

## Identification and Molecular Profiling of A Family I Cystatin Homologue from *Sebastes schlegeli* Deciphering Its Putative Role in Host Immunity

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**Abstract :** Cystatins are a large superfamily of proteins which act as reversible inhibitors of cysteine proteases. Papain proteases and cysteine cathepsins are predominant substrates of cystatins. Cystatin superfamily can be further clustered into three groups as Stefins, Cystatins, and Kininogens. Among them, stefines are also known as family 1 cystatins which harbors cystatin Bs and cystatin As. In this study, a homologue of family one cystatins more close to cystatin Bs was identified from Korean black rockfish (*Sebastes schlegeli*) using a prior constructed cDNA (complementary deoxyribonucleic acid) database and designated as RfCyt1. The full-length cDNA of RfCyt1 consisted of 573 bp, with a coding region of 294 bp. It comprised a 5'-untranslated region (UTR) of 55 bp, and 3'-UTR of 263 bp. The coding sequence encodes a polypeptide consisting of 97 amino acids with a predicted molecular weight of 11kDa and theoretical isoelectric point of 6.3. The RfCyt1 shared homology with other teleosts and vertebrate species and consisted conserved features of cystatin family signature including single cystatin-like domain, cysteine protease inhibitory signature of pentapeptide (QXVXG) consensus sequence and N-terminal two conserved neighboring glycine (<sup>6</sup>GG<sup>9</sup>) residues. As expected, phylogenetic reconstruction developed using the neighbor-joining method showed that RfCyt1 is clustered with the cystatin family 1 members, in which more closely with its teleostan orthologues. An SYBR Green qPCR (quantitative polymerase chain reaction) assay was performed to quantify the RfCytB transcripts in different tissues in healthy and immune stimulated fish. RfCyt1 was ubiquitously expressed in all tissue types of healthy animals with gill and spleen being the highest. Temporal expression of RfCyt1 displayed significant up-regulation upon infection with *Aeromonas salmonicida*. Recombinantly expressed RfCyt1 showed concentration-dependent papain inhibitory activity. Collectively these findings evidence for detectable protease inhibitory and immunity relevant roles of RfCyt1 in *Sebastes schlegeli*.

**Keywords :** *Sebastes schlegeli*, family 1 cystatin, immune stimulation, expressional modulation

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