A potential vaccine to control bacterial coldwater disease (CWD)

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Background

- Etiological agent is *Flavobacterium psychrophilum*
- Originally isolated in 1948 in Washington state from coho salmon at 10°C
  - Was referred to as “low temperature disease”, “coldwater disease”, and “peduncle disease”
- Taxonomic status of this bacterium has changed due to DNA technology
  - Formerly known as *Cytophaga psychrophila* and *Flexibacter psychrophilus*
Host Susceptibility

- **Salmonid Species**
  - Coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*) are particularly susceptible to *F. psychrophilum* infection
  - Atlantic salmon and others affected

- **Non-salmonid species**
  - Eel (*Anguilla anguilla*)
  - Tench (*Tinca tinca*)
  - Carp (*Cyprinus carpio*)
  - Crucian carp (*Carassius carassius*)
  - Ayu (*Plecoglossus altivelis*)
  - Sea Lamprey (*Petromyzon marinus*)
F. Psychrophilum distribution

- Found throughout North America, Europe, Korea, and Japan
- Identified in Atlantic salmon in Chile and Australia
Clinical Signs of CWD

- Fry and fingerlings
  - Lesions of the caudal peduncle
  - Erosion and fraying of fins
  - Dark coloration
  - Loss of appetite
Significance

CWD considered number one bacterial disease in the Pacific NW

– Commercial aquaculture losses in Idaho alone are estimated at $9.6 million dollars per year
  • Does not include treatment, egg replacement, and market devaluation for deformities of survivors

– Public aquaculture (WA state facilities) losses estimated at $4 million dollars per year
  • Does not include treatment, fish and egg replacement
Control options for CWD?

1. Good management/culture practices!
2. Antibiotic treatments
   – Aquaflor approved under VFD
3. Egg disinfection (vertical transmission)
   – Reduces surface bacteria but can’t eliminate intra-ovum bacteria
4. Culling program? (ongoing research)
5. Probiotics? (promising)
6. Vaccination
   – There are no commercially available vaccines for CWD
   – A standard bacterin does not work well – not a “silver bullet” as in the case of ERM
1. **Immune response** - Antibody development important for disease protection

2. **Tested vaccine formulations** - Whole-cell “killed” bacteria (standard bacterin) not effective

3. **Identified many bacterial genes/proteins associated with immunity** – Developed recombinant and DNA vaccines and immunized fish: Limited protection

![Image of gel electrophoresis with bands at 18-28 kDa, 41-49 kDa, and 70-100 kDa]

![Graph showing cumulative percent mortality over days post challenge with different protein bands at 18-28 kDa, 41-49 kDa, and 70-100 kDa]
Other options??

Alternative: Can we develop an efficacious live attenuated vaccine?

– Numerous studies on different fish pathogens
– Stimulate both innate and specific immune responses

Three live fish vaccines approved by USDA-APHIS-CVB

– RENIGEN (*Arthrobacter* live culture) – *Renibacterium salmoninarum*: Bacterial Kidney Disease
– *AQUAVAC-ESC™* - *Edwardsiella ictaluri*: Enteric Septicemia of Catfish
– *AQUAVAC-COL™* – *Flavobacterium columnare*: Columnaris
Attenuated Vaccine Development

AQUAVAC-ESC™ and AQUAVAC-COL™ were developed using a rifampicin-resistant strategy

– Originally used for the development of a live attenuated *Brucella abortus* vaccine for cattle in the US

• Bacteria are passed in presence of increasing concentrations of the antibiotic rifampicin
  • Results in attenuation of the pathogenic bacteria

Isolation of rifampicin resistant *Flavobacterium psychrophilum* strains and their potential as live attenuated vaccine candidates

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Attenuation of *F. psychrophilum*

• Generation of rifampicin-resistant strains
  – FP 259-93 (virulent) used as parent isolate
  – A single colony was passed to TYES agar containing 5 µg ml\(^{-1}\) rifampicin
  – Two colonies were selected and independently passed on increasing concentrations of rifampicin
    • 259-93A.16: passed 16 times to 200 µg ml\(^{-1}\) RIF
    • 259-93B.17: passed 17 times to 250 µg ml\(^{-1}\) RIF
Methods

- Assessment of attenuation
  - Rainbow trout (mean weights of 5.0 and 15.0 g)
  - Subcutaneously injected 2 doses of FP 259-93, 259-93A.16, and 259-93B.17
  - Mortality monitored for 28 d and CPM determined
Results

- Experimental CWD challenges demonstrated attenuation of both resistant strains
  - Complete attenuation of the 259-93B.17 strain

