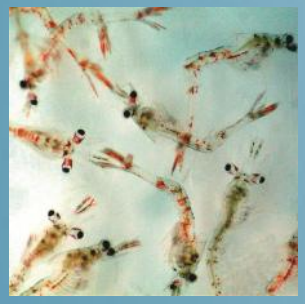
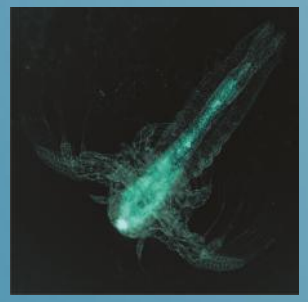
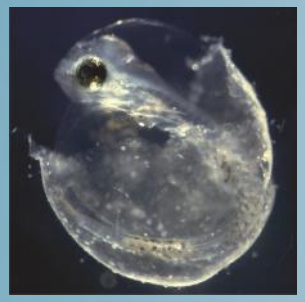
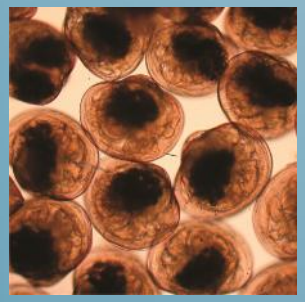




larvi 2013

6th fish & shellfish larviculture symposium

Control of bacterial disease in cultures of marine larvae and live feed by a probiotic bacterium

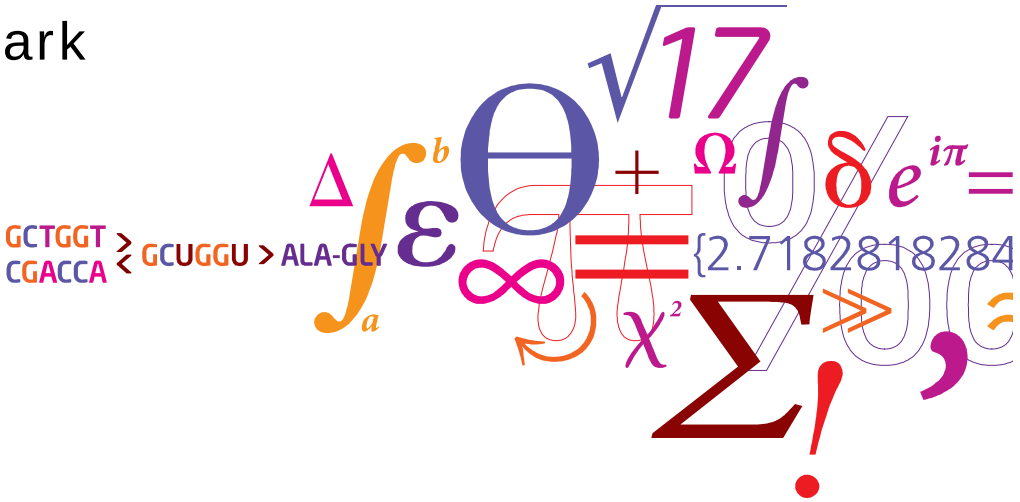
Paul D'Alvise

ghent university, belgium, 2-5 september 2013

Control of bacterial disease in cultures of marine larvae and live feed by a probiotic bacterium

