0514

Comprehensive expression trials for recombinant larval cement related proteins of the barnacle *Megabalanus rosa* using *Escherichia coli* expression system

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Summary

Barnacle settles to man-made substrata such as ship hulls and pipelines of the power stations, causing serious troubles, so-called biofouling. The settlement of barnacles is initiated from the release of the proteinaceous adhesives (cement), from the cyprid, which is the final and settlement–competent larval stage. The long term goal of our study is to understand how many proteins are involved in the cement and how they work.

The major obstacles for the researches are the size of the larva (less than 1 mm) and the absolute shortage for the cement amount released from the larva.

Therefore, we have been sequencing and characterizing clones expressed specifically in the cement glands of the cypris larvae of the barnacle *Megabalanus rosa* to identify the larval cement proteins. With the supports from the Salt Science Research Foundation (0314 and the current support), we have successfully identified the genes encoding Ccg-57K, 36K, BSP, ASP, TRP with no homology to known genes, and LOX and several protease inhibitor homologues as the candidates involving in larval cementation process.

pET41b and pET32a (Novagen) vectors were used to express recombinant *M. rosa* larval cement related proteins in *E. coli*. So far, we have succeeded in expressing 57K (C terminal parts), 110K candidates (parts), LOX (C terminal part), ASP, TRP, and three protease inhibitors. Expression of 36K and BSP was still not successful. Large scale production of the cement related proteins is going to be used to produce the corresponding antibodies which should help to identify the mechanisms of cementation in the cyprids, and to perform functional assays.

Overall, the understanding of the cementation mechanism could help to develop anti-fouling technology for barnacles, and to develop new synthetic adhesives working in salt water environment.