



SIP-tracking of protein synthesis in anaerobic methanotrophic communities

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Black Sea microbial mats, capable of anaerobic oxidation of methane (AOM), were investigated to identify functional proteins, to shed light on the metabolic diversity in this consortium. We used ^{13}C and ^{15}N labelled substrates to follow their incorporation into functional proteins such as the mcrA-reductase (MCR) and the APS-reductase (APR). Both enzymes are believed to be key enzymes in anaerobic methanotrophy coupled to sulfate reduction. While the MCR is assumed to catalyse the initial methane attacking step, the APR is involved in the second step of sulfate reduction. For this, homogenates of ANME-1 or -2 dominated mats were fed with ^{15}N labelled ammonia, nitrate or dinitrogen. All substrates were incorporated in the bulk biomass in amounts of up to