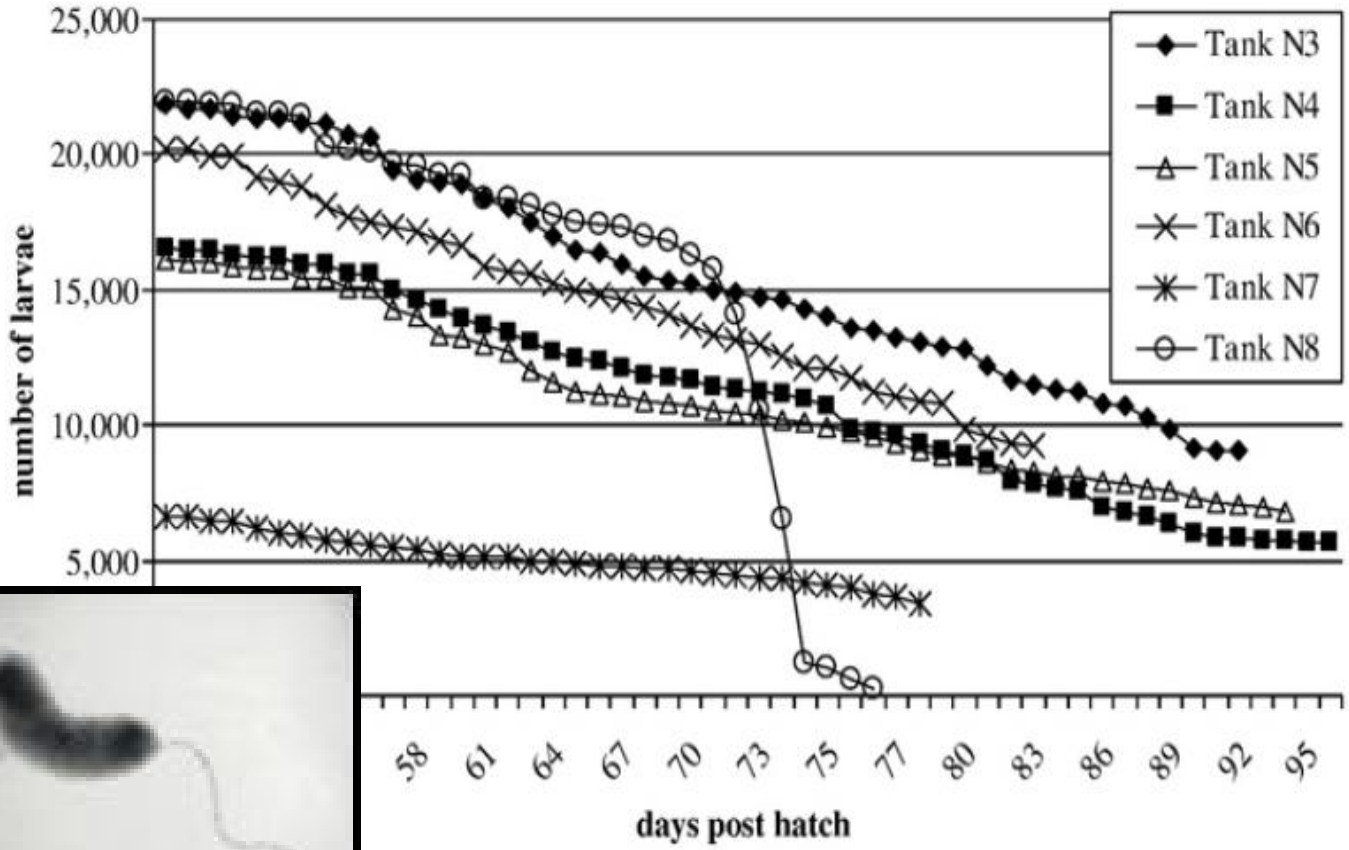
Paul D'Alvise

Technical University of Denmark



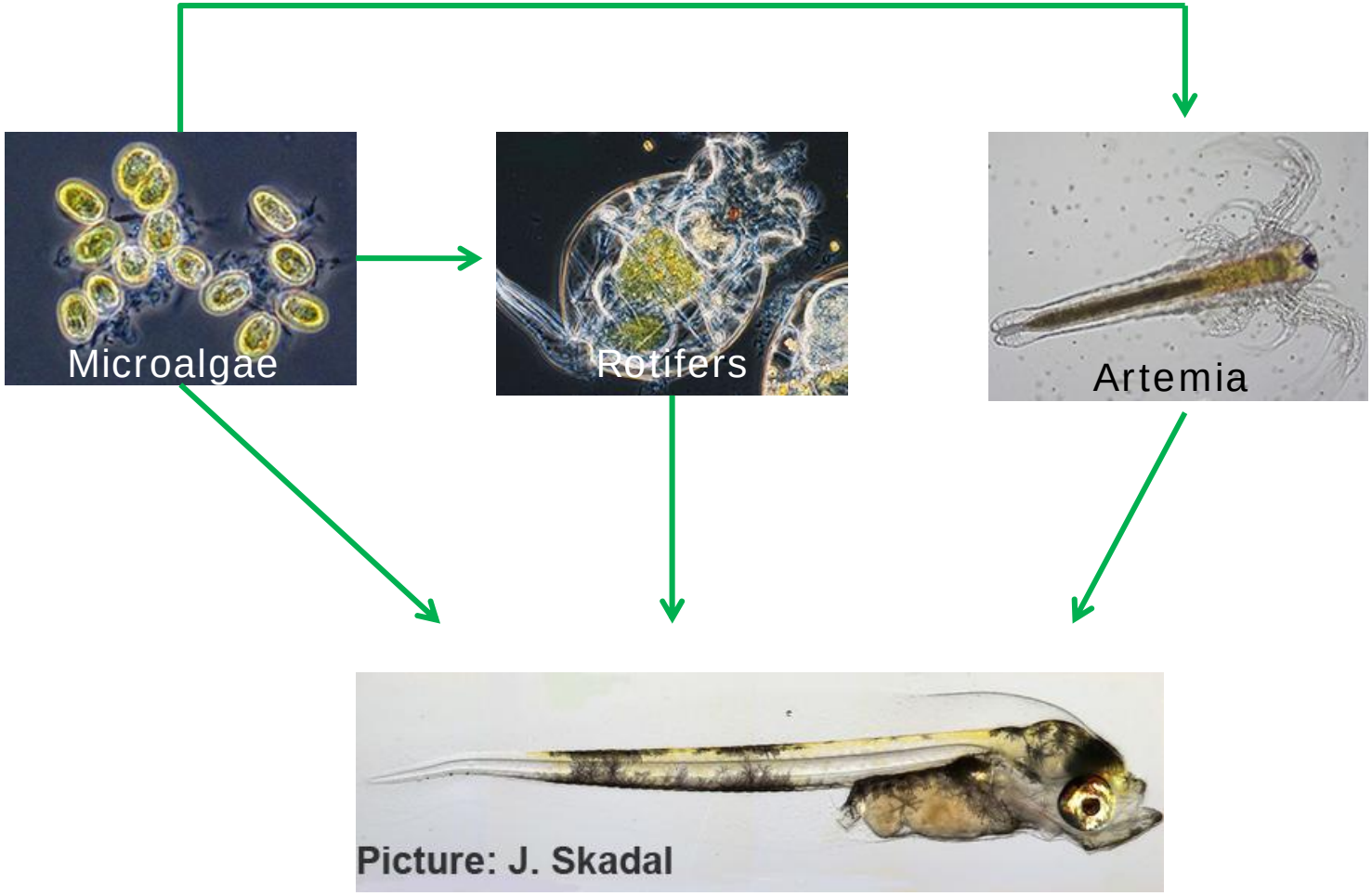
DTU Systems Biology
Department of Systems Biology

Mortalities in fish larvae cultures

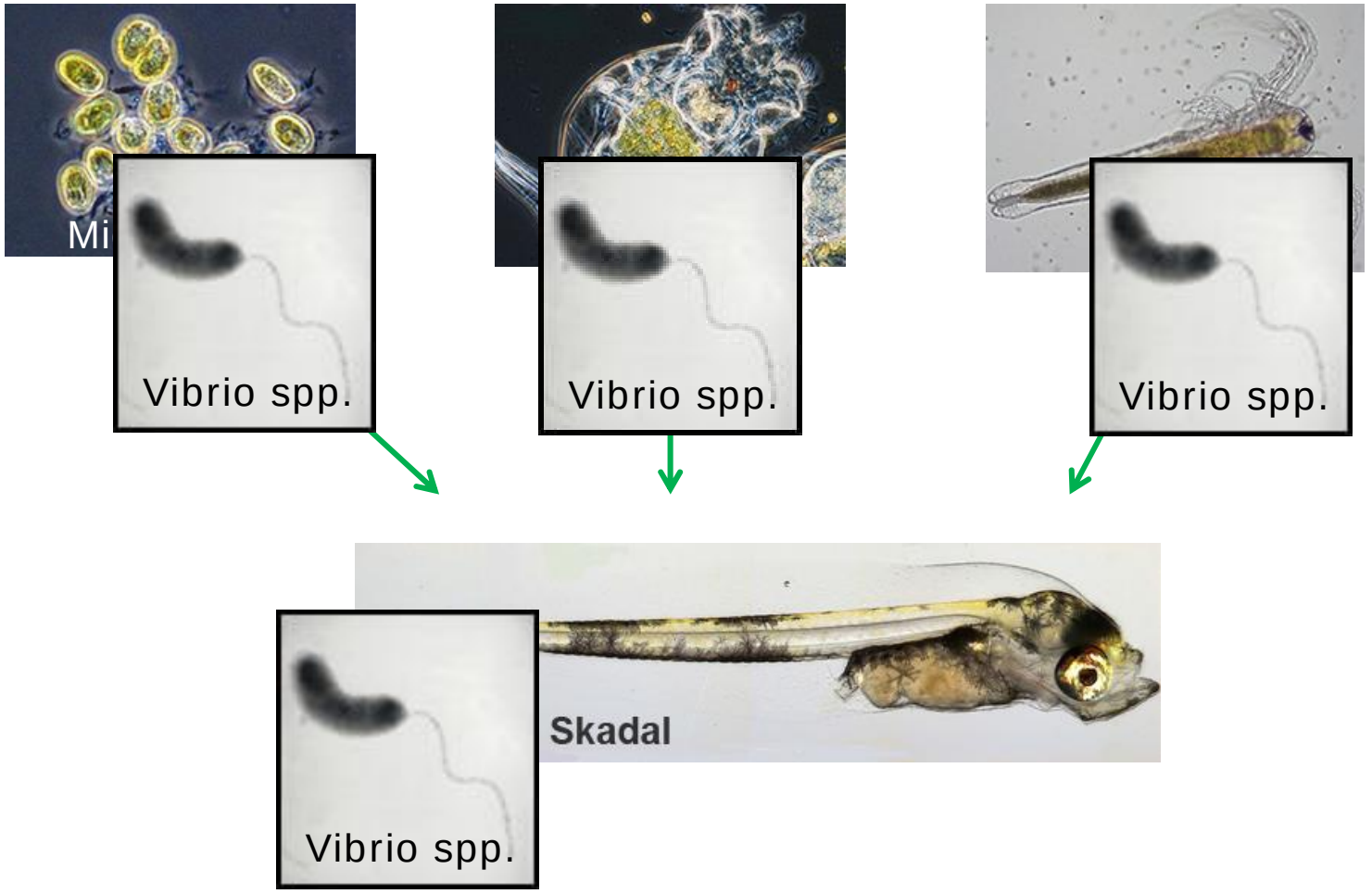


Reid et al. (2009) Aquaculture

Green water larviculture



Green water larviculture



Probiotic bacteria – Mechanism of action?

In the animal

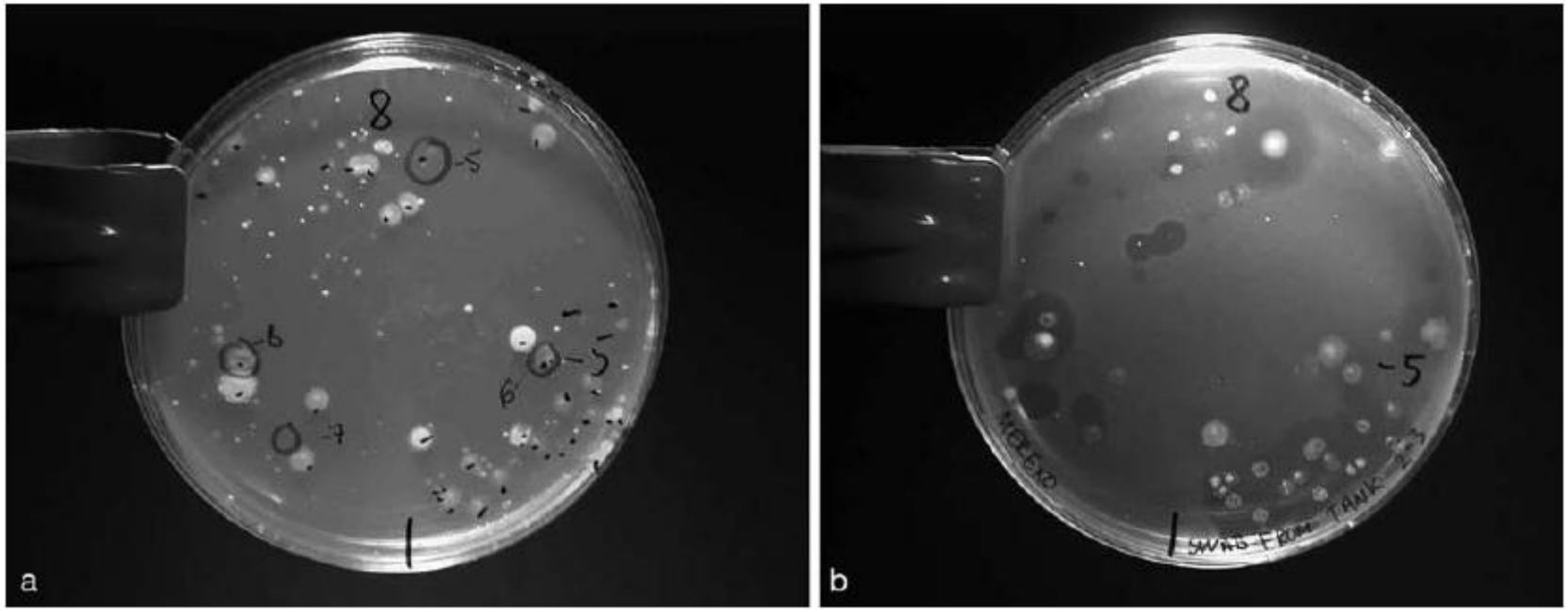
- Improved digestion
- Enhancement of immune response
- Antagonism against pathogens

