Improvement of the quality of pearls produced by the oyster *Pinctada margaritifera* in French Polynesia: characterization of cellular and molecular processes from the grafting to the pearl formation.

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1. **INTRODUCTION**

Tahitian pearl farming industry plays a major socio-economic role in French Polynesia with more than one hundred million euros worth of pearls exported in 2007. In the context of a more and more competitive market where the production of high quality pearls becomes essential, research can contribute to secure and ensure durable production. The objective of the "Laboratory of Biotechnology and Pearl Quality" of Ifremer (French Research Institute for Exploitation of the Sea) in close collaboration with the "Service de la Perliculture" ("pearl farming office", a French Polynesian government agency) is to study by a global approach the mechanisms underlying pearl formation and defects occurrence in *Pinctada margaritifera*. Researches range from the complete exam of the grafting process to the characterization of the cellular and molecular mechanisms of the pearl formation with a specific focus on pathology, grafting optimization and biomineralization. The aim, in accordance with pearl farmers concerns, is to propose methods and tools that will increase superior pearl quality production.

2. **PEARL CULTURE**

In French Polynesia, cultured pearls are the product of grafting and rearing of *P. margaritifera* in their natural environment. Pearl culture includes several stages. To start with, the pearl oysters are collected and raised to serve either as donor or receiver. The grafting process then takes place following a surgical operation during which the graft, a small piece of mantle epithelium (about 4mm of tissue), is inserted into the “pearl pouch” of the receiving oyster together with a nacre bead known as the nucleus (Figure 1). Once inserted into the receiving oyster, the epithelial edge of the graft multiplies to carpet the pearl pouch and form a pearl sac around the nucleus. The pearl sac then starts to deposit nacre (aragonite) layers onto the nucleus. This is the starting point for the future pearl. Over the first 45 days or so, mortalities and nucleus rejection occur at variable rates. A rearing period of about 18 months is needed to finally produce a pearl with a sufficiently thick layer of nacre (0.8 mm) (Figure 1). Generally, with oysters that have produced a high quality pearl, a second graft can be carried out. Another nucleus of the size of the harvested pearl is introduced into the existing pearl sac without any addition of a second graft tissue.

3. **GRAFTING PROCESS OPTIMIZATION**

The grafting operation is a key step to obtain a high quality pearl that has been performed in Polynesia since the 1960s. The grafting techniques are however far from being standardized. The choice of donor oysters, quality of the graft tissue as well as the physiology of the receiving oysters are decisive factors for success, but these factors are not sufficiently tested or technically controlled. From graft surgery to harvest, pearl culture is still done in an empirical way, depending on the methods of each particular grafter or pearl farmer. Such differences lead to highly variable levels of post-operative mortality, nucleus retention and pearl quality. Few studies have been published on post-graft mortalities and nucleus rejection. Comps *et al.* (1998) [1] suggested that some histological anomalies, found when there were post-graft mortalities, could indicate infections by a pathological agent or be due to grafting practices. Thus, the use of sterile procedures, in and after grafting surgery, significantly decreases the risk of bacterial infection.

Based on grafting procedures analyses, the Laboratory of "Biotechnology and Pearl Quality" of Ifremer has developed in close collaboration with the "Service de la Perliculture" research projects in order to (1) better understand the causes of graft failure (nucleus rejection, mortality or bad quality pearls), (2) identify factors determining the success of the grafting and the pearl quality, (3) propose standardization of the graft (nucleus choice, surgery, cutting and insertion of the graft, graft hygiene).
4. MOLECULAR ANALYSES OF TISSUES INVOLVED IN PEARL FORMATION

The aim of this work is to investigate the molecular mechanisms underlying biomineralization processes during pearl formation in *P. margaritifera*. In pearl oysters, shells are subdivided in two calcium carbonate crystal structures: an outermost calcite prismatic layer and an inner nacreous aragonite layer. Both are embedded in an organic matrix framework. The mantle tissue is responsible for the secretion of the organic components necessary for mineralization of the shell as is the graft for the pearls. As central effectors of crystal development, previous studies have mainly focused on the purification of matrix proteins, identification of their primary structure and evaluation of their functions in shell formation. We still know however comparatively little about how the genes coding for these proteins are expressed in the mantle and how the grafting process affects gene expression through pearl formation.

To understand this process, we have developed two complementary approaches: (1) a candidate gene approach to elucidate the presence of conserved matrix gene and (2) a genome-wide approach to unravel regulation and transcriptomic data during pearl formation (figure 2).

4.1 Functional characterization of mineralization markers

This first approach allowed us to identify three gene families representing the first homologues of calcium carbonate organic matrix genes found in *P. margaritifera*. Pattern expression analyses of the first gene, called “perline” [2], and immunological detection of the protein in mineralized tissues seem to show its involvement in aragonite formation. The “calcine” and “aspéine” genes exhibit an expression pattern specific to mantle cells thought to be the source of the prismatic layer matrix organic molecules. Those genes thus represent good candidates for mineralization biomarkers and the first opportunity to get tools to improve pearl quality. Finally, transcriptomic expression analyses of all these genes through the grafting process show a strong heterogeneity underlying the high level of complexity of pearl formation.

Figure 1: the different steps of the grafting process and pearl formation.

Figure 2: scheme of the molecular and cellular events studied to understand pearl formation.
4.2 Identification of biomineralization markers by transcriptomic SAGE approach
The goal of this second approach is to get a global view of transcriptomic events taking place in mineralizing tissues involved in pearl formation. Therefore, a transcriptome analysis using the SAGE method (Serial Analysis of Gene Expression) and the construction of an EST library (Expressed Sequence Tag) were developed in parallel on mantle cells in order to characterize graft cell genes differentially expressed. Expression profiles of 48,000 genes have been established and more than 280,000 ESTs have been sequenced allowing the constitution of 47,000 clusters. These results represent an important set of genomic data for this organism, and have allowed the selection of a combination of genes, which characterization was undertaken by real-time PCR. Correlation between the expression level of these potential biomarkers and the quality of pearls is now assessed in the course of experimental graftings. This work will lead to the identification of a set of genes in relationship with the nature of calcium carbonate deposits of the pearls. The evidenced biomineralization markers will be used to gain original tools (bio-assays) for the professionals to assess and select donor oysters with higher mineralization capacities.

5. CONCLUSION
Pearl farming holds an essential place in French Polynesian economy. Thus, the Laboratory of “Biotechnology and Pearl Quality” of Ifremer collaborates with the “Service de la Perliculture” in Tahiti in order to deepen the knowledge concerning the mechanisms underlying pearl formation and defects occurrence in *P. margaritifera*. Researches range from the complete exam of the grafting process to the characterization of the cellular and molecular mechanisms of the pearl formation. This work is therefore closely linked to the concerns of the professionals and the aim is to propose methods and tools that will increase superior pearl quality production. A part of this research is achieved through a large collaborative project regarding the “Improvement of the Pearl Quality” (GDR ADEQUA) and intend to support the sustainable development of pearl farming in French Polynesia.

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7. REFERENCES