Characterization of two new genes implicated in the mineralization process of *Pinctada margaritifera*

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Introduction

P. margaritifera as the other mollusk shells consist of an internal nacreous layer (figure 1-1) and an external prismatic layer (figure 1-2). Both layers are composed of two different forms of calcium carbonate crystal (aragonite in the nacre and calcite in the prismatic layer) and different structural proteins. The mantle is involved in the formation of the shell. However, the exact role of mantle epithelium, and particularly the edge cells, in the formation of aragonite or calcite is not well understood. Pearl oyster, *P. margaritifera* produces pearls following a graft process. The pearl is composed of the aragonite crystal and organic matrices. One of the key steps for pearl formation resides in the formation of the pearl bag, as functional relations between the coating nature of the pearl and the cellular organization of the pearl bag are know to exist (Hui, 2001).
In this study, we have characterized two partial complementary DNA encoding putative aragonite and calcite shell matrix protein from the mantle epithelial tissues of *Pinctada margaritifera*. These two proteins, which we have named Perline and Calcine, exhibit a specific pattern of expression and may have role in pearl mineralization.

Material and methods

RNA purification and complementary DNA synthesis

Live individual of *P. margaritifera* were collected in the lagoon of Tahiti. Total RNA from mantle, muscle, gills and digestive gland were isolated by classical method (TRIzol©) and poly(A)$^+$ RNA were prepared by oligo(dT). Poly(A)$^+$ RNA (1µg) was applied as template for reverse transcription to prepare cDNA

cDNA amplification and gene expression analysis

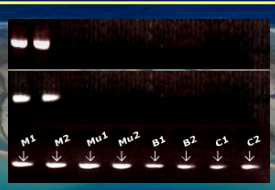
Using CLUSTALW multiple alignment program, conserved regions were identified by comparison, for each gene, with cDNA sequences from *P. fucata* and *P. Maxima* (accession N° AB326122.1, AB023254.1, AB020779.1, AB32613.1, D83523.1), Putative specific primers for Perline and Calcine were designed. Primer pairs used for perline and calcine are respectively PeS4 5'- GAC ATA GAG AGA GAC AGA TAT GA-3' / PeAS1 5'-CCA TTG CCA TTT CCG TTA-3' and NaS3-5' GAG ACA ATC ACC AAT CAA CAT-3' / NaAS1 5'-ACC AAA TGA GCC TCC AT-3'. PCR products were sequenced and alignments were performed with BLAST program in GenBank, National Center for Biotechnology Information. Tridimensional structure of Calcine partial deduced amino acid sequence was performed with Geno3D and Rasmol programs.

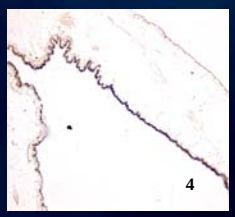
In situ hybridization

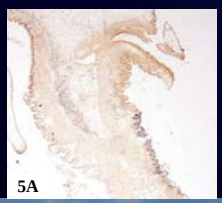
Putative primers were used to develop *in situ* hybridization. Probes were labelled with digoxigenin incorporation by PCR. Perline and Calcine RNA expressions were assessed on paraffin-embedded tissus sections of mantle, graft and pearl bag of *P. margaritifera*

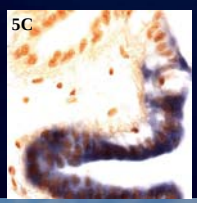
Results : genes expression analyses

As expected expression analysis of Perline and Calcine matrix protein genes showed that transcripts are specifically expressed in the edge of the mantle cells but not in muscle (Mu) gills (B) and heart (C) tissues. Actin was included as positive control



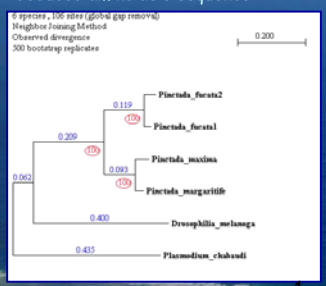






Results : partial cDNA and deduced amino acid sequences

Phylogenetic relationship of Perline deduced amino acid sequence



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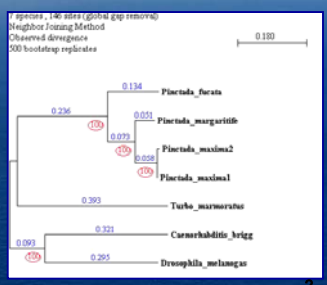
Phylogenetic relationship of Calcine deduced amino acid partial sequence



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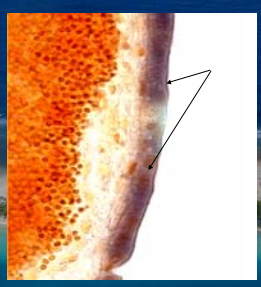
Two partial cDNA sequences were obtained, respectively 387pb and 586 pb for Perline and Calcine genes. High homology rates were observed for nucleic and deduced amino acid sequences between *P. maxima*, *P. fucata and P. margaritifera*, respectively 70 and 67% for Perline sequences and 67 and 65% for Calcine sequences. Phylogenetic analyses show great homology between *P. maxima, P. fucata* and *P. margaritifera* sequences (figures 1, 2). Partial calcine amino acid sequence exhibited greatest identity with carbonic anhydrase isolated from the nacreous layer of *P. fucata* and with human carbonic anhydrase CA Ii.

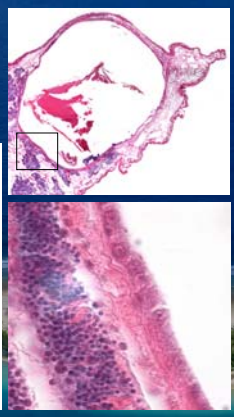
Figure 3 : Tridimensional protein structure of Calcine

Obtention of tridimensional protein structure confirms this result and suggest its participation in calcium carbonate crystal formation (figure 3).

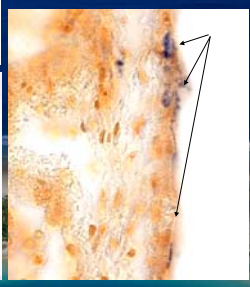
In situ hybridization shows that the pallial mantle outer epithelial cells express Perline messenger RNA (figures 5 A, B, C), and that the edge mantle outer epithelial cells express Calcine mRNA (figure 4). These findings suggest that the proteins in the pallial form the nacreous layer and that the proteins from the edge are involved in prismatic layer. *In situ* hybridization allow to localize pearl bag cells involved in the formation of aragonite layer. Both mRNA are expressed in pearl bag however Perline expression seems more significant and homogenous than Calcine expression.

Perline expression on pearl bag





Calcine expression on pearl bag



Epithelial cells of pearl bag (X1000)

Discussion and conclusion

We report identification and characterization of the first shell matrix protein genes of *P. margaritifera,* named Perline and calcine . Our results suggest that they may have important roles in calcium carbonate mineralization and that they were respectively involved in aragonite and calcite formation layers. Both genes are expressed in graft compartments (mantle, graft tissue [not shown], and pearl bag). Perline and Calcine genes could be useful markers to quantify graft tissue expressions and pearl bag development. A mantle cartography expression of Perline and Calcine is currently underway to study the distribution of both genes. The ultimate goal is to better characterize and select grafts to optimize the pearl structure.

References

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