Development of an antiseptic procedure to improve retention rates during the grafting process of black-lip oyster, *Pinctada margaritifera*

NATHALIE COCHENNEC-LAUREAU[1], PHILIPPE HAFFNER[1], PEVA LEVY[1], DENIS SAULNIER[2], A. FOUGEROUSE[3] AND SANDRA LANGY[4]

In French Polynesia, black pearl cultivation now takes place on about 30 of the 118 atolls or islands. This activity has largely replaced copra, coconut oil and fishing as a source of income. Around 1,000 pearl farms, generally small ones of less than 5 ha, are registered. The pearl farms are mainly owned by families. In 1998, about 7,000 persons were employed in the entire pearl industry, from production to retailing. In value, this activity represents the first exportation of French Polynesian products. From 1988 to date, exports increased exponentially before leveling. Starting with less than two kilograms in 1972, exports have now stabilized at 11 tons of "black" pearls.

Obtaining a pearl is an arduous process. For every 100 oysters implanted, 10 do not survive the grafting operation, 30 reject the nucleus and only a few of the remaining 60 will produce a flawless or "perfect pearl." The grafting process consists of the insertion of a mantle tissue graft and a nucleus, which is a small, round piece of shell (bead) used by the grafter for implanting into the pearl pocket. This process, which takes one or two minutes, is the beginning of the creation of a cultured pearl. During the following weeks, the implanted graft grows to form a pearl sack. Then, concentric layers of nacre are secreted around the nucleus, covering it totally. If the wound created by the initial surgery heals quickly and if good development or growth conditions are met, a round pearl will more easily develop. Heterogeneity in the pearl rejection rate and poor pearl quality are both limiting factors for producers but the reasons behind the problems are not really understood.

Cultured black pearl

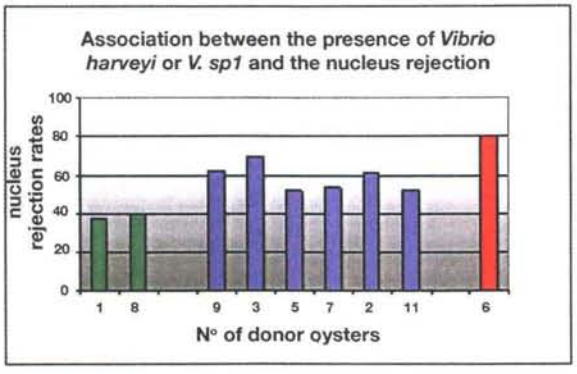

Fig. 1. Comparison of rejection rates between the different donor oysters used in this experiment. Green: Absence of Vibrio *types belonging to the bacterial groups defined as* V. *sp1 and* V. harveyi*; Blue: isolation of* V. *sp1 from graft and/or pearl pocket. Red: isolation of* V. harveyi *from pearl pocket.*

As part of a global research project devoted to the improvement of the grafting process, an attempt is being made to analyze the causes of nucleus rejection through bacteriological and histological approaches. Two different processes were tested to determine their effect on the efficiency of the grafting procedure, retention and mortality rates: 1) the standard process using lagoon water for washing solutions and, 2) an antiseptic process using Iodine Polyvadone and antibiotic solution for washing tools, tissue grafts or recipient oysters prior the inclusion of the nucleus. Six hundred three year old oysters were grafted in the Tuamotu archipelagos and tagged to allow the researchers to follow individual oysters. The recipient oysters were placed in small mesh bags

(Continued on page 57)

to protect them from predators and were inspected daily for mortality and rejection. The bags enabled the farmer to determine if one or more of the implants were expelled by the oysters. Mortality and nucleus rejection were monitored daily. Bacteria associated with the grafting process were isolated, identified and characterized. Histological examinations of grafts and pearl pockets were made.

Most of the mortality was observed during the first two weeks after the grafting process. That mortality may correspond to oysters that didn't survive the surgical procedure. Nucleus rejection was immediately observed after the grafting process and was still noticeable 72 days later. Comparison of cumulative mortality and nucleus rejection between improved and standard procedures did not show a great difference: 14 and 11 percent for cumulative mortality and 42 and 46 percent for cumulative rejection, respectively. However, it is obvious that the improved process allows for significantly reduced total bacterial counts in the graft tissue and the grafted pearl pocket. Similarly, a significant decrease in total bacteria counts was observed on wash solutions used for the antiseptic protocol compared to the standard method. These results suggest that the improved process was effective in reducing bacterial contamination.

