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Methodological Considerations and Comparisons of Measurement Results for Extracellular Proteolytic Enzyme Activities in Seawater

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Microbial extracellular hydrolytic enzymes that degrade organic matter in aquatic ecosystems play key roles in the biogeochemical carbon cycle. To provide linkages between hydrolytic enzyme activities and genomic or metabolomic studies in aquatic environments, reliable measurements are required for many samples at one time. Extracellular proteases are one of the most important classes of enzymes in aquatic microbial ecosystems, and protease activities in seawater are commonly measured using fluorogenic model substrates. Here, we examined several concerns for measurements of extracellular protease activities (aminopeptidases, and trypsin-type, and chymotrypsin-type activities) in seawater. Using a fluorometric microplate reader with low protein binding, 96-well microplates produced reliable enzymatic activity readings, while use of regular polystyrene microplates produced readings that showed significant underestimation, especially for trypsin-type proteases. From the results of kinetic experiments, this underestimation was thought to be attributable to the adsorption of both enzymes and substrates onto the microplate. We also examined solvent type and concentration in the working solution of oligopeptide-analog fluorogenic substrates using dimethyl sulfoxide (DMSO) and 2-methoxyethanol (MTXE). The results showed that both 2% (final concentration of solvent in the mixture of seawater sample and substrate working solution) DMSO and 2% MTXE provide similarly reliable data for most of the tested substrates, except for some substrates which did not dissolve completely in these assay conditions. Sample containers are also important to maintain the level of enzyme activity in natural seawater samples. In a small polypropylene containers (e.g., standard 50-mL centrifugal tube), protease activities in seawater sample rapidly decreased, and it caused underestimation of natural activities, especially for trypsin-type and chymotrypsin-type proteases. In conclusion, the materials and method for measurements should be carefully selected in order to accurately determine the activities of microbial extracellular hydrolytic enzymes in aquatic ecosystems; especially, low protein binding materials should be chosen to use at overall processes of the measurement.

Keywords: extracellular hydrolytic enzyme, protease, activity measurement, microbial loop, organic matter degradation, low protein binding microplate, MCA substrate

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