Estuarine, Coastal and Shelf Science 182 (2016) 235–242

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Effect of electrolysis treatment on the biomineralization capacities of pearl oyster *Pinctada margaritifera* juveniles

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ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form
11 May 2016
Accepted 19 June 2016
Available online 21 June 2016

Keywords and regional index terms:
Pearl oyster
Pinctada margaritifera
Electrolysis
Biomineralization
Gene expression

ABSTRACT

The present study investigated the effect of electrolysis on the biomineralization capacities of juveniles of the mollusk *Pinctada margaritifera* for the first time. Size-selected individuals from two groups, "Medium" and "Large", from a multi-parental family produced in a hatchery system were subjected to electrolysis under a low voltage current over a nine-week experimental period. The growth of the juveniles was individually monitored and assessed weekly by wet weight and shell height measurements. At the end of the experiment, mantle tissue was sampled for biomineralization-related gene expression analysis. Electrolysis significantly increased pearl oyster growth in terms of shell height and wet weight for Large juveniles from the 5th and the 2nd week, respectively, until the end of the experiment. However, differences were only significant for Medium individuals from the 7th week for shell height and from the 9th week for wet weight. Furthermore, transcriptional analysis of six known biomineralization genes coding for shell matrix proteins of calcitic prisms and/or nacreous shell structures revealed that five were significantly overexpressed in the mantle mineralizing tissue under electrolysis: three in common between the two size class groups and two that were expressed exclusively in one or the other group. Finally, we found no statistical difference of the shell thickness ratio between individuals undergoing electrolysis and control conditions. Taken together, our results indicate, for the first time in a calcifying marine organism, that electrolysis influences molecular mechanisms involved in biomineralization and may stimulate some parameters of pearl oyster growth rate.

1. Introduction

The cultured pearl industry, with around US$784 million worth of production in 2005 (Tisdell and Poirine, 2008), is of great economic importance for a number of countries in tropical and subtropical regions. In French Polynesia, the black-lip pearl oyster *Pinctada margaritifera* "Linnaeus 1758" is the top aquaculture species and the basis of the mass production of a unique gem built by a living organism. Not only is pearl culture the second highest economic resource of French Polynesia (65 million Euros export value in 2013, customs statistics, Wane, 2013), but it also represents an important source of employment (nearly 5000 people employed on 487 farms in 2013) (Ky et al., 2014). However, since the early 2000s, this industry has suffered a severe crisis, mainly due to overproduction and a slowdown of the world economy, leading to a dramatic fall in mean pearl value per gram. Pearl size and quality are among the most important factors that go into determining pearl value (Blay et al., 2014). Increasing cultured pearl quality, through cultural practices and/or genetic selection, is the biggest challenge for research and development.

Production of cultured pearls is achieved starting with a surgical operation called "grafting" carried out by skilled technicians. A small piece of mantle tissue is removed from a donor oyster to be inserted into the gonad of a recipient oyster, along with a spherical

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http://dx.doi.org/10.1016/j.ecss.2016.06.014
0272-7714/

nucleus made of mollusk shell or synthetic material (Kishore and Southgate, 2014; Taylor and Strack, 2008; Cochennec-Laureau et al., 2010). *P. margaritifera* recipient oysters are used for graft operations when their shell height has reached 11 cm, at approximately two years of age (Gervis and Sims, 1992). An additional 18- to 24-month period is required to produce a pearl with a sufficiently thick layer of nacre (0.8 mm) for harvest. In French Polynesia, *P. margaritifera* shell growth increments are highly variable, with higher growth rates in island lagoons and the open ocean compared with the atoll lagoons where they are usually reared (Pouvreau and Prasil, 2001). Improving pearl oyster growth and reducing the length of the culture time needed to reach a suitable size for graft operations would contribute significantly to increase the cost-effectiveness of the industry. Moreover, recipient pearl oyster shell increments are correlated with the pearl nacre deposition rate (Coeroli and Mizuno, 1985; Le Pabic et al., 2016). Thus, producing larger pearl oysters would potentially lead to the formation of thicker nacre layers.

P. margaritifera shell growth relies on the formation of a mineral phase composed of layers of calcium carbonate and an organic matrix containing mostly proteins, glycoproteins, lipids and polysaccharides (Joubert et al., 2010; Levi-Kalisman et al., 2001). This organic matrix, secreted by the epithelial cells of the external mantle, controls nucleation, orientation, growth, and the polymorphism of the calcium carbonate crystals formed as aragonite or calcite (Mann, 1988; Belcher et al., 1996). Shell matrix proteins play a major role in the shell biomineralization process. Some genes encoding matrix proteins have been identified and are known to be specifically involved in the formation of the nacreous layer and/or prismatic layer (Joubert et al., 2010; Montagnani et al., 2011; Marie et al., 2012). For example, the genes *Pif 177* and *MSI60* are involved in shell nacreous layer formation by regulating aragonite crystal growth (Suzuki et al., 2009; Sudo et al., 1997). Shematrin proteins are secreted into the prismatic layer where they are thought to establish a structure for calcitic prism formation (Yano et al., 2006). Prismalin 14 controls calcitic prism calcification (Suzuki et al., 2004), and Aspein is thought to play a key role in calcite precipitation (Isowa et al., 2012). In contrast, some proteins such as Nacrein are involved in both the aragonite and calcite mineralization processes (Miyamoto et al., 2013).