most studies: Lactic acid bacteria

In the environment

- Nutrient uptake and remineralization
- Disruption of bacterial quorum sensing → Bacillus spp.
- Antagonism against pathogens

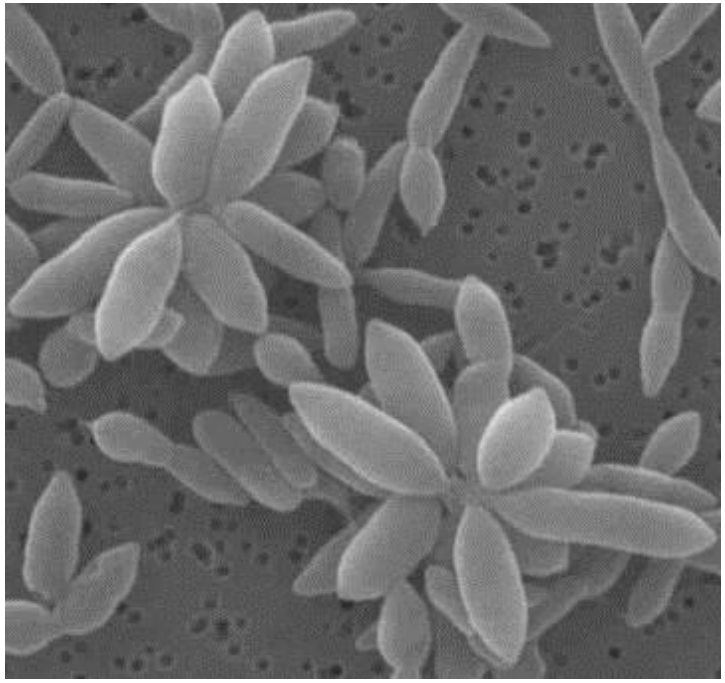
Indigenous probiotics from larviculture

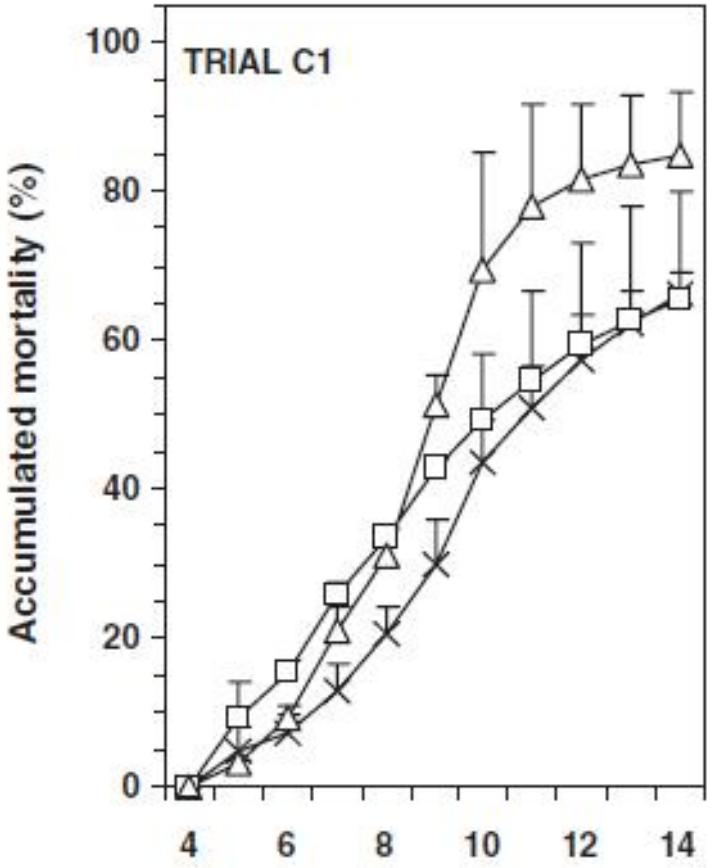


Phaeobacter sp. isolates from biofilms in a turbot hatchery inhibit the fish-pathogen Vibrio anguillarum

Hjelm et al. (2004) Syst. Appl. Microbiol.

Phaeobacter gallaeciensis / P. inhibens

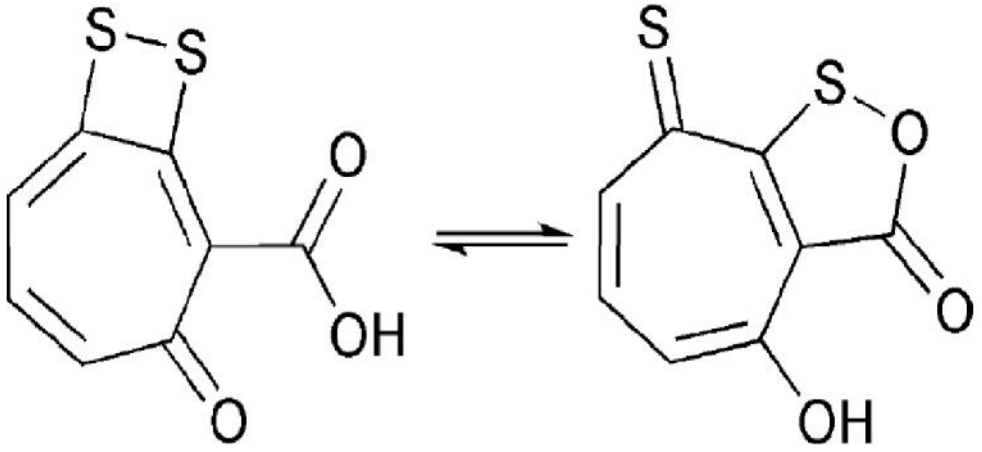




Bruhn et al. 2005 AEM

Planas et al. 2006 Aquaculture

How does Phaeobacter inhibit Vibrio spp.?

