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
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)


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


AQUAPHAGE team:

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.

**Secondments:**

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:


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:

2. Christiansen et al: Aquatic Virus Workshop, Holland, 2011 (Poster)
5. Middelboe: AQUAPHAGE workshop, Crete, 2011 (Oral)
8. Laigaard et al: KU, Section seminar, 2012 (Oral)
9. Middelboe et al.: Seminar at Chr. Hansen A/S, 2012 (Oral)
DGREA analysis of *F. psychrophilum*

**Danish Strains**

<table>
<thead>
<tr>
<th>Strain Code</th>
<th>Origin</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td></td>
<td>Trout</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Chilean Strains**

<table>
<thead>
<tr>
<th>Strain Code</th>
<th>Origin</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Coho salmon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rainbow trout</td>
</tr>
</tbody>
</table>

**Sensitivity and Specificity**

- **Sensitivity:** 99.8%
- **Specificity:** 99.6%

**Discussion**

The analysis revealed high sensitivity and specificity, indicating a robust diagnostic tool for identifying *F. psychrophilum*. Further studies are needed to validate the results in field conditions.
Clustering of 32 *F. psychrophilum* phage isolates based on their host ranges of infectivity against 89 bacterial isolates.

### Danish phages

- **#5**: Denmark, 48 kb, Siphoviridae
- **#6**: Denmark, 48 kb, Siphoviridae
- **#8**: Denmark, 48 kb, Siphoviridae
- **#10**: Denmark, 48 kb, Siphoviridae
- **#9**: Denmark, 48 kb, Siphoviridae
- **#11**: Denmark, 48 kb, Siphoviridae
- **#17**: Denmark, 12 kb, Podoviridae
- **#3**: Denmark, 90 kb, Podoviridae
- **#4**: Denmark, 90 kb, Podoviridae
- **#21**: Denmark, 12 kb, Podoviridae
- **#13**: Denmark, 12 kb, Podoviridae
- **#15**: Denmark, 12 kb, Podoviridae
- **#19**: Denmark, 8 kb, Podoviridae
- **#20**: Denmark, 8 kb, Podoviridae
- **#1**: Denmark, 90 kb, Podoviridae
- **#2**: Denmark, 90 kb, Podoviridae
- **#16**: Denmark, 12 kb, Podoviridae
- **#18**: Denmark, 10 kb, Podoviridae
- **#22**: Denmark, 10 kb, Podoviridae
- **#7**: Denmark, 48 kb, Siphoviridae
- **#12**: Denmark, 12 kb, Siphoviridae
- **#14**: Denmark, 12 kb, Siphoviridae

### Chilean phages

- **#P2**: Chile, 50 kb, Siphoviridae
- **#P3**: Chile, 50 kb, Siphoviridae
- **#P4**: Chile, 50 kb, Siphoviridae
- **#P1**: Chile, 50 kb, Siphoviridae
- **#P5**: Chile, 50 kb, Siphoviridae
- **#1H**: Chile, 50 kb, Siphoviridae
- **#6H**: Chile, 50 kb, Siphoviridae
- **#23T**: Chile, 50 kb, Siphoviridae
- **#2P**: Chile, 50 kb, Siphoviridae
- **#2A**: Chile, 50 kb, Siphoviridae
Phage 6H genome organization

Integrase

Tail protein

Hypothetical protein 1

Hypothetical protein 2
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

Presence ORFs prophage 6H in genome *Flavobacterium psychrophilum* isolated in Chile

Presence ORFs prophage 6H in genome *Flavobacterium psychrophilum* 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

All Danish phage-resistant strains had lost the 6H prophage
Complete host range analysis including all Chilean and Danish *F. psychrophilum* strains and phages.
Complete host range analysis including all Chilean and Danish *F. psychrophilum* strains and phages

### Chilean strains

### Danish Strains

### Danish and Chilean Strains
Resistance against some phages provides increased sensitivity to others.
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E.g.: FpV-15

<table>
<thead>
<tr>
<th>Strain</th>
<th>Burst size</th>
<th>latency time (hr)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>950106-1/1: WT</td>
<td>9 ± 1</td>
<td>5</td>
<td>3x10^6</td>
</tr>
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<td>9 ± 1</td>
<td>5</td>
<td>3x10^6</td>
</tr>
<tr>
<td>950106-1/1c: Cured</td>
<td>195 ± 10</td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td>v3-5: Resistant</td>
<td>125 ± 20</td>
<td>3</td>
<td>&gt;10^{10}</td>
</tr>
<tr>
<td>v3-16: Resistant</td>
<td>183 ± 9</td>
<td>3</td>
<td>5x10^9</td>
</tr>
</tbody>
</table>
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 in resistant strains

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Function</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein A</td>
<td>Product</td>
<td>Variant A</td>
<td>Phenotype A</td>
</tr>
<tr>
<td>Protein B</td>
<td>Function B</td>
<td>Variant B</td>
<td>Phenotype B</td>
</tr>
</tbody>
</table>

Diagram:

- 950106.1/1
- V2-20
- V1-20
- V3-5
- V4-28
- V4-33
- V4-24

Key:
- Cell surface protein
- Biosynthesis
- Genetic expression
- Hypothetical protein
Genetic basis for resistance: Deletions and insertions in resistant strains

| Probable cell surface protein | Lysine-rich repeat protein | Probable cell surface protein | Lysine-rich repeat protein | Probable cell surface protein | Lysine-rich repeat protein | Probable cell surface protein | Lysine-rich repeat protein | Probable cell surface protein | Lysine-rich repeat protein | Probable cell surface protein | Lysine-rich repeat protein | Probable cell surface protein | Lysine-rich repeat protein | 10S ribosomal protein S2 | 5S ribosomal protein L20 | Threonyl-tRNA synthetase | Phenylalanine tRNA synthetase | Histidinol deaminase | L-glutamine synthetase and dihydroorotase synthase |
|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|---------------------------|---------------------------|-------------------------------|---------------------------|

| NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrogenase | NADH dehydrog
CRISPR resistance in *F. psychrophilum*

1. Endonuclease Csn1 family protein
2. Crispr associated cas1 family protein
3. Crispr associated cas2 family protein

- **Repeat**
- **Spacer**

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- **F. psychrophilum 950106-1/1**
- Phage-resistant V1-20
- Phage-resistant V2-20
- Phage-resistant V3-5
- Phage-resistant V4-24
- Phage-resistant V4-28
- Phage-resistant V4-33
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