

Identification of Disease-specific Gene Caused Softness Syndrome of the Sea Squirt, *Halocynthia Roretzi*, by differential display PCR

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Introduction

The sea squirt, *Halocynthia roretzi* is commercially cultured in the southern coast of Korea. In 2004, Korean has consumed approximately \$35 million sea squirt as aquaculture food. Due to an unidentified death syndrome, large numbers of sea squirts in the farming areas died during last 20 years and the industry is in bad shape. The dying sea squirts usually exhibited thinning of the tunics and the meat become quite soft and dissolving. It has been not known the causes involved in this disease. Until now, researchers have proposed several possible syndrome-inducing factors, including environmental pollutants, microorganisms infection, and abnormal temperature changes. To find out the truth of the disease, differential display polymerase chain reaction was carried out. And up-regulate or down-regulate genes were selected in sea squirt with softness syndrome. One of them was Mp. We would prove an attack the diseases by over-expression of Mp.

Materials & Methods

Differential display polymerase chain reaction (ddPCR) was implemented in this study to contribute to the current understanding of this mechanism at the genetic level and to identify novel genes. And We carried out 5'-amplification using partial sequence of metalloproteinase (MP) to find open reading frame of MP. We carried out real-time PCR to confirm MP over-expression in softness syndrome sea squirt.

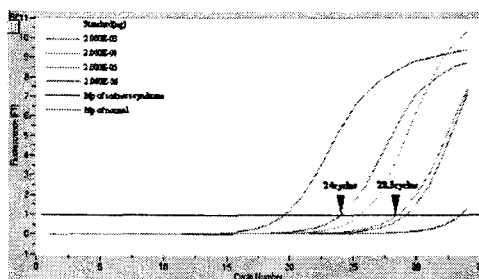


Fig 53. Different copy number of metalloproteinase in softness syndrome and normal sea squirt.

Results & Discussion

Metalloproteinase was up-regulate in two years life of *Halocynthia roretzi* liver than one year life that. We carried out 5'-amplification using partial sequence of metalloproteinase (MP). As a result, the MP of *Halocynthia roretzi* composed of 472 amino acids and belong to astacin family (MCKAY *et al.*, 1995). The MP conserved domain displayed an amino acids identity about 40% with other proteinases. By the real-time PCR experiment (Philip S *et al.*, 2002), it is revealed that MP in softness syndrome sea squirt was highly expressed. To find factors caused MP over-expression in softness syndrome sea squirt, we will carry out single nucleotide polymorphism of MP promoter.

References

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