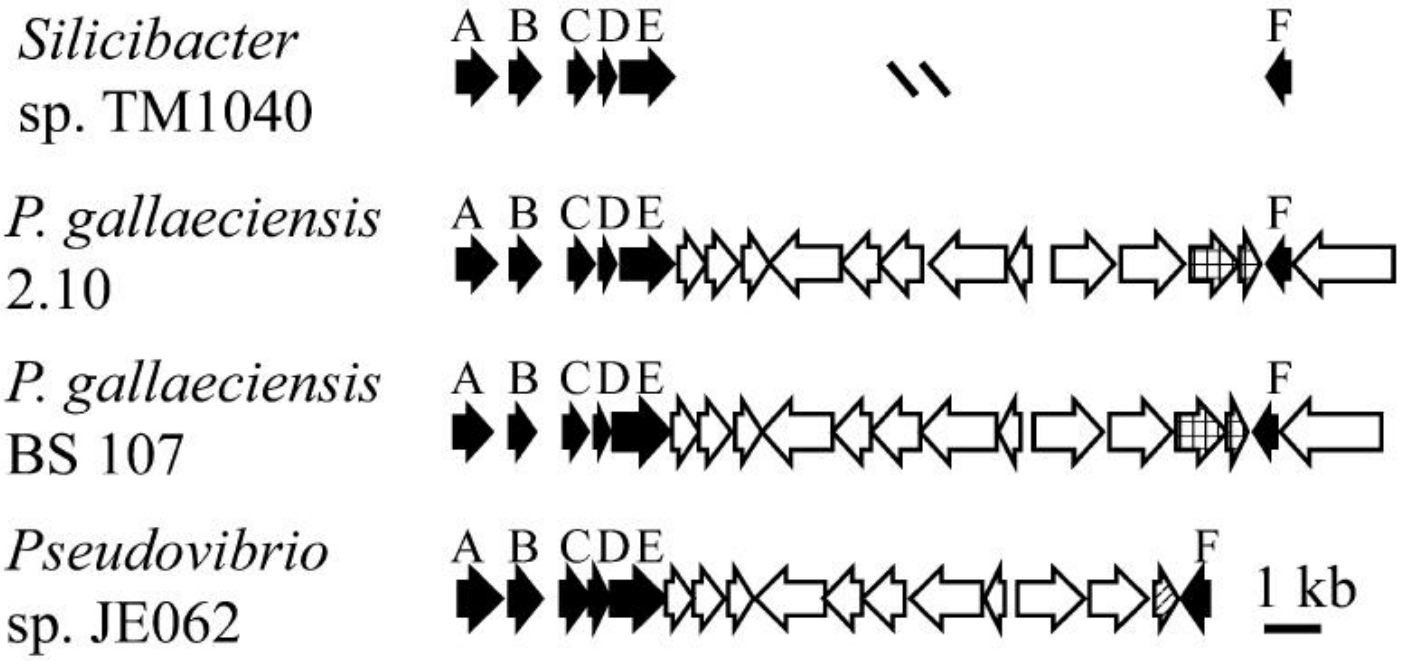


Tropodithietic acid (TDA) <-> Thiotropocin

Mechanism of action?

Chemical analyses by
Kristian Fog Nielsen,
DTU BioSys

Biosynthesis of TDA?



All TDA-producing bacteria share tdaA,B,C,D,E,F

Geng et al. 2010 J.Bact.

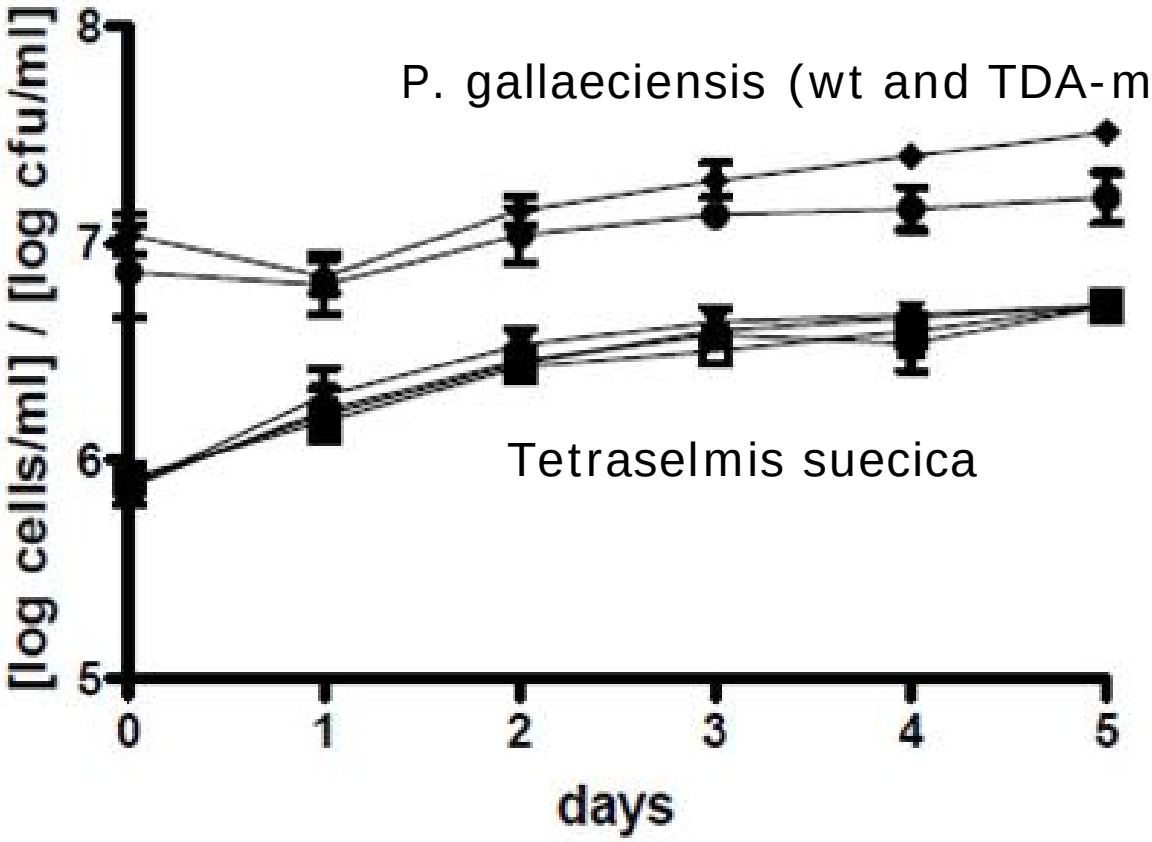
Hypotheses

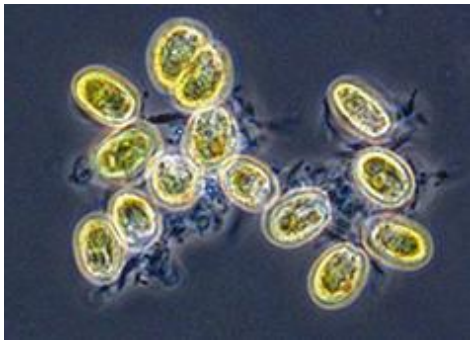
1. Since Phaeobacter thrives in cultures of fish larvae and their feed organisms, it could be used to reduce pathogen concentrations in the environment of the larvae

2. Since Phaeobacter strains produce the antibacterial compound tropodithietic acid (TDA), this could be the in vivo mechanism of action

