Evelien De Swaef

UNIVERSITEIT GENT

FACULTY OF VETERINARY MEDICINE
approved by EAEVE

Marlien Schaeck

Gnotobiotic models for seabass (*Dicentrarchus labrax* L.) and Dover sole (*Solea solea* L.): the chain is only as strong as its weakest link…

De Swaef Evelien[1], Schaeck Marlien[1], Van Den Broeck Wim[1], Bossier Peter[2], Dierckens Kristof[2], Decostere Annemie[1]

[1]Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

[2]Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Ghent University, Rozier 44, 9000 Ghent, Belgium

Despite the fast expansion of aquaculture in the last decennia, major losses during larval production still torment the industry. These losses are mainly caused by bacterial diseases, and combatting these diseases by means of antimicrobial agents causes an increase in acquired antimicrobial resistance. The use of probiotics is a promising alternative treatment technique, although their working mechanism is still poorly understood. To unravel their mode of action, there is a great need for a **gnotobiotic model**.

The advantage of working with a **gnotobiotic model** is that the microbial community is known, eliminating the interference by unknown microbiota.

Dover sole larva

Seabass larva

Seabass (*Dicentrarchus labrax*) and **Dover sole** (*Solea solea*) are both important species for the European aquaculture industry, of which the larviculture poses a major challenge hence the justification for the creation of a gnotobiotic model. For seabass, a gnotobiotic model was developed by Dierckens *et al*. (2009). Regarding sole, a gnotobiotic model currently is non-existing. Pinpointing/developing a gnotobiotic model for both species, without having to house the larvae in antibiotics, is a major challenge. During the development of such a model, many different **pitfalls** can be encountered, as listed below.

1. All used material should be sterile and all manipulations should be carried out under strict sterile conditions

Use of sterile gloves, autoclaved material, autoclaved seawater

Sterilisation procedure and manipulations in a laminar flow

2. Finding the sterilisation protocol with a good balance between axenity and hatchability of the eggs

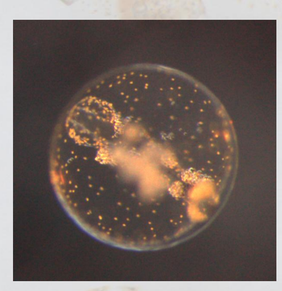
Seabass

Best working protocol:
1% H_2O_2 (5 min) + ozone (3 min)
Overall: good tolerance to different disinfectants, good hatchability

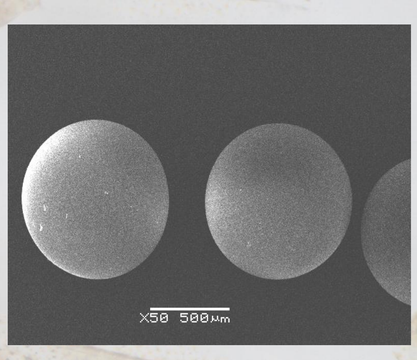
Many different protocols and products are tested:
H_2O_2, ozone, glutaraldehyde, antibiotic mixtures, plasma sterilisation, …
Combinations of these products

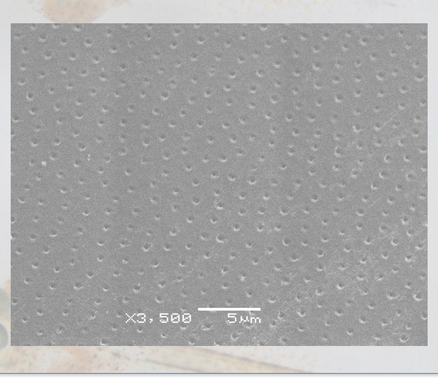
Sole

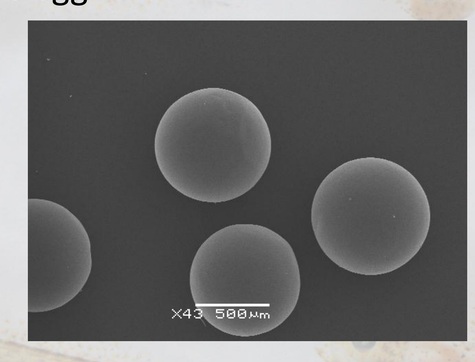
Most promising protocol:
1% H_2O_2 (3 min) + 400 ppm glutaraldehyde (2.5 min) + antibiotic mixture (rinsed before hatching)
Overall: low tolerance to disinfectants, lower hatchability

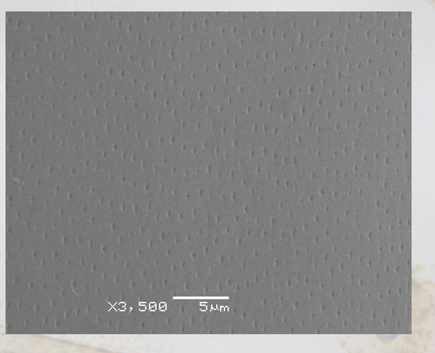


Why the difference in tolerance?
Ultrastructure of the egg



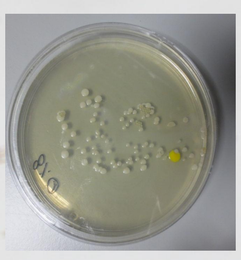


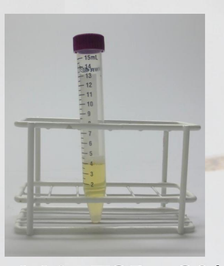




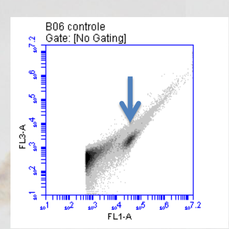
3. Evaluating the axenity of the retrieved egg/larva in a quick and reliable way

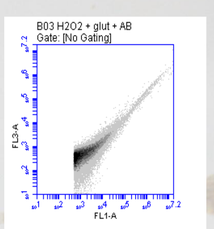
- culture dependent techniques:
  + widely used
  - selective media and need for a long incubation period
- culture independent techniques:
  + also non-culturable bacteria, quick method
  - difficult to interpret





Culture dependent techniques: TCBS, MA, TSB+ 2% NaCl





Control (bacteria: arrow) vs most promising treatment

Culture independent techniques: Flow cytometry

4. Maintaining axenity of the larvae during development

Larvae are housed in well plates

Wells are placed in a glove box

Food (*Artemia*) has to be axenic

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Reference: **Dierckens, K. *et al.*** (2009). Development of a bacterial challenge test for gnotobiotic sea bass (*Dicentrarchus labrax*) larvae. *Environ. Microbiol. 11(2)*: 526-533.

Evelien.DeSwaef@ugent.be