Selection study of potential probiotic bacteria for shrimp hatcheries in New Caledonia

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Shrimp farming in New Caledonia

- Commercial production since 1983
- Semi-intensive culture from *Litopenaeus stylirostris* captive broodstock
- 2500 tons in 2005, less than 1200 tons in 2010
- 2 seasonal *Vibrio* pathogens in grow-out ponds (*V. penaeicidae* and *V. nigripulchritudo*)
Hatchery constraints in New Caledonia

• Most of postlarvae demand concentrated in warm season (October to January)

• Increasing use of antibiotics to stabilize production

• Research project for evaluating alternative prophylactic treatments in hatchery such as probiotics
Experimental approach

Potential probionts selection

- Strains characterization / identification
- *In vitro* growth-inhibition test
- Innocuousness test
- Effectiveness test in larval rearing
- Impact on animal status
Potential probionts selection

493 isolates sampled in extreme marine environments in New Caledonia

Screening for antagonistic activity against *V. nigripulchritudo*.

Selection of 7 isolates: NC72, NC197, NC201, NC203, NC204, NC257 and NC297
5 isolates: *Pseudoalteromonas* genus

- NC201 close to *P. maricaloris*
- NC257, NC297, NC203, NC204, NC197 close to *P. piscicida*

1 isolate: *Vibrio* genus

- NC72 belonging to *Harveyi* clade

Bacteria group including pathogens and probiotic strains for marine invertebrates

Several species with antibacterial, antifungal and antialgal properties
Inhibitory test on *V. harveyi* ORM4-GFP growth

Fluorescence reading after 48h

1

x 3

10^5 CFU.mL\(^{-1}\)

29°C

2

V. harveyi GFP

V. harveyi GFP + Isolate

Fluorescence (arbitrary unit)
Inhibitory test on *V. harveyi* ORM4-GFP growth

Fluorescence reading after 48h

- Weak inhibition activity from NC257
Inhibitory test on *V. harveyi* ORM4-GFP growth

Fluorescence reading after 48h

- Very strong inhibition activity from NC72
- Strong inhibition from NC197, NC201, NC203, NC204, NC297

Excellent antagonistic activity from 6 out of the 7 isolates towards *V. harveyi* ORM4-GFP

![Graph showing fluorescence readings for different isolates](image)

1. **V. harveyi** GFP  
2. **V. harveyi** GFP + Isolate

- x 3  
- 10^5 CFU.mL^{-1}  
- 29°C

- **ORM4**
- **GFP**

- **Fluorescence** reading after 48h

- **NC72**
- **NC197**
- **NC201**
- **NC203**
- **NC204**
- **NC297**

- **Excellent antagonistic activity from 6 out of the 7 isolates towards V. harveyi ORM4-GFP**
Harmfulness of probiotic candidates

Pathogenicity test towards Mysis 2 and PL9
- Mysis 2 in duplicates for 48 hours
- PL9 in triplicates for 72 hours
- Isolates final concentration of $10^5$ CFU/mL

- No survival alteration from any isolates in both stages
- No abnormality in larvae and postlarvae behaviour with all isolates

No pathogenicity detected for any isolates
Probiotic effectiveness in larval rearing: from nauplius to mysis

- No treatment (NT)
- Antibiotic treatment (AB) at D3, D5, D7 and D9 at 2.5g/m³
- 5 probiotic treatments: *Vibrio* NC72 and *Pseudoalteromonas* NC197, NC201, NC203, NC297 every day administration at $10^5$ CFU/ml

Survival and growth

![Image showing survival and growth graph]
Probiotic effectiveness in larval rearing: from *nauplius* to *mysis*

![Survival and growth](image)

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**Survival and growth**

![Graph showing survival rates](graph)

Similar or better survival rates with probiotic treatments
Probiotic effectiveness in larval rearing: from *nauplius* to *mysis*

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- 5 probiotic treatments: *Vibrio NC72* and *Pseudoalteromonas NC197, NC201, NC203, NC297* every day administration at $10^5$ UFC/ml

**Better growth with probiotics compared to antibiotic**
Probiotic effectiveness in larval rearing: from *nauplius* to *mysis*

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- Antibiotic treatment (AB) at D3, D5, D7 and D9 at 2.5g/m³
- 5 probiotic treatments: *Vibrio* NC72 and *Pseudoalteromonas* NC197, NC201, NC203, NC297 every day administration at $10^5$ UFC/ml

Survival and growth

Selection of NC201 for further trials
NC201 effectiveness in larval rearing: from *nauplius* to P9

Posology: every day (NC201) vs each other day (NC201^{1/2})

- Survival
- Growth
NC201 effectiveness in larval rearing: from *nauplius* to P9

Posology: every day (NC201) vs each other day (NC201\(^{1/2}\))

- Survival
- Growth

Same survival enhancement of NC201 each other day compared to every day supply
NC201 effectiveness in larval rearing: from nauplius to P9

Posology: every day (NC201) vs each other day (NC201<sup>1/2</sup>)

NC201 each other day as effective as NC201 every day
NC201 effectiveness in larval rearing: from *nauplius* to P9

Single administration vs combination of two probiotics

- Survival
- Growth
NC201 effectiveness in larval rearing: from nauplius to P9

Single administration vs combination of two probiotics

- Survival
- Growth

No survival improvement with probiotics combination
No improvement with probiotic combination compared to single administration

NC201 effectiveness in larval rearing: from *nauplius* to P9

Single administration vs combination of two probiotics
Probiotic impact on animal status

Three parameters were evaluated:
  Vibrio load in animal
  AMP gene expression
  Resistance to salinity stress test
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Estimation of *Vibrio* concentration at D9 and D19 in animals on TCBS media.
Probiotic impact on animal status

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- Vibrio load
- AMP gene expression
- Resistance to salinity stress test

Estimation of *Vibrio* concentration at D9 and D19 in animals on TCBS media.

Could this load drop be due to higher antimicrobial peptide action?

Lower *Vibrio* concentration in animal at D19 with probiotics
Probiotic impact on animal status

Three parameters were evaluated:
- Vibrio load in animal at D9 and D19
- Lysozyme gene expression at D9 and D19
- Resistance to salinity stress test

Sampling of pooled 20 animals at D9 and pooled 10 animals at D19 for each triplicate.
Gene expression profile analysed by quantitative RT-PCR, using EF1 as housekeeping gene.
Probiotic impact on animal status

Three parameters were evaluated:
1. Vibio load in animal at D9 and D19
2. Lysozyme gene expression at D9 and D19
3. Resistance to salinity stress test

Sampling of pooled 20 animals at D9 and pooled 10 animals at D19 for each triplicate
Gene expression profile analysed by quantitative RT-PCR, using EF1 as housekeeping gene.

No detection of significant modulation of Lysozyme expression with probiotics
Three parameters were evaluated:
- Vibrio load in animal at D9 and D19
- AMP gene expression at D9 and D19
- Resistance to salinity stress test

Direct transfer from 27 ppt to 17-25 ppt in 5 days-old post-larvae from three different batches reared with or without probiotics -> survival after 24h
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Resistance enhancement of postlarvae with probiotics
Conclusions

• Possible alternatives to antibiotic treatments in Caledonian hatcheries
• *Pseudoalteromonas* genus is a good probiont candidate
• NC 201 in single administration each other day improves zootechnical performances in larval rearing
• Probiotics can improve global health status of postlarvae
• More studies are necessary to optimize the use of probiotics in larval rearing:
  • imprinting time
  • administration way
  • mode of action
• Better knowledge by experimenting:
  • bacterial challenge in larval and postlarval phases
  • probiotic effect on juveniles and adults
Thank you for your attention

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