   → TDA-negative mutant

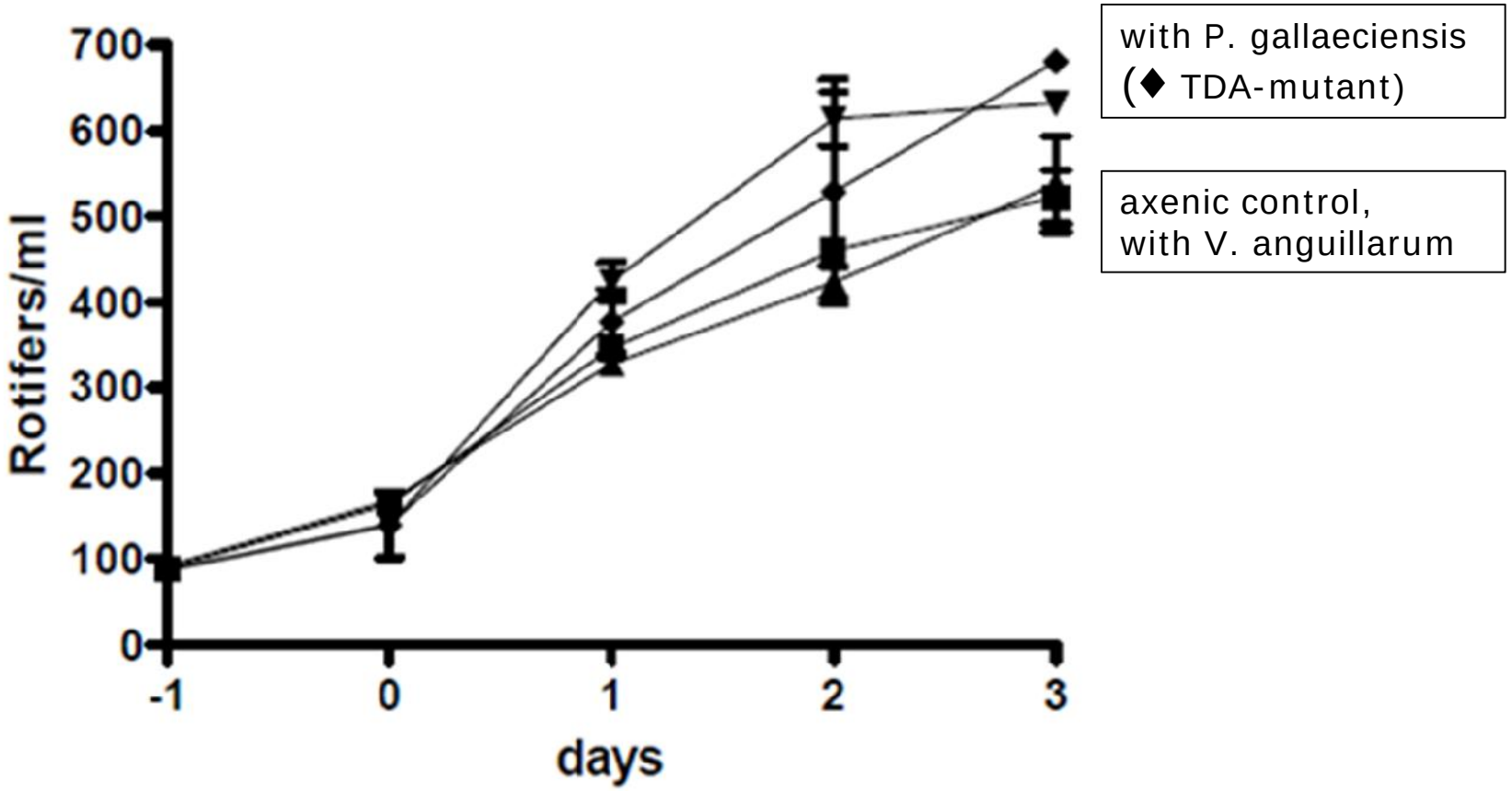
Phaeobacter in algae cultures





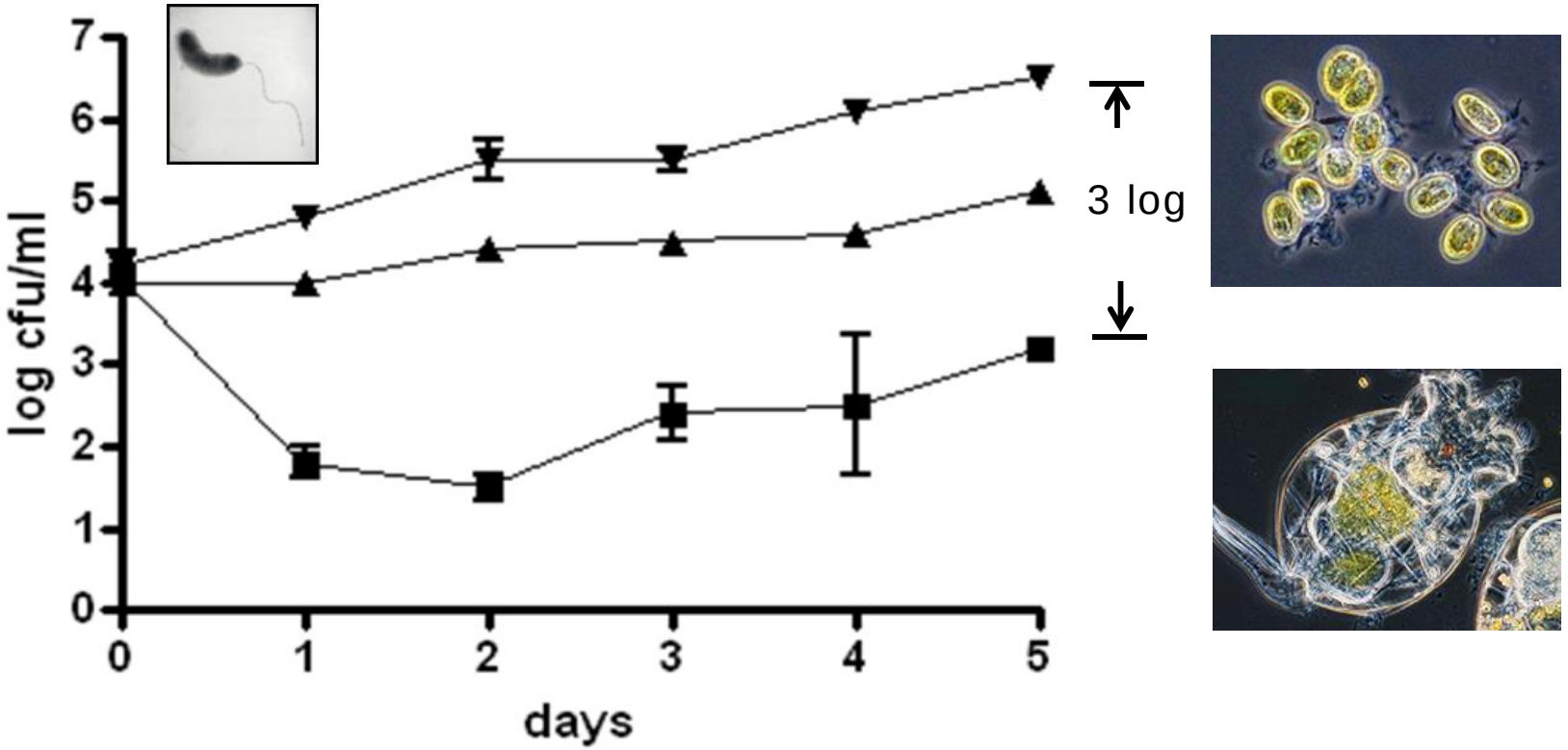
D'Alvise et al. 2012 PLoS One

Rotifer growth in presence of Phaeobacter



D'Alvise et al. 2012 PLoS One

Pathogen reduction in algae and rotifer cultures



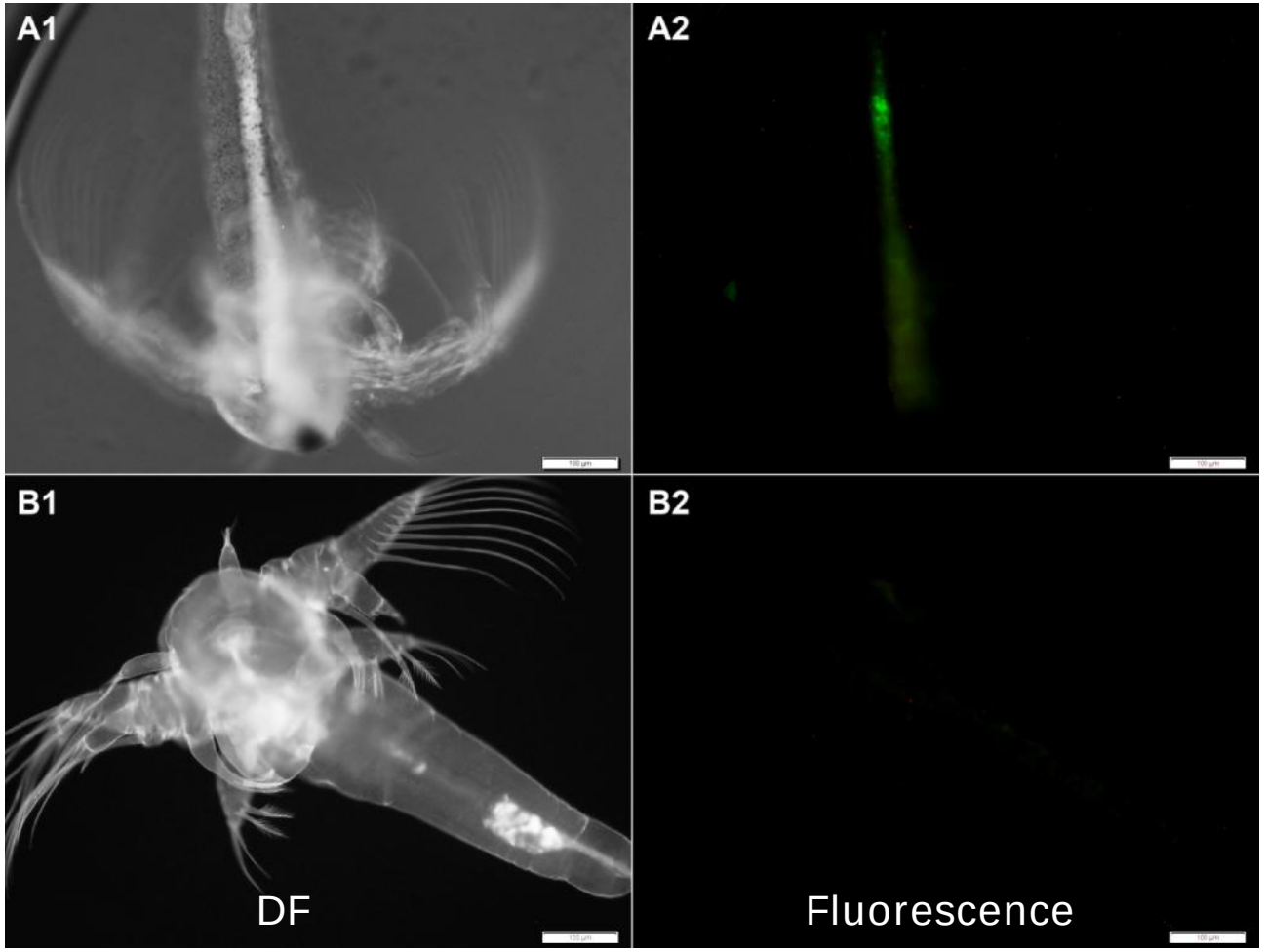
Counts of Vibrio anguillarum without Phaeobacter (▼), with wt (■) and with TDA- mutant (▲)

