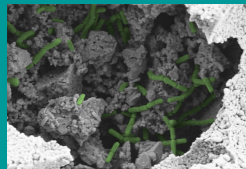


Improving larvae rearing of the marine fish *Amphiprion ocellaris* by beneficial biofilms

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Background

Marine fish farming in aquaculture becomes more and more important, triggered by exploitation of fish stocks all over the world (Bostock *et al.*, 2010). One of the major problems in aquaculture is the infection of fish egg clutches by pathogenic microorganisms, subsequently interfering with the development of larvae, which also are highly sensitive to water and food quality, as well as environmental parameters (MOORHEAD & ZENG, 2010). This cooperation project, including enterprises, research

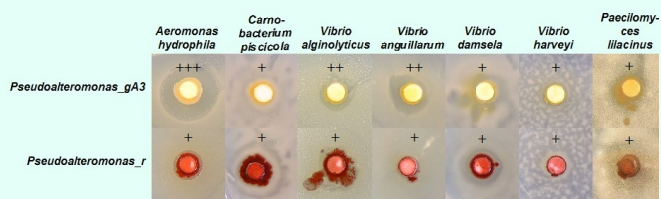
and development partners, aims to address this bottleneck. As a model *A. ocellaris*, the false clown anemonefish was selected, a substrate spawner and one of the most traded marine ornamental fish. Thereby, innovative biofilm formation techniques with beneficial bacteria are investigated to prevent fish egg clutches from infestation by fish-pathogenic microorganisms and reach qualitatively and quantitatively high-value rearing of marine fish larvae.

Goals of MiBiLab Dr. M. Lohmeyer

Investigate and (im)prove the **development of biofilms by beneficial bacteria** on special spawning tiles provided by *Advanced Ceramics* to protect fish egg clutches of *A. ocellaris* from infestation by fish-pathogenic microorganisms.

Results

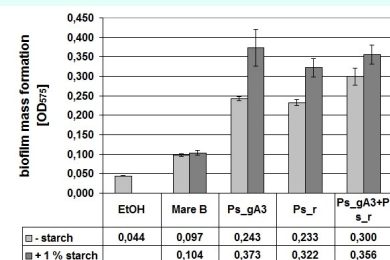
1. Isolation and selection of beneficial bacteria



- Isolation of **2 *Pseudoalteromonas* strains** from ZMT sea water
- **4 selection criteria** for potential beneficial bacteria: inhibition of fish-pathogens + *Paecilomyces lilacinus* (fungus isolated from infested egg clutch), growth rate, biofilm mass formation, and no mutual inhibition

Clear zone formation of *Pseudoalteromonas_gA3* and *Pseudoalteromonas_r* towards fish-pathogens and *P. lilacinus* indicated a beneficial feature.

2. Optimize biofilm mass formation with starch



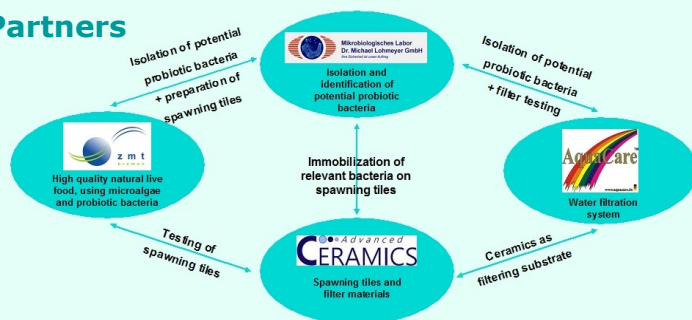
- biofilm quantification modified according to O'Toole (2011): the higher the optical density, the more biofilm mass is formed (ethanol and Marine bouillon = negative control)
- different media and starch concentrations were tested (0.5 %, 1 %, 2 %, 5 %) after starch hydrolysis by beneficial strains was observed
- > **biofilm formation was performed with Marine broth and 1 % starch**

Addition of 1 % starch to the inoculated marine broth enhanced biofilm mass formation of beneficial bacteria.

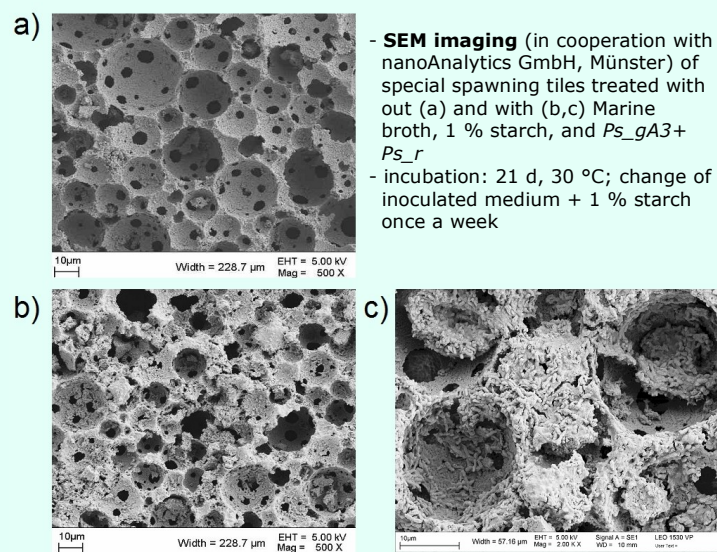
Conclusion

Two strains of *Pseudoalteromonas* (*Pseudoalteromonas_gA3* and *Pseudoalteromonas_r*) were suitable for biofilm formation investigations according to their inhibitory ability towards selected fish-pathogens, growth rate and biofilm mass formation. As *Ps_gA3* and *Ps_r* did not inhibit mutually (data not shown), these strains were selected for multicultural biofilm formation on the special spawning tiles. Furthermore, SEM images of the treated spawning tiles demonstrated a biofilm formation only

Partners



3. Multicultural biofilm formation on special spawning tiles



A multicultural biofilm consisting of *Pseudoalteromonas_gA3* + *Pseudoalteromonas_r* covered the special spawning tiles.

with co-cultivated *Ps_gA3* and *Ps_r*. Neither *Ps_gA3*, nor *Ps_r* cultivated individually with spawning tiles formed such masses of biofilm. Biofilm mass formation was enhanced by the addition of 1 % starch to the inoculated marine broth. *In vivo* tests of these beneficial biofunctionalised spawning tiles are in preparation. The developed biofilms are suggested to protect fish egg clutches in a probiotic manner from infestation by fish-pathogenic microorganisms, which interfere with larvae rearing.

References: Bostock *et al.*, Philos. Trans. R. Soc. Lond. B. Biol. Sci., 2010, 365(1554): 2897-2912
 Moorhead & Zeng, Rev. Fish. Sci., 2010, 18(4): 315-343
 O'Toole G.A. (2011). Microtiter Dish Biofilm Formation Assay. JoVE. 47. <http://www.jove.com/details.php?id=2437>, doi: 10.3791/2437