** Indicates a significant difference in CPM compared to 259-93 control (P < 0.05)
Methods

• Immunization study
  — Injection: Two groups of 350 rainbow trout (mean weight, 2.4 g) were injected intraperitoneally with:
    • PBS and 259-93B.17 (8.3 x 10^6 cfu fish^-1)
    • Boosted at 5 weeks
    • Challenged at 8 weeks
  — “Pilot” immersion: Three groups of 100 rainbow trout (mean weight, 3.4 g) were vaccinated by immersion (1 h):
    • 259-93B.17 diluted into water
      — With adipose fin removal
      — Without adipose fin removal
      — Booster at 4 weeks
      — Challenged at 10 weeks
Vaccination Results

** Indicates a significant difference in CPM compared to controls (P < 0.05)

Injection: RPS = 45%

Immersion: RPS = 28 and 45%
# Coho salmon vaccine trial results

<table>
<thead>
<tr>
<th>Delivery method</th>
<th>Treatment</th>
<th>Ab Titer 4 weeks</th>
<th>Ab Titer 6 weeks</th>
<th>Ab Titer 12 weeks</th>
<th>CPM</th>
<th>RPS</th>
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<tbody>
<tr>
<td><strong>Injection</strong></td>
<td>PBS</td>
<td>40 ± 7</td>
<td>40 ± 7</td>
<td>200 ± 55</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>259-93 B.17</td>
<td>800 ± 278*</td>
<td>2720 ± 697*</td>
<td>8960 ± 1568*</td>
<td>7^</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>259-93 B.17 w/ DPD</td>
<td>490 ± 90*</td>
<td>1640 ± 374*</td>
<td>14720 ± 4703*</td>
<td>1^</td>
<td>98</td>
</tr>
<tr>
<td><strong>Immersion</strong></td>
<td>TYES</td>
<td>&lt; 50</td>
<td>&lt; 50</td>
<td>140 ± 24</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>259-93 B.17</td>
<td>1480 ± 315#</td>
<td>1760 ± 261#</td>
<td>4480 ± 784*</td>
<td>29</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>259-93 B.17 w/ DPD</td>
<td>1680 ± 278#</td>
<td>880 ± 80#</td>
<td>5440 ± 1998*</td>
<td>15§</td>
<td>73</td>
</tr>
</tbody>
</table>
Summary

• Rifampicin-resistant strategy resulted in complete attenuation of the 259-93B.17 strain – potential vaccine candidate

• Immunization with the live attenuated 259-93B.17 strain resulted in protective immunity (RBT and Coho)
  – Injection delivery
  – Immersion delivery

• Alternative growth conditions for B.17 may enhance efficacy

• **Speculation** – Protection should be enhanced during a natural outbreak compared to laboratory injection challenge
Field trials
(Glenwood State Fish Hatchery)

Methods:
• 140,000 rainbow trout (0.93 g initial wt) designated as test fish
• Group split and vaccinated 30 days post feeding
• Primary and booster vaccination (at 14 days) – 1.5 min dip
• Vaccinates = 69,984; Controls = 69,984
• Two additional unhandled groups from same lot were also monitored.
Results

![Graph showing daily mortality with Aquaflor treatment]

- **Vaccinates**
- **Controls**

Date:
- 16-May
- 18-May
- 20-May
- 22-May
- 24-May
- 26-May
- 28-May
- 30-May
- 01-Jun
- 03-Jun

Aquafior treatment
### Mortality from 5/16 – 6/13

<table>
<thead>
<tr>
<th></th>
<th>Vaccinates</th>
<th>Controls</th>
<th>Others (2 ponds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>284</td>
<td>1316</td>
<td>3727</td>
</tr>
<tr>
<td>Percent</td>
<td>0.41%</td>
<td>1.88%</td>
<td>1.86%</td>
</tr>
<tr>
<td>%per day</td>
<td>avg. 0.02%/day</td>
<td>0.08%/day</td>
<td>.08%/day</td>
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<tr>
<td>RPS</td>
<td>0.784194529</td>
<td>-</td>
<td>-1.832066869</td>
</tr>
</tbody>
</table>
Summary/Observations

- CWD outbreak confirmed
  - Colony growth for controls = very concentrated with enlarged spleens
  - Colony growth for vaccinates = limited
- RPS in vaccinated fish = 78%
  - Mortality never exceeded expected natural mortality rates (0.02%/d)
- Two additional ponds (110,000 fish) broke and mortality mirrored controls
- Note: medicated feed administered due to stocking needs
- Trial demonstrates both safety and efficacy of the vaccine
- Replicate trials at other locations are necessary
Current status

• Established partnership with private company (Aquatic Life Sciences, Inc.) for field evaluation at hatcheries in Utah, ID, Co, etc.
• ALS will have first option to license patent rights from UI
• Initial field trials appear promising
• Alternative growth conditions for B.17 may enhance efficacy
Acknowledgements

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Questions?