D'Alvise et al. 2012 PLoS One

Pathogen reduction in Artemia cultures

Artemia with V. anguillarum (gfp)

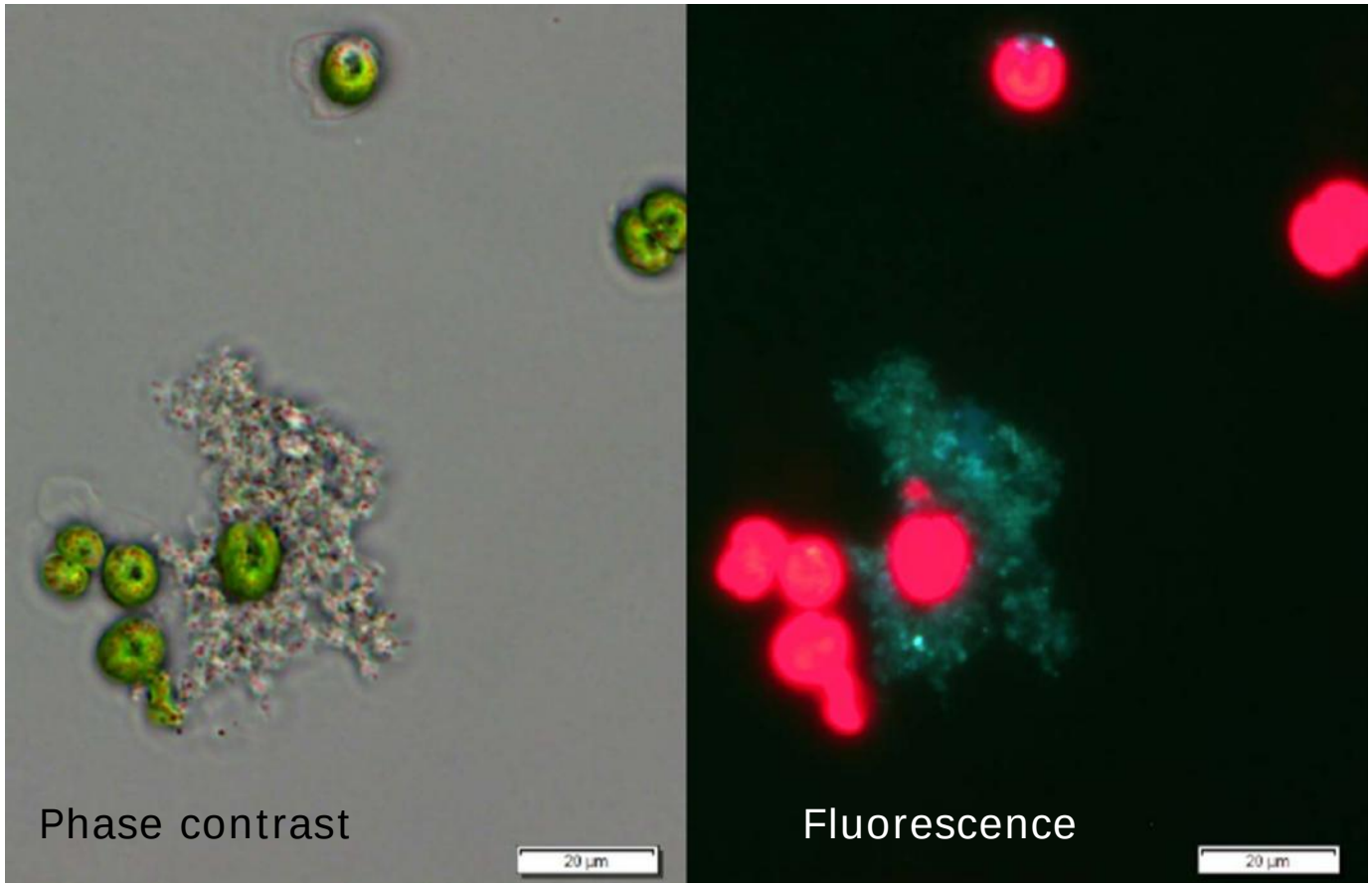
Artemia with V. anguillarum (gfp) and P. gallaeciensis



Praest, Lauridsen, D'Alvise; unpublished

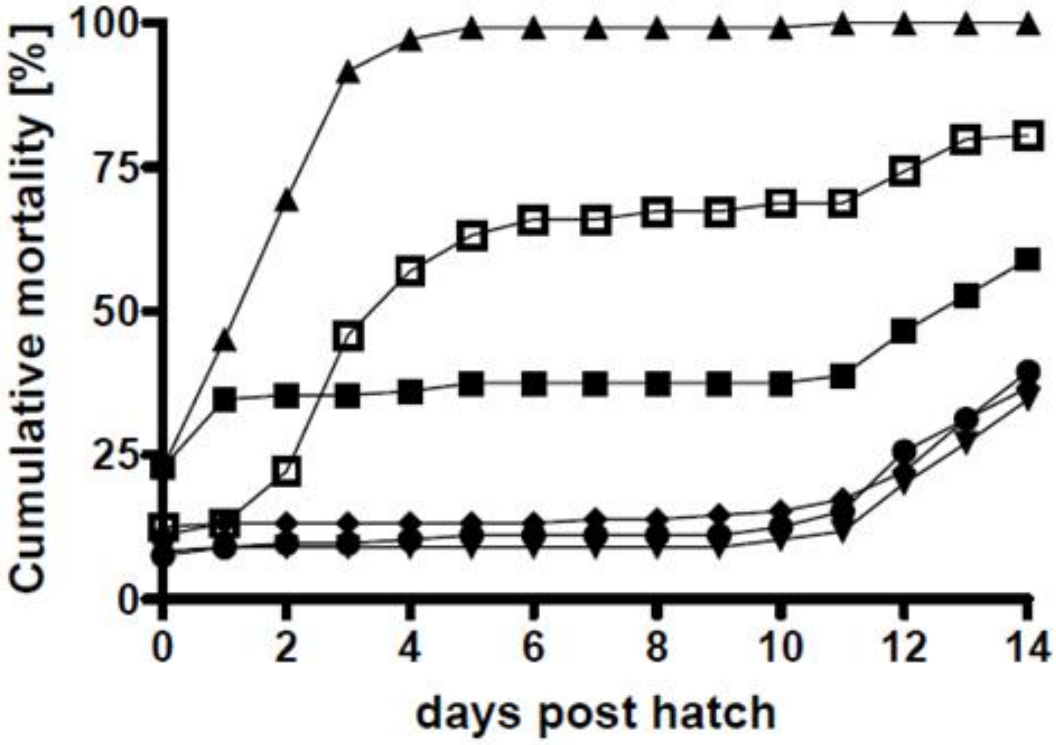
Localization and in situ TDA-production

pPDA11: pRK415 (PtdaC::gfp)



