Probiotics as disease control in marine larviculture

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Outline

• Introduction
  – Aquaculture, marine fish larvae
  – Fish probiotic bacteria
• Results
  – Isolation of probiotic bacteria
  – *Phaeobacter* strains as probiotic bacteria
  – Biofilters and biofilms
• Conclusions and Perspectives
The rise of aquaculture
Larval mortalities

Mortalities in cod larvae cultures

Reid et al. (2009) Aquaculture
Prevention of fish (larval) diseases

- Management of water quality / environment
- Vaccination
- Antibiotics
- Probiotics
  - Live microorganisms that exert a beneficial effect on host health
  - Inhibitory to pathogens
Isolation of antibacterial bacteria

1. culturing

2. Copy colonies to filter paper

3. Copy from paper to *Vibrio*-agar

4. Incubate

5. Read: inhibitory clearing zones
Searching for antibacterial bacteria in aquaculture
Aquaculture antibacterial culturable bacteria

- Stolt, Spanish turbot farm (Hjelm et al. 2004)
  - *Roseobacter* clade bacteria + *Vibrio* spp.

- Maximus, Danish turbot farm (Porsby et al. 2008)
  - *Roseobacter* clade bacteria + *Vibrio* spp.

- Venøsund, Danish flounder/halibut farm (Grotkjær, unpubl)
  - *Roseobacter* clade bacteria + *Vibrio* spp. + other

- Selonda, Greek seabass/seabream farm (Grotkjær, unpubl)
  - *Roseobacter* clade bacteria + others
Isolation of inhibitory roseobacters

Plating / replica

Enrichment
The *Roseobacter* clade

- Major part of the marine microbiota
- *Phaeobacter* / *Ruegeria* spp.
- Ecological generalists
- Algae-associated

Newton et al. 2010 ISMEJ
DNA from a *Roseobacter* relative (SAR11)
Roseobacters can be cultured from everywhere

Gram et al. 2010
Phaeobacter gallaeciensis inhibit Vibrio

Bruhn et al. 2005 AEM
How does *Phaeobacter* inhibit *Vibrio* spp.?

Mechanism of action?

Resistance?

Chemical analyses by Kristian Fog Nielsen
Probiotic bacteria – Mechanism of action?

In the animal
- Improved digestion
- Enhancement of immune response
- Antagonism against pathogens

most studies: Lactic acid bacteria

In the environment
- Nutrient uptake and remineralization
- Disruption of bacterial quorum sensing
- Antagonism against pathogens

Bacillus spp.

Roseobacter clade species
The larviculture food web

Microalgae → Rotifers → Artemia → Copepods → Picture: J. Skadal
**Phaeobacter** in algae cultures

*P. gallaeciensis* (wt and ΔTDA mutant)

*Tetraselmis suecica*

D’Alvise *et al.* 2012 PLoSOne
Pathogen reduction in algae cultures

Counts of *Vibrio anguillarum* without *Phaeobacter* (▼), with wt (■) and with TDA- mutant (▲)

D’Alvise *et al.* 2012 PLoSOne
Pathogen reduction in *Artemia* sp

*Artemia* with *V. anguillarum* (gfp)

*Artemia* with *V. anguillarum* (gfp) and *P. gallaeciensis*
## Survival of infected *Artemia* sp

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% <em>Artemia</em> survival</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>V. anguillarum</em></td>
</tr>
<tr>
<td>Axenic control</td>
<td>54 ± 0</td>
</tr>
<tr>
<td>Pathogen only</td>
<td>8 ± 3.2</td>
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<tr>
<td>Pathogen + <em>P. inhibens</em> DM17395 alone</td>
<td>89 ± 1.01</td>
</tr>
<tr>
<td>Pathogen + <em>P. inhibens</em> MJGG6</td>
<td>45 ± 12.2</td>
</tr>
<tr>
<td>Pathogen + <em>Phaeobacter</em> S26</td>
<td>77 ± 11.4</td>
</tr>
<tr>
<td>Pathogen + <em>Phaeobacter</em> S60</td>
<td>55 ± 3.8</td>
</tr>
</tbody>
</table>

Grotkjær et al. submitted
Cod larvae challenge trials

Mortality of cod larvae infected with *V. anguillarum* +/− *Phaeobacter* wt +/− *Phaeobacter* TDA- mutant.

D’Alvise et al. 2012 PLoSOne
TDA production as mechanism of action

• Replace antibiotics by a antibiotic-producing bacterium?
  – Based on the system ecology
  – resistance to TDA not seen (Porsby et al. 2011, AAC)

• Which factors influence TDA production?
  – Culture conditions (biofilm); c-di-GMP
  – Media components (iron, amino acids)
Probiotics on/in biofilters

Bio Max ceramics

Prol-Garcia et al. 2012
Probiotics on filters

Growth of *V. anguillarum* (upper) and *V. splendidus* (lower) in the presence of ceramics with probiont (●) or without probiont (○).

Levels of probiont (square)
Conclusions

• *Roseobacter (Phaeobacter)* strains could be used as prophylactic treatment against bacteria-induced larvae mortalities in cultures of marine larvae and their feed organisms

• TDA is likely the *in vivo* mechanism of action

• TDA production is facilitated by attachment and may be enhanced in biofilms
ProAqua 2013 - 2017

Objective:
Reducing the need for antibiotic treatment in the production of marine fish larvae by developing and combing two probiotic strategies (*Phaeobacter* and *Vibrio* bacteriophages)

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