

## **Induction of metacaspase expression and caspase-like activities during senescence in the toxic dinoflagellate, *Karenia brevis***

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*Karenia brevis*, a toxic dinoflagellate, is responsible for near annual harmful algal blooms (HABs) in the Gulf of Mexico causing extensive ecological and economic losses. Evaluation of bloom termination in other bloom forming phytoplankton has implicated a role for metacaspases, metazoan caspase orthologs, in regulating programmed cell death (PCD). Molecular mechanisms governing *K. brevis* bloom demise have remained largely uninvestigated; therefore identification and characterization of *K. brevis* metacaspases may lead to possible targets for bloom control strategies. We have identified five metacaspases (KbMC 1 – 5) in *K. brevis* all containing the well-conserved caspase catalytic diad and p20 domain previously identified in other unicellular organisms. Metacaspase expression over a growth curve, characterized by immunoblot with a polyclonal antibody raised against a recombinant *Emiliania huxleyi* metacaspase protein, revealed an induction of both metacaspase type and quantity in stationary phase/senescence. Metacaspase expression was further characterized in logarithmic and stationary phase cultures using a peptide antibody developed against the histidine active site of KbMC 1 and 2. Preliminary results indicate an induction of full-length KbMC1 and 2 in stationary phase cultures, as well as activated p20 domain cleavage products, suggesting both an increase in metacaspase protein expression and activation in senescent cells. Caspase-like activities over a growth curve were characterized by quantifying the cleavage of fluorogenic canonical caspase tetrapeptides, and demonstrated that *K. brevis* exhibits a significant increase in activity during the transition into stationary phase and peaks in late stationary. Together these data indicate that *K. brevis* may be upregulating and utilizing PCD machinery during late senescence prior to culture demise. Further characterization of the involvement of metacaspases in cell death may lead to the identification of molecular biomarkers for *K. brevis* bloom termination.

*Funding provided by the NOAA Oceans and Human Health Initiative Pre-Doctoral Fellowship, NOAA National Ocean Service's Center for Coastal Environmental Health and Biomolecular Research -- Marine Biotoxins Program, and the Medical University of South Carolina -- Marine Biomedicine and Environmental Sciences Department.*