The mineral accretion method, based on the electrolysis of seawater, involves a low-voltage direct electrical current through two submerged electrodes to induce deposition of dissolved minerals on conductive substrates (Hilbertz, 1979). Seawater is split into hydrogen gas H_2 and hydroxide ion HO^-, leading to an increase of the pH in the vicinity of the cathode. Calcium ions Ca^{2+} from seawater combine with dissolved bicarbonate HCO_3^- to precipitate as aragonite $CaCO_3$ and magnesium ions Mg^+ with hydroxide ions to precipitate as brucite $Mg(OH)_2$. Several experiments have been conducted to study the effect of this mineral accretion method on survival and growth rate of marine calcifying organisms, such as corals and oysters (Borell et al., 2010; Piazza et al., 2009; Sabater and Yap, 2002, 2004; van Treeck and Schuhmacher, 1997). Results vary considerably, since some studies on the effect of the mineral accretion method report increased survival rate of coral transplants (van Treeck and Schuhmacher, 1997; Sabater and Yap, 2002) and enhanced coral growth rate (Sabater and Yap, 2004) whereas other studies show lower growth rates for juvenile oysters (Piazza et al., 2009) and no effect or a negative effect on coral survival (Borell et al., 2010).

Surprisingly, studies on the effect of electrolysis on mollusk and coral biomineralization have only focused on biometric analysis of calcifying tissues. Indeed, to our knowledge, no molecular approaches have yet been explored to characterize biomineralization processes under electrolysis treatment. With the advent of proteomic, transcriptomic, and genomic technologies, several biomineralization-related proteins, referred to as the biomineralization "toolkit" have been recently identified in the pearl oyster *P. margaritifera* (Marie et al., 2012).

This study is the first aiming to investigate the effect of electrolysis on the biomineralization capacities of the black-lipped pearl oyster *P. margaritifera*. Some growth parameters (shell thickness, height, animal weight) and the expression level of six biomineralization-related genes were measured in juvenile *P. margaritifera*.

2. Materials and methods

2.1. Biological material

A multi-parental family was produced in the Ifremer hatchery facilities in Vairao (Tahiti, French Polynesia) using a cross between three female and six male broodstock oysters. Artificial spawning, larval rearing, and oyster culture were conducted as described in Ky et al. (2013). Juveniles were reared in the same natural environment, in Aquapurse® plastic trays suspended on long lines located in Vairao lagoon (Tahiti). At 180 days post fertilization, oysters were categorized into two groups according to their shell size: 40 "Medium" size (mean shell height of 3.8 cm ± 0.4 and mean wet weight of 5.64 g ± 1.47) and 30 "Large" size (mean shell height of 5.1 cm ± 0.4 and mean wet weight of 12.44 g ± 3.42). All pearl oyster juveniles were transferred by airplane from Vairao lagoon to Bora Bora lagoon (GPS location, 16.528553 S, 151.768184 E, French Polynesia).

2.2. Experimental design

Two conditions were tested for an experimental period of nine weeks in the lagoon of Bora Bora using a total of 70 pearl oysters: electrolysis using low-voltage electric current and control conditions (no electrolysis). Twenty Medium and 15 Large juvenile pearl oysters were randomly selected and subjected to each condition. These pearl oysters were randomly hung on chaplets (ropes) in two Aquapurse® plastic trays to prevent predation from shellfish and fish (Fig. 1). Pearl oysters under electricity were placed on a steel structure subjected to a low-voltage current of 3.7 V, flowing between the positively charged anode and the negatively charged cathode. The electrolysis structure was switched on every other hour from 4 a.m. to 7 p.m. alternating with periods of an hour with no current. This structure was used two months prior to the oyster experiment so that mineral accretion occurred at the cathode where calcium carbonate and magnesium hydroxide were deposited. Both electrically charged structures and the identical uncharged control structures were fixed to pillars at 3.5 m depth set 20 m apart from one another.

Tagged juvenile pearl oysters were individually measured weekly for shell height and live weight. For each individual, absolute cumulative shell growth and wet weight gain were calculated by the formula $PR = (100 \times (V_W - V_{W0}))/V_{W0}$, where PR is the percent change, V_W the present value by week "W" and V_{W0} the initial value by week W0 when oysters were placed on the charged and uncharged (control) structures. After nine weeks of monitoring, all the pearl oyster juveniles were collected.

2.3. Mantle gene expression

For gene expression analysis, mantle tissue samples from four to five randomly chosen individuals were pooled for each of the tested conditions (electrolysis versus control), resulting in three and five pools per condition for Large and Medium oyster batches,