D'Alvise et al. 2012 PLoS One

Cod larvae challenge trials



No Phaeobacter

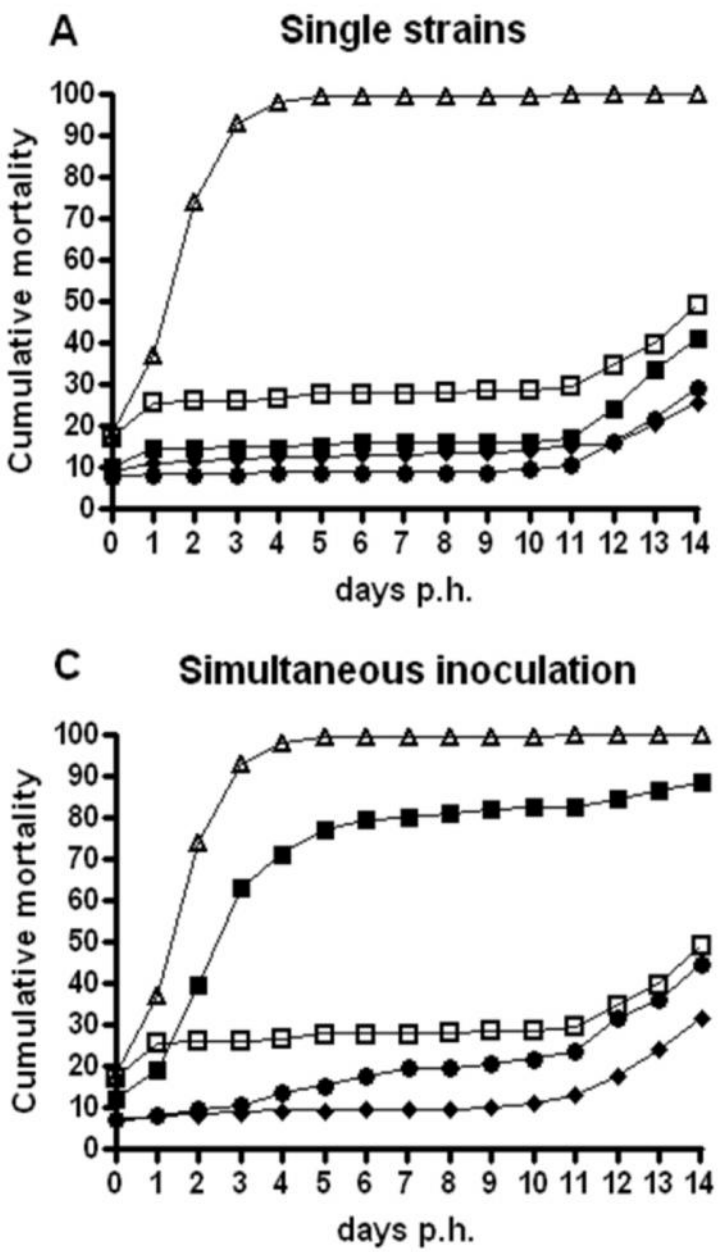
TDA-mutant

No pathogen/no probiont

Phaeobacter wildtype

Mortality of cod larvae infected with V. anguillarum + / - Phaeobacter

D'Alvise et al. 2012 PLoS One



Δ only
V. anguillarum

□ no added bacteria

■ *Phaeobacter* sp. 27-4

●*P. gallaeciensis* DSM17395

◆*Phaeobacter* sp. M23-3.1

D'Alvise et al. 2013 Aquaculture

TDA and resistance?

- Does it make sense to replace antibiotics by an antibiotic-producing bacterium?
  - Based on the system ecology…

Porsby et al. 2011 Antimicrob. Agents Chemother.

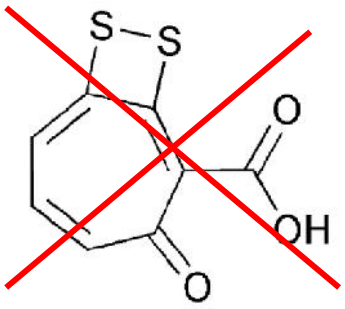
Attempt to select TDA-resistant mutants

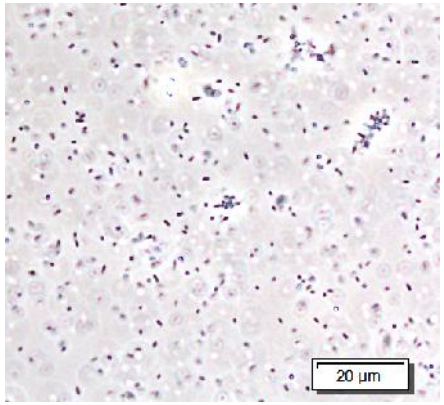
- single TDA exposure (different methods)
- screening a Tn-mutant library
- prolonged exposure to sub-inhibitory TDA concentrations

→ No resistant mutants were found

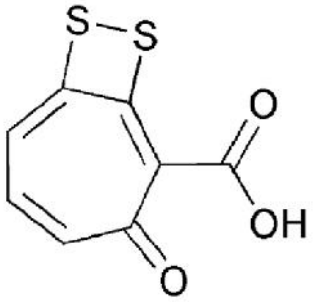
TDA production and biofilm formation

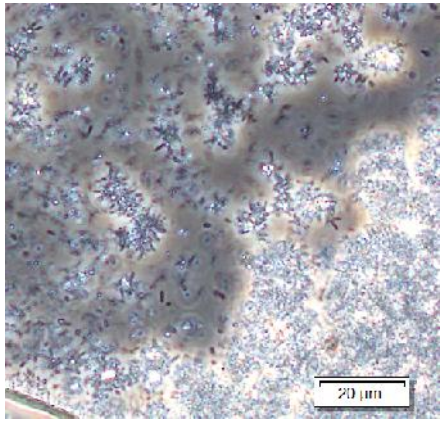
Shaken culture



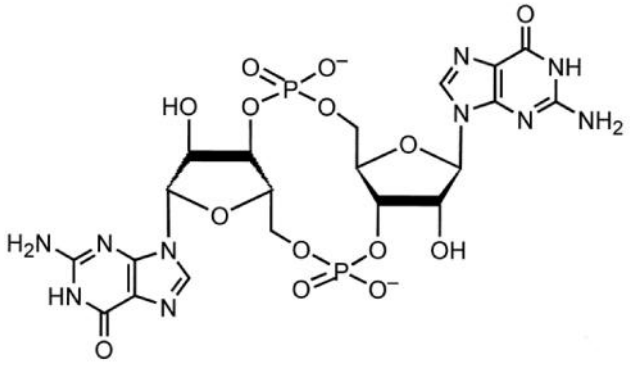


Static culture

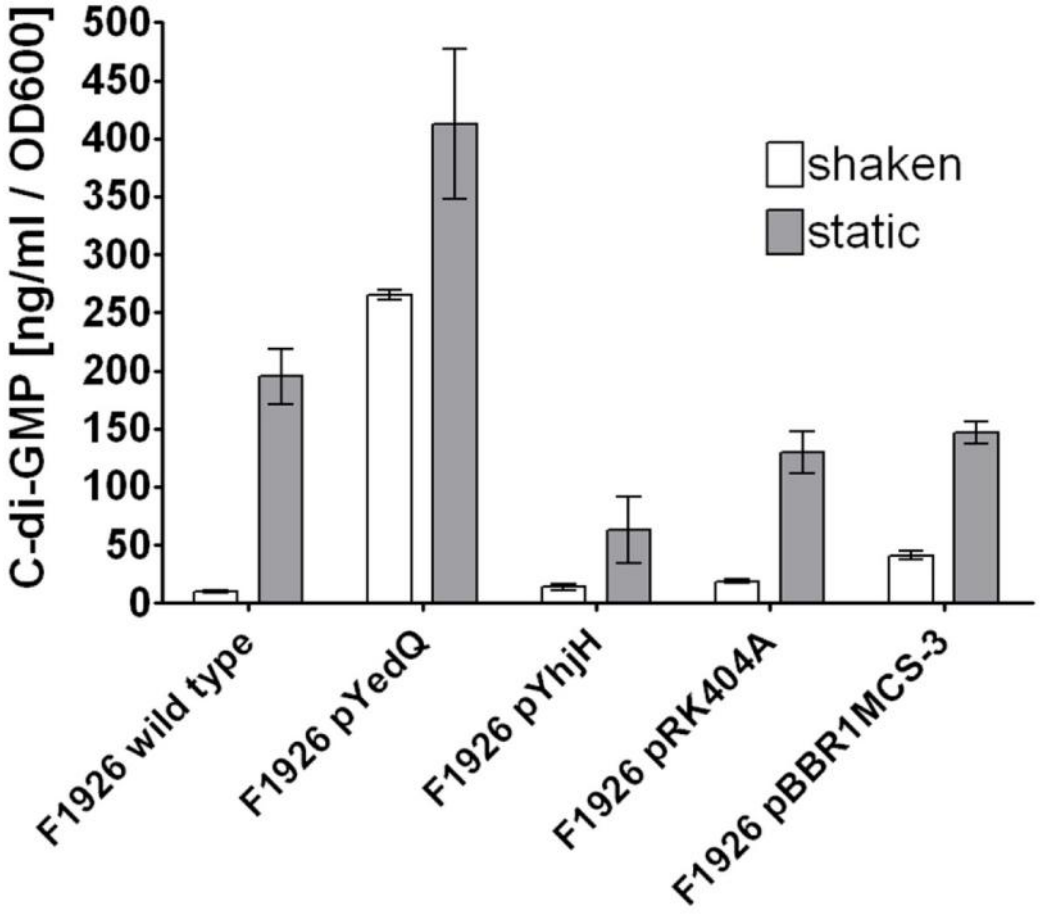




Transition from motile to sessile life is mediated by intracellular c-di-GMP levels

