Characterization of *Pollicipes pollicipes* cement protein-100K gene

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BBE - Blue Biotechnology and Ecotoxicology

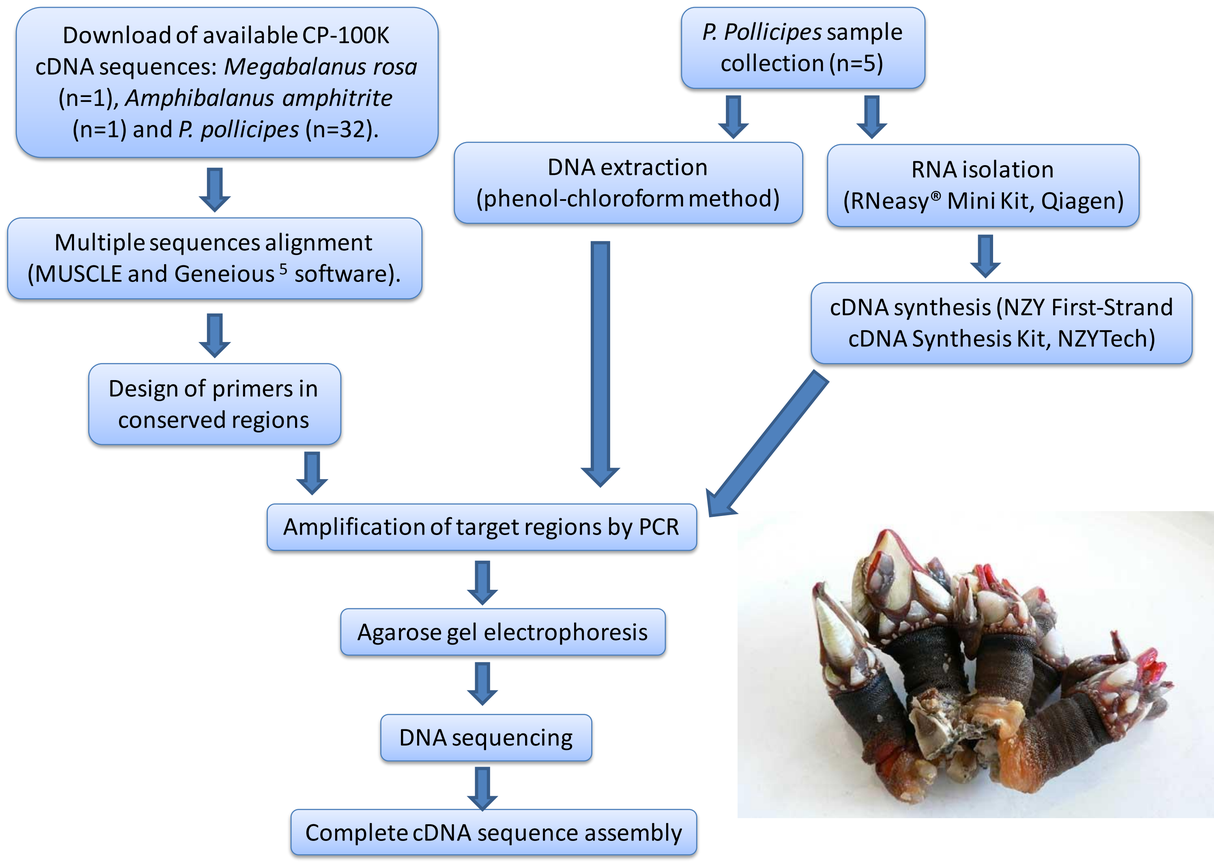
1. Introduction

Pollicipes pollicipes (Gmelin, 1789) is a goose barnacle (Crustacea: Pedunculata) abundant on exposed rocky shores, and distributed in Western Europe and on the North African coasts of the eastern Atlantic from France to Senegal[1]. They are important fouling organisms that adhere to underwater surfaces through singular cement proteins[2,3]. However, it is not fully understood how the mechanisms of fouling and adhesion work. Moreover, the sequence and function of most *P. pollicipes* genes remains unknown.

2. Objectives

Our aim was to characterize the cement protein-100K gene of *P. pollicipes*, which is one of the most abundant cement proteins weighting 100 kDa[4]. The long-term objective of this work is to provide the necessary data to be used on antifouling studies and induction of settlement, with possible industrial applications.

3. Materials & Methods



4. Results & Discussion

- ❖ The CP-100K cDNA sequence of *P. pollicipes* was completely sequenced and compared with available EST's collected from public databases allowing us to provide the first complete CP-100K cDNA sequence (Figure 1 and 2).
- ❖ We have amplified the CP-100K gene in five partially overlapping regions by PCR using cDNA as template (Figure 3). The amplified product had the expected length when compared with the related *Megabalanus rosa* and *Amphibalanus amphitrite* CP-100K cDNA sequences. The amplification of CP-100K fragment located at the 5' region of the cDNA (fragment 1) was not achieved in genomic DNA, which may indicate the presence of an intron in this region.
- ❖ The sequence obtained in our samples had some differences relative to available EST's. Most notably, we detected several erroneous insertions and deletions in available EST's (fragment 2) that were not compatible with the translation of a functional protein. This may be explained by the fact that EST's are only sequenced once, therefore being highly error prone[6].
- ❖ We have also detected four heterozygotic positions across the DNA sequence, one of them resulting in an amino acid change (Figure 4).

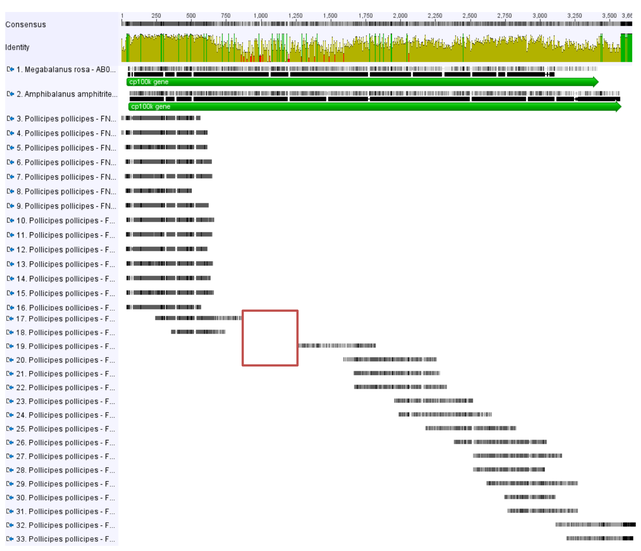


Figure 1 – Alignment of CP-100K cDNA sequences of *Megabalanus rosa* and *Amphibalanus amphitrite* with the *P. pollicipes* EST's collected from public databases. The red square indicates a region not covered by the available EST's.

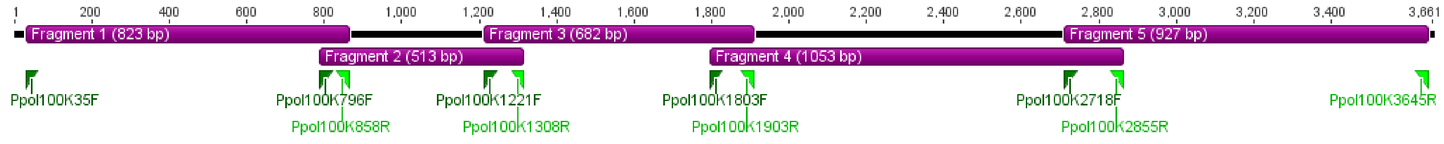


Figure 2 – Schematic representation of CP-100K cDNA. The primers are represented by green arrows and the purple bars correspond to the overlapping regions amplified by PCR.

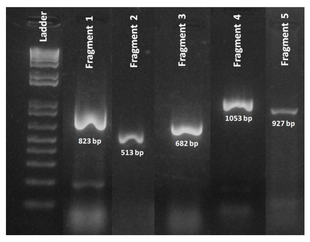


Figure 3 – Amplified CP-100K cDNA fragments separated on 2% agarose gel electrophoresis.

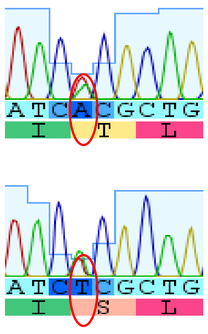


Figure 4 – Example of an heterozygotic position on CP-100K fragment 3 on cDNA (top image) and genomic DNA (bottom image). The two alternative bases (indicated by the red circles) originate two different amino acids: threonine and serine.

5. Conclusions

- ❖ The complete sequence of the *P. pollicipes* CP-100K cDNA was obtained, providing useful data to design functional primers and gene expression studies. For example, it will allow us to test different compounds with potential to induce/inhibit larval settlement. Our work also demonstrated that the available EST's sequences for this gene are not fully correct. In addition, several polymorphic regions were identified, which may be useful for phylogenetic and population studies.
- ❖ This work will set the grounds for the sequencing of other cement protein genes (e.g., CP-19K, -20K and -52K) and other genes involved in *P. pollicipes* attachment (e. g., SIPC).

6. References

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Acknowledgements

This study was funded by the Project MARBIOTECH (reference NORTE-07-0124-FEDER-000047) within the SR&TD Integrated Program MARVALOR - Building research and innovation capacity for improved management and valorization of marine resources, supported by ON.2 Program and by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and national funds through FCT – Foundation for Science and Technology, through the project "PEst-C/MAR/LA0015/2013" .
NOVOMAR (reference 0687-NOVOMAR-1-P), supported by the European Regional Development Fund.