Detection of shrimp pathogen *Vibrio nigripulchritudo* in sediments of a New-Caledonian grow-out pond during a drying period

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Introduction

Since 1980, shrimp pond farming in New Caledonia is a developing industry, based on the culture of the domesticated Pacific blue shrimp, *Litopenaeus stylirostris*. However, the profitability of the New Caledonian shrimp industry is threatened by seasonally recurring mortality outbreaks associated with two different *Vibrio* species: *V. penaeicida*, the causative agent of a disease occurring in winter and referred to as "syndrome 93" (1) and ***V. nigripulchritudo***, implicated since 1997 in a hot season disease known as "**summer syndrome**" (2).

Experimental infections together with epidemiological studies have shown that pathogenic and non-pathogenic isolates of *V. nigripulchritudo* co-existed in shrimp farm environment (2, 3). Moreover, obtained results also demonstrated that the "summer syndrome" was caused by a single, possibly emerging, cluster of virulent strains. Consequently, it was hypothesized that pathogenic strains of *V. nigripulchritudo* may persist from one year to the next in the shrimp farm environment and re-develop inside the grow-out system at the following rearing cycle (3). This study was therefore aimed at determining whether *V. nigripulchritudo* isolates may survive, or not, in a shrimp pond bottom soil during a 18-week drying period. To this end, *V. nigripulchritudo* mapping was performed with recently developed molecular tools and classical culture-dependent techniques.

Material & Methods

Sampling : bottom soil samples were collected from 15 sediment stations (Fig. 1) selected to cover the whole surface of a 7.5 ha earthen pond (Ferme Aquacole de la Ouenghi, FAO).

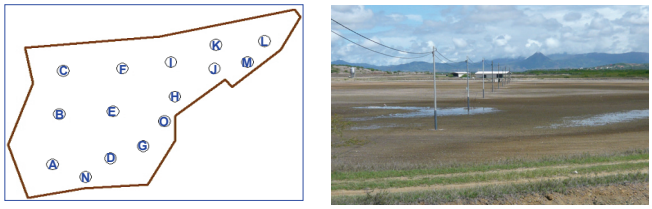
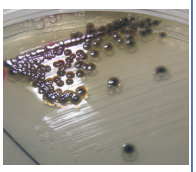


Fig. 1: schematic representation of the pond and location of the 15 stations

For each station, three 5-cm deep sediment cores were collected, pooled in a sterile plastic tube and brought to the laboratory. Sampling was performed once every 3 weeks during the whole duration of the drying period (i.e. 18 weeks).

Sediment enrichment step : 5-g of wet sediments were incubated with 10 ml of enrichment solution (Marine broth half diluted with sterile saline) at 120 rpm and 28°C for 24 h, as previously described (4).

***V. nigripulchritudo* enumeration** : the enrichment solution was 10-fold diluted using sterile saline. A 100-µl volume was then plated at 28°C on solid Marine Agar with 2% glycerol (w/v) for 72 hours. Colonies showing a black coloration were considered as presumptive *V. nigripulchritudo* isolates.



PCR detection of *V. nigripulchritudo*: following enrichment, 250 mg of wet sediments were extracted using the MO BIO Power Soil DNA Isolation kit. *V. nigripulchritudo* detection by PCR was performed using specific primers VnF (5'-GTGTGAATTTAATAGATGCACATT-3') and VR (5'-CGCATCTGAGTGTCAGTATCT-3'). To assess the quality of extraction, recovered DNA was used as template for 16S rRNA gene amplification.

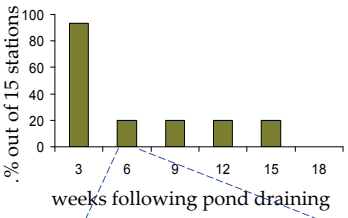
Organic matter (OM) concentration: organic matter concentrations in pond bottom soil was determined according to (5).

Results

Comparison of culturable bacteria count and PCR detection of *V. nigripulchritudo* in bottom soil samples

Both techniques (PCR vs culturable bacteria count) used in this study to detect *V. nigripulchritudo* in pond sediments were found to correlate well ($P < 0.05$)

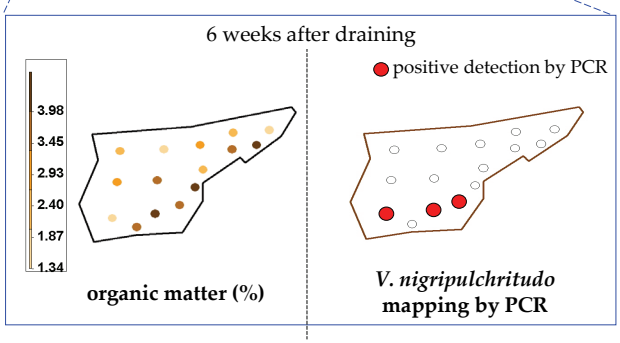
PCR detection of *V. nigripulchritudo* during the drying period



V. nigripulchritudo was detected in 14 out of 15 stations 3 weeks after draining. Afterwards, detection occurred only in 3 stations out of 15. At the last sampling date, this species was not detected.

Fig. 2: PCR detection of V. nigripulchritudo during the drying period

Effect of organic matter concentration on *V. nigripulchritudo* detection in pond bottom soil samples



Six weeks after draining, the organic matter concentration was quantified. Statistical analyses demonstrated that *V. nigripulchritudo* detection was highly correlated with this parameter ($P < 0.05$)

Discussion & conclusion

Data obtained during the course of this study show that the presence of *V. nigripulchritudo* in a pond bottom soil dramatically decreased from week 3 onwards and until the end of the drying period, becoming then undetectable. However, when an additional station was sampled at this date (18 weeks after draining), in an area containing black organic sludge and located at the periphery of the pond, this bacterial species could be evidenced by PCR (data not shown).

Supporting the initial hypothesis of Goarant *et al* (4), this study demonstrates thus that the shrimp pathogen *V. nigripulchritudo* is able to **survive** in shrimp pond sediments from one year to the next and preferentially in areas with a high organic matter content. This persistence may help to explain how this bacterial species develops inside the grow-out system during a rearing cycle.

1. Costa R, Mermoud I, Koblavi S, Morlet B, Haffner P, Berthe F, Le Groumellec M, Grimont P. 1998. Aquaculture 164: 297-309.
2. Goarant C, Ansquer D, Herlin J, Domalain D, Imbert F, De Decker S. 2006. Aquaculture 253: 105-113
3. Goarant C, Reynaud Y, Ansquer D, de Decker S, Saulnier D, le Roux F. 2006. Syst Appl Microbiol. 29 (7): 570-80.
4. Walling E, Vourey E, Ansquer D, Goarant C, Beliaeff B. 2009 (submitted)
5. Ayud M & Boyd C.E. 1994. Journal of the World Aquaculture Society 25 (2): 322-325

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