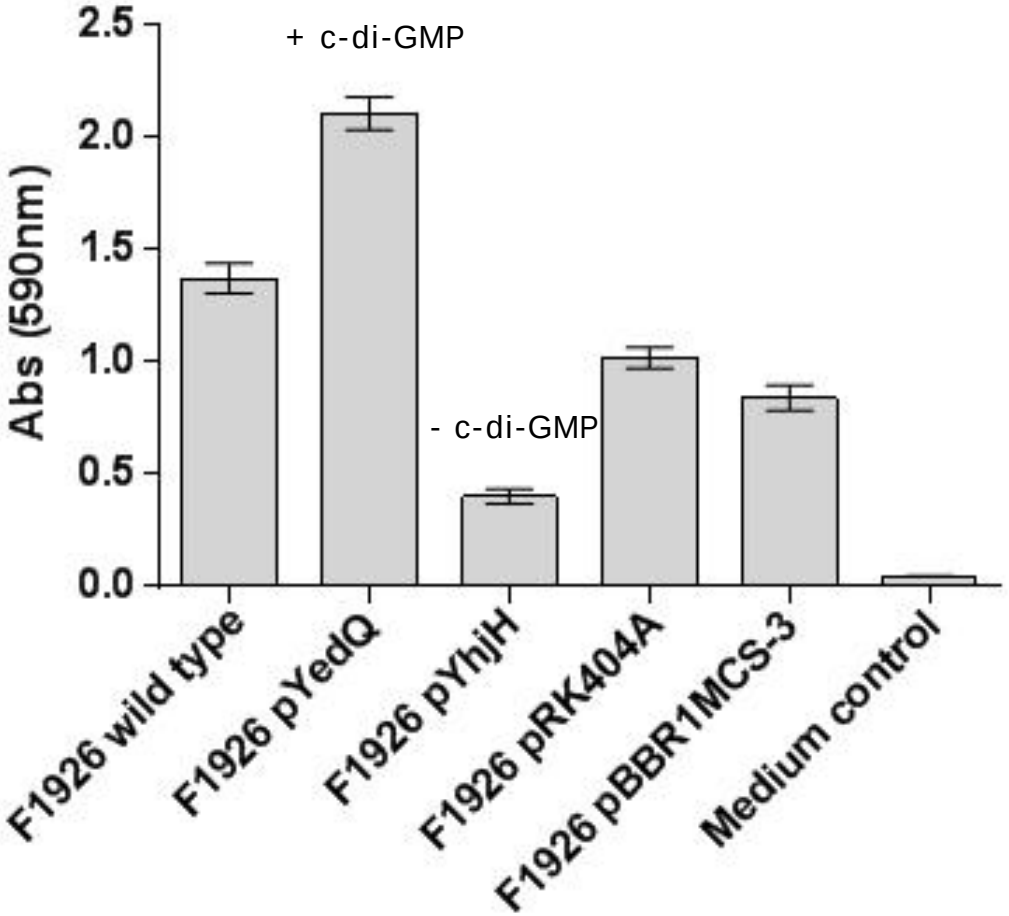


Cyclic dimeric guanosinmonophosphate



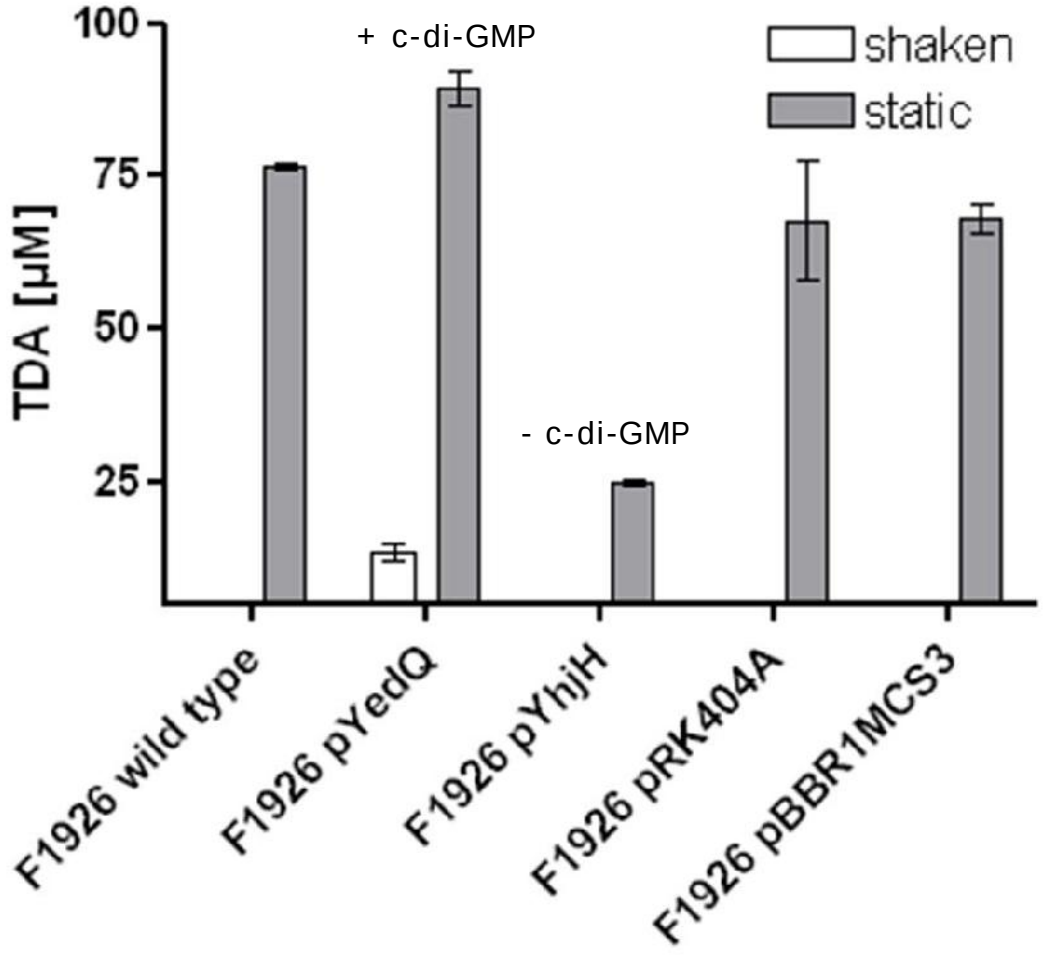
D'Alvise et al. 2013 Env. Microbiol.

C-di-GMP influences biofilm formation



D'Alvise et al. 2013 Env. Microbiol.

C-di-GMP influences TDA production



D'Alvise et al. 2013 Env. Microbiol.

Conclusions

- Phaeobacter strains could be used as probiotic bacteria to reduce pathogenic Vibrio spp. in live feed cultures
- Phaeobacter can prevent vibriosis in fish larvae
- Prophylactic application is most effective
- Efficacy of strains depends on TDA production
- TDA production requires attachment (biofilms, aggregates)

Acknowledgements

- Det Frie Forskningsråd - Teknologi og Produktion (FTP)

- Lone Gram - PhD supervisor
- Øivind Bergh, Siril Lillebø, Heidrun Wergeland - Cod larvae trials
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- Debra Milton
- Tim Tolker Nielsen

Thank you for listening!