What method alternative to the antibiotic treatment for bivalve larval culture?

Philippe Miner,
J. L. Nicolas
(Ifremer, PFOM, UMR-PE2M Brest,)
Régis Lasbleiz, Benoît Sérive
Tinduff hatchery
Introduction

- Generally the culture of bivalve larvae does not require antibiotic treatment except Pectinidae (example *Pecten maximus*, *Argopecten purpuratus* (Chile). *Placopecten magellanicus*...). These species are very sensitive to bacterial infections (Vibriosis or undetermined bacteria).

- Antibiotic is preventively used in these cases. However they present adverse effects, mainly the increase of bacterial (plasmidic) resistant to antibiotics and residues in tissues which can induce different troubles at long term exposures (chloramphenicol aplastic anaemia in human). If chloramphenicol is banned since 1994 some antibiotics are still authorized for aquaculture purposes including flophenicol, flumequine. However the veterinary preparations contain excipient or diluent (50% or more) such as lactose.

- Is it possible to replace antibiotic by probiotic bacteria or by other methods?
What criterions to choose a probiotic?

- Efficient against several targets
- Not toxicity for larvae
- Reliable
- Easy to produce and to distribute

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Among 887 marine bacteria the most active ones (6%) belonged to *Roseobacter group* or *Pseudoalteromonas*.

Phylogenetic trees constructed from sequences of 16S rDNA.
Antagonism activity of these marine probiotics

Test on marine agar

Roseobacter galleaciensis (A020, BS1, X34, X129) and the Pseudoaltermonas X153 displayed a broad spectrum of activity against vibrio
What method and concentration to add probiotic into larval culture?

Live bacteria is necessary and at least $10^6$ cell ml$^{-1}$ to exert an antagonism in seawater (It is complex to keep alive marine bacteria)

Antagonism against *V. anguillarum*
Protection of scallop larvae by the probiotic X34

The probiotic X34 cells were broken by ultrasonic treatment before addition to larval culture.
Method of production

In axenic algae Association with the probiotic X34 lasted several months without contamination. X34 reached about the same concentration as T-iso cells ($10^7$ cell ml$^{-1}$).

In casamino-acid medium ($1g$ L$^{-1}$) to add directly to larval tank.
Protection and stimulation of growth

*Pecten maximus* larvae fed on a mixture of 3 algal species of which one (T-iso) was axenic and associated with X34. X34 was at 10^4 cell ml\(^{-1}\) and X129 at 10^6 cell ml\(^{-1}\).

Growth

Mortality at day 16

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Protection of larvae facing bad quality of sea water

- *C. gigas* larvae at day 8
- X34 associated with axenic T-iso

Growth

Mortality
Summary about marine probiotic

- Probiotic bacteria were selected from a large number of isolates.
- They belonged mainly to *Roseobacter* and *Pseudoalteromonas*
- *Pseudoalteromonas* (H6, X153) were more active but they were toxic to bivalve larvae.

- Probiotic cultured in Marine Broth, broken by ultra-sonic treatment protected efficiency scallop larvae (too expensive for hatchery)

- The probiotic X34 associated with axenic algae protected oyster larvae (*C. gigas*) and sometimes scallop (*P. maximus*) larvae. It increased larval growth of scallop larvae. (insufficient protection and reliability)

- The probiotic X129 cultured in Casamino-acid protected more efficiently scallop larvae than X34 (expensive)

- Combination of 2 probiotics appeared necessary to replace antibiotic in larval culture of scallop. They appear insufficiently reliable.
Other methods

- Probiotic preparations used for cattle, fishes, shrimps
- Clay particles to adsorb the organic matter and toxic compounds, Stimulation of filtration and digestion
- Improvement of growth by a better nutrition
- Decrease of organic matter by active charcoal
- Decrease the stress of rearing:
  - Change of rearing method: continuous culture
  - Change of tank design and aeration
Lactobacillus and marketed probiotic preparations

Mortality to Day 15

% mortality

Distribution of lactobacillus $10^4$ ml$^{-1}$ at every renewal of sea water

$Lactobacillus$ or probiotic preparations based on $Lactobacillus$ were not adapted to scallop larvae.
Clay (Montmorillonite) particles (mean size 8µm) slow down the mortalities and slightly the growth rate. However, larvae are not protected during metamorphosis which is a crucial phase.
Elimination of dissolved organic matter

DOM is at low level in sea water. Charcoal can retain it only when it exceeds 1.5 mg L⁻¹. It can be useful to eliminate some toxins from harmful algae.

No improvement neither adverse effect were observed.
Improvement of larval fitness

Scallop larvae fed with different mixture of microalgae: P: *Pavlova lutheri*, T: T-iso, C: *Chaetoceros gracilis*, R: *Rhodomonas salina*

A better adapted diet allows to improve the growth rate. However the scallop larvae receiving *Rhodomonas salina* did not survive without antibiotic treatment.
Aeration in large tank is necessary to avoid settlement of larvae but increase the stress.

In recent experiment without aeration and with another type of tank, larvae were able to metamorphose without antibiotic treatment in continuous culture system. However the output was not sufficient and the handling was important.
Conclusion

• The restrictive use of antibiotic as preventive method obliges to change the method of rearing, particularly in taking account the behavior of larvae.

• In combining probiotic associated with algae, continuous larval culture (towards a complete recycling seawater system), change of tank design, better nutrition (Rhodomonas), we hope to solve the problem of survival in Pectenidae without antibiotic and considerable additional cost.