It was found that 75 percent of the total bacteria isolated from grafts and/or grafted pearl pockets, three days after the procedure, were gram-negative. Among those bacteria, 28 percent are included in the family Vibrionacae. Two primary types: *Vibrio harveyi* and a new one *Vibrio* sp1, were characterized using biochemical and molecular analyses. Those two were isolated from seven of the nine donor oyster samples.

To assess the pathogenicity of the two bacteria types, experimental injections of 10^8 bacteria per oyster were performed in the adductor muscles of 60 *P. margaritifera* maintained in laboratory quarantine facilities. Significant mortalities, from 50 to 82 percent, were observed for *V.* sp1 and *V. harveyi* 48 h after injection. In negative controls, one gram-positive bacterial strain and sterile seawater, mortality was only 15 percent.

Comparison of cumulative rejection and mortality rates between carrier and non-carrier donor oysters showed a significant difference. The absence of *V.* sp1 or *V. harveyi* in grafts and/or grafted pearl pockets was associated with a rejection rate of 38.7 percent. On the other hand, the presence of *V. harveyi* and *V.* sp1 resulted in 80 percent and 58.7 percent, cumulative rejection rates (Figure1).

During histological examination of graft samples from each donor oyster prepared for the procedure, some showed signs of necrosis associated with the presence of large numbers of bacteria, thus confirming a true bacterial infection. These data suggest one possible way of contamination of recipient oysters.

Many lesions were observed on recipient oyster tissues adjacent to the grafted pearl pocket after nucleus expulsion, the presence of numerous hemocytes within the pearl pocket and inflammation along the insertion area and nucleus. However, no bacteria were observed in association with the lesions after expulsion. The observations are in agreement with those of experimentally infected muscles. Many lesions and particularly the presence of numerous lacunas were observed in the muscle of injected oysters. Some rare occurrences of bacteria were observed on adjacent connective tissue and not in association with the muscular lesions.

The grafting process is an extremely complex phenomenon and we can speculate that failure to obtain a pearl is due to many factors acting simultaneously or in cascade. Mortality and rejection rates may be due, at least in part, to bacterial contamination. The presence of bacteria may influence the immune system of oysters and, if so, oysters become more susceptible to the stress of the grafting process. An association between the presence of bacteria, particularly *V. harveyi* and *V.* sp1, and their potential influence on nucleus rejection was revealed. Confirmation of our hypothesis is important because of the pathogenicity of *V. harveyi* in many mollusc species. The antiseptic process used in this experiment clearly reduced the risk of bacterial contamination from oyster to oyster during the grafting process and is recommended.

Further work is being conducted to complete the molecular characterization of these bacteria types and to develop an experimental infection method to assess their respective virulences.

Notes

[1] Ifremer – BP7004, Taravao, 98719 Tahiti, Polynésie Française. E-mail ncochenn@ifremer.fr

[2] Ifremer – Laboratoire de Génétique et Pathologie, 17390 La Tremblade

[3] Service de la Perliculture – BP 9047 Motu Uta, 98715 Tahiti, Polynésie Française

[4] Ministère de la promotion des Ressources Naturelles, BP2551, Papeete, 98713 Tahiti, Polynésie Française

New Literature

Malison, J.A. and C.F. Hartleb (editors). 2005. **Best Management Practices for Aquaculture in Wisconsin and the Great Lakes Region.** University of Wisconsin Sea Grant Institute. Madison, Wisconsin USA. 125 p. US$40.00

Kelly, A.M. and J. Silverstein (editors). 2005. **Aquaculture in the 21st Century.** American Fisheries Society Symposiumn 46. Bethesda, Maryland USA. 643 p. US$69 (nonmembers), US$48 (members).

Ray, R.C. (editor). 2005. **Microbial Biotechnology in Agriculture and Aquaculture.** Volume I. Science Publisher, Inc., Enfield, New Hampshire USA. 495 p. US$97.50.

[Note: This volume is primarily dedicated to microbial biotechnology in agriculture.]