distribution of broad-host range phages specific to *Flavobacterium psychrophilum* – from local to global scales.

Major discoveries:

1) Distinct clusters of pathogens and phages BUT …
2) Global scale distribution of phenotypic traits related with phage-susceptibility
3) Global scale distribution of a specific prophage,
4) Fast and highly dynamic changes in phage susceptibility in the global *F. psychrophilum* community.
5) Identification and characterization of phage resistance mechanisms

=> *Despite the spatial separation of the phage-host communities, the individual communities in Denmark and Chile has maintained host range and susceptibility patterns that are adapted to the global community.*

=> *On a local scale, the susceptibility properties are highly dynamic and diverse*

Thanks to:

Daniel Castillo
Rói Hammershaimb Christiansen
Helle Kjær Laigaard
Romilio Espejo
Inger Dalgaard
Lone Madsen
Jeanett Hansen
Eric Johansen