AQUAEXCEL

Aquaculture Infrastructures for Excellence in European Fish Research

Project number: 262336

Combination of CP & CSA
Seventh Framework Programme
Capacities

Deliverable D9.3

Phenotypic analysis of G1 offspring in salmon and A1 offspring in seabass and carp

Addendum D9.3A: Disease resistance in different seabass families

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Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
Dissemination Level	
PU Public	**X**
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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Glossary

AQUAEXCEL:	Aquaculture Infrastructures for Excellence in European Fish Research
Gnotobiotic:	An environment in which all organisms are known

Summary

Objectives of this deliverable
To determine which sea bass family has a higher resistance to *Vibrio anguillarum* during the early larval stage.

Rationale:
Through the use of a gnotobiotic (an environment in which all organisms are known) set-up, one can avoid uncontrolled microbial communities which can include pathogens. Through egg disinfection, one can get bacteria-free sea bass larvae that are maintained in sterile conditions up to 15 days after hatching. A pure strain of V. anguillarum is added to the culture water and the survival of the sea bass larvae is monitored daily. Bacteria-free non-challenged sea bass larvae serve as a control.

Teams involved: INRA has transported sea bass eggs that were produced by Ifremer and originated from one female and that were separately fertilized with sperm from five different males.
UGent has disinfected the eggs, monitored the effect of disinfection on the hatching percentage and egg development rate and check the efficiency of the disinfection.

Geographical areas covered: All Europe

Phenotypic analysis of A1 offspring in seabass

Resistance to *Vibrio anguillarum* in seabass larvae with different genetic back ground.

Introduction:

The establishment of isogenic seabass lines could not be obtained during the course of the Aquaexcel project. Two experiments were set up in order to search for genetic information that is linked to a better resistance to pathogens in sea bass larvae at the facilities of Ghent University.

Material and methods:

In preparation of the each experiment, 40 0.5L-bottles were autoclaved and filled with 300 mL of filtered (0.2 µm) autoclaved natural sea water. The same amount of caps with one glass in- and one outlet, 80 0.2µm air-filters, 25 1L glass beakers, 25L filtered sea water, erlenmeyers and micropipette tips were autoclaved. Marine agar was prepared and poured into petridishes. Marine broth was prepared to culture the pathogen. Small plastic vials to stock the sea bass larvae were UV sterilized overnight.

One clutch of eggs originating from one female was divided into five groups and fertilized by sperm of five different males at Ifremer. The fertilized eggs were transported by train to Ghent by INRA, where they were acclimatized and rinsed for 6 hours in a temperature (16 ± 1°C) and light controlled (10 candela steradian m^{-2}) room. Subsequently, the each group of eggs was disinfected according to the protocol described in Dierckens et al. (2009). In short, the eggs were treated with glutaraldehyde, washed and incubated in pre-aerated autoclaved sea water in sterile 0.5L bottles. Each bottle was equipped with a filter-sterilized (0.2 µm) air in- and outlet (Fig. 1). To avoid the risk of having a bacterial contamination during the incubation, ampicillin and rifampicin, both at 10 mg/L were added to the incubation medium.

Fig. 1: Incubation bottles equipped with filtered air in- and outlet. The bottles are filled with 400 mL autoclaved, filtered sea water with 10 mg/L rifampicin and 10 mg/L ampicillin added. Each bottle contains 600 eggs. The eggs from each family were spread over 8 bottles.

For each bottle, 30 eggs were collected 24 hours after disinfection to check for axenity. The eggs were homogenized under sterile conditions and 50 µL was spread plated (Spiral plater TM, Spiral systems USA) on marine agar (marine broth; Difco Laboratories, Detroit, USA + 15% agar; Bacteriological Grade, MP Biomedicals). The rest of the sample was used to perform a PCR with specially designed universal primers detecting bacteria without interference of eukaryotic DNA (Bakke et al., 2011) to check for non-culturable bacteria.

To test the effect of the disinfection procedure on the hatching, two groups of 96 eggs from each family were put individually into sterile 96-well plates. Half of one group of eggs was disinfected while the second half underwent the same procedure, but without the chemicals. The multiwell plates were put in the same temperature and light controlled room.

For the second experiment trial, Ifremer provided again 5 batches of sea bass eggs originating from 1 female and separately fertilized by sperm of 5 different males. Ugent made all preparations. Half of each batch of eggs was disinfected using the standard procedure (Dierckens et al., 2009).

Results:

In both experiment, there was no detection of bacteria on any of the eggs of the different families.

Due to undefined reasons, the eggs from 4 out of 5 families did not hatch, either in the incubation bottles or in the multi-well plates. This was also the case for the non-disinfected eggs. As only 1 family remained, comparison for better disease resistance between families was not possible.

However in the second experiment as well, the hatching of the eggs was too low in 4 out of 5 families.

References:
Bakke, I., De Schryver, P., Boon, N., Vadstein, O., (2011). PCR-based community structure studies of Bacteria associated with eukaryotic organisms: A simple PCR strategy to avoid co-amplification of eukaryotic DNA. JOURNAL OF MICROBIOLOGICAL METHODS 84 (2): 349-351. DOI: 10.1016/j.mimet.2010.12.015
Dierckens, K., Rekecki, A., Laureau, S., Sorgeloos, P., Boon, N,. Van den Broeck, W., Bossier, P. (2009). Development of a bacterial challenge test for gnotobiotic sea bass (Dicentrarchus labrax) larvae. ENVIRONMENTAL MICROBIOLOGY 11 (2):526-533. DOI 10.1111/j.1462-2920.2008.01794.x

Conclusion

Unfortunately, due to unexpected problems, the comparison for a difference in V. anguillarum resistance could not be made.