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Cloning perlucin gene from abalone (*Haliotis discuss*) and its protein expression in *Escherichia coli*

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Introduction

The mechanism of mollusk shell formation has attracted many attentions (Kamat et al., 2000), for its outstanding mechanical strength and nanoscale microstructure. Especially, the nacre (mother of pearl) growth mechanism has great economic interests. As the fundamental protein involving in pearl formation, perlucin was isolated from abalone nacre and well characterized (Weiss et al., 2000). Perlucin is a C-type lectin like water soluble protein and can nucleate calcium precipitation and make aragonite crystal which is the main ingredient of pearl. In this study we cloned perlucin gene from our abalone EST cDNA library and induced this protein expression in *E. coli* Rosetta gammi (DE3). The success of perlucin in vitro expression will obviously promote the understanding of shell bio-mineralization mechanism and provide important molecular foundation for further biotechnology application to artificial pearl production.

Materials and method

After cDNA library first round short gun sequencing, we selected 6 clones and did completed sequencing based on their sequence identity to known perlucin sequence. Then we designed the specific primers and did PCR amplification. All the PCR products was inserted into expression vector pMAL-c2X and transformed into 10G cells for sub-cloning. Purified plasmid was transformed into *E. coli* Rosetta gammi (DE3) for protein expression. A volume containing 5 ml of starter culture was inoculated into 100 ml Luria broth with 100 mg/ml ampicillin and 10 mM glucose and kept at 37℃ with 180 rpm until OD_{600} approached 0.5~0.8. Then we may start protein induction with 1.5 mM IPTG. After 6 hrs of induction, the cells were harvested and stored in -70℃ overnight. After thawing, the bacterial cells were placed in an ice-water bath and sonicated. Having centrifuged at 9000 x g for 30 min, the supernatant was loaded into maltose binding resin column. Through the

maltose binding resin, we our protein can be purified and become ready for activity assay. SDS-PAGE was performed to confirm our protein expression and purification. Protein concentration was calculated by Bradford method. Then we used the appropriate concentration and purity expressed protein to do the calcium carbonate precipitation and crystallization test as previous research mentioned.

Results and Summary

We sequenced and cloned six cDNA clones which share high identity with known perlucin sequence. Although their size is shorter than known sequence, owing to the remnant function motif, finally two of them can successfully express proteins with good activity. In calcium precipitation test, those proteins obviously accelerate the pH decrease of saturated calcium carbonate solution. And the aragonite crystallization assay result is also similar to the previous activity test of perlucin from abalone nacre. That means our protein expression is successful and our gene at least contained the function motif for calcium binding. Obviously the confirmation of perlucin gene will promote the research of pearl formation mechanism in abalone and make it possible to enhance pearl production by the modification of pearl formation gene or gene transfer.

Reference

Kamat S., X. Su, R. Ballarini and A.H. Heuer, 2000. Structural basis for the fracture toughness of the shell of the conch *Strombus gigas*, *Nature*, 405: 1036~1040.

Weiss I.M., S. Kaufmann, K. Mann and M. Fritz, 2000. Purification and identification of perlucin and perlustrin, two new proteins from the shell of the mollusc *Haliotis laevigata*. *Biochemical and Biophysical Research Communications*, 267: 17–21.