Thesis Title Development of DNA probe for the RFLP analysis of DNA from Macrobrachium rosenbergii (de Man)

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ABSTRACT

Macrobrachium rosenbergii, the so call giant freshwater prawn, is presently important for economic of Thailand. Genetic studies and group classification of M.rosenbergii have never been explored. Nowaday, RFLP pattern analysis has been applicable for classification of bacteria, plants and animals. In this study, the DNA of M.rosenbergii was prepared by proteinase K-phenol/chloroform extraction at 55°C, 2hr from hepatopancreas, heart and hemocyte (blood cell). The extracted DNA has T_m (melting temperature) about 86°C corresponding to 43 % G-C content. The genomic library of M.rosenbergii was constructed from Sau3AI-digested hepatopancreas DNA of a male prawn and ligated to BamHI site of pUC18. The transformants were selected through ampicillin resistant and their colorless characteristic on the medium containing IPTG and X-gal and screening by hybridization with total DNA of either male or female DNA. 20 recombinant colonies were selected for RFLP analysis. 13 clones contained repetitive sequences and could generate 120 bp repeating fragments as the common RFLP pattern of M.rosenbergii.

The repetitive sequences were divided into 2 types based on their sequence homologies. 61 prawns collected from 3 provinces were classified into 13 groups based on their HinfI-RFLP patterns against pMR3 probe and suggested strain variation of M.rosenbergii. MR 3-A fragment of pMR3 could generate HinfI-RFLP patterns and showed homology to DNA of P.monodon and P.merguiensis, whereas, MR 3-B and MR 3-C hybridized specifically to M.rosenbergii DNA.