Shrimp pathogen detection in the farming environment: analysis of pond water and sediment samples using molecular tools

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INTRODUCTION

Recurrent disease outbreaks during the warm season have been described in New Caledonian *Litopenaeus sytlirostris* shrimp farms (Goarant et al., 2006a). The study of bacterial strains isolated from septicaemic moribund shrimp previously revealed the emergence a highly virulent genotype of *Vibrio nigripulchritudo* (Goarant et al.2006b). As of year 2007, this strain was diagnosed in 5/19 farms and remained localized in a specific area of New Caledonia. In order to evaluate the occurrence of this pathogen in the farm environment, molecular tools using PCR were developed to analyze pond water and sediment samples. These tools will allow to:

- Better understand the life cycle of pathogenic *Vibrio* strains during growout and dryout periods.
- Study how pond management can help reduce the risk of disease.

MATERIALS AND METHODS

Sampling

A 20 by 80 m experimental shrimp pond was studied. 42 sediment samples were collected at two dates of the 112-day-long growout (day 56 and 107). For each location, two 3-cm deep sediment cores were collected underwater using 50-mL syringes cut open and brought to the laboratory in sterile 150-ml plastic jars. Water samples were collected in sterile 500 mL bottles and analyzed immediately.

Sediment enrichment step

5 g of wet sediments were placed in a 50-mL centrifuge tube and enriched with 10 mL of enrichment solution (Marine broth half diluted with Lewis water). The tubes were incubated 24 h at 28°C with a 120 rpm agitation.

DNA extraction from water samples

50 ml of water were filtered on a sterile cellulose membrane (47 mm diameter, porosity 0.2 µm). Particles retained by the filter were washed with 2 ml TE buffer (**T**ris-Hcl 10 mM, **E**DTA 1 mM pH 8) in a 30-mLcentrifuge tube. Particles were finally collected by centrifugation in a 2-mL microtube and DNA was extracted using the QIAGEN QIAamp DNA Stool Mini Kit.

DNA extraction from fresh or enriched sediments

250 mg of wet sediments were extracted using the MO BIO Power Soil DNA Isolation kit according to the manufacturer's instruction.

PCR reactions

PCR primers and conditions were described previously (Goarant et al., 2007)

RESULTS

Optimal sample volume

DNA extraction from sea water was done on cells collected from 20 mL, 50 mL and 100 mL. The strongest PCR signal was obtained with 50 mL.

Detection Limits, see table I.

Pathogen mapping in sediments using PCR : see figure 1.

TABLE I. Estimated detection limits of different analytical tools using a decreasing concentration range of inoculated cells of *Vibrio nigripulchritudo*.

	Sea water	*Sediment (wet weight)*	*Enriched sediment (wet weight)*
Plating on Marine Agar with glycerol	100 CFU / mL but identification of black colonies uneasy	10^4 CFU / g	Sup. to 10^4 CFU / g (enrichment leads to plate saturation with biomass)
V. nigripulchritudo species specific PCR	1 CFU / mL	10^3 CFU / g	100 CFU / g

Legend :

Presence of the species *V. nigripulchritudo*

Quantification of the pathogenic subspecies by real time PCR (estimated cells / mL)

- > $4x10^3$
- $2x10^3$ – $4x10^3$
- < $2x10^3$
- Not detected

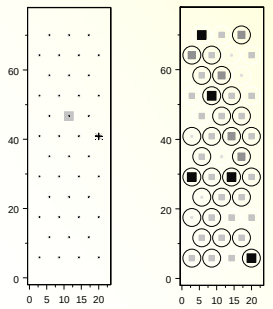

FIGURE 1.*Vibrio nigripulchritudo* mapping at the species and subspecies level, using PCR on fresh sediments from shrimp ponds.

Detection of *V. nigripulchritudo* at the species and subspecies level is more frequent at day 107 than at day 56. This shows that the sediments become an ecological niche favorable to potential shrimp pathogens.

CONCLUSION

The advantages of PCR compared to culture techniques for pathogen detection are precision and rapidity and the possibility to detect non-culturable organisms. The diagnostic is species- or strain-specific when the genetic sequences are documented. The tools presented here will also be of particular interest when the bacterial species or strain to be detected can not be characterized on a selective medium.

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