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Succession of protease activity in seawater and bacterial isolates during starvation in a mesocosm experiment

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ABSTRACT: Protein biodegradation in the marine environment is caused by proteases derived from various organisms, including bacteria, which are considered to be a major source of these enzymes. We investigated the succession of bacterial proteases in seawater to determine the variation in protease activity over time. The potential activities of proteolytic enzymes in stored seawater and isolated bacteria were studied using 19 different synthetic oligopeptide substrates for aminopeptidase, trypsin, chymotrypsin and elastase. In time-course experiments carried out over 112 d, aminopeptidase activity increased, whereas trypsin activity decreased over time. Aminopeptidase activity was mainly found in unfiltered seawater containing bacterial cells, whereas trypsin activity was mainly found in 0.2 µm seawater filtrates. Individual bacterial isolates showed different proteolytic properties but all exhibited aminopeptidase activity. Members of the *Gammaproteobacteria* and *Bacteroidetes* showed high trypsin and chymotrypsin activities. Based on these results, we conclude that protein degradation in seawater occurs via the combined action of various bacterial proteases.

KEY WORDS: Bacterial protease · Aminopeptidase · Trypsin · Cell-associated enzymes · Dissolved fraction · Seawater

INTRODUCTION

Heterotrophic bacteria are major degraders of organic matter in seawater and numerous studies have shown that they play an important role in carbon flux in the marine ecosystem (Cottrell & Kirchman 2000, Azam & Worden 2004, Nagata 2008). Most of the bioavailable dissolved organic matter in seawater is present as high molecular weight molecules (Benner 2002). These need to be hydrolyzed by extracellular enzymes prior to bacterial uptake, as transport of organic molecules across the bacterial cell membrane is limited to monomers or small polymers (Nikaido & Vaara 1985, Hoppe et al. 2002).

Proteins are important macromolecules for the structure and function of all living organisms. In the seawater environment, proteins comprise 5 to 20 % of dissolved organic nitrogen and 3 to 4 % of dissolved organic carbon (Sharp 1983, Thurman 1985) and are correlated with bacterial growth (Rosso & Azam 1987, Benner et al. 1992, Keil & Kirchman 1993). A wide variety of proteolytic enzymes are consistently found in the marine environment and play a role in the degradation and modification of marine proteins prior to cellular uptake (Hoppe et al. 2002).

Bacterial proteases are either released into the marine environment or present as ecto-enzymes (cell-associated forms). Previous studies have reported dissolved enzyme activity in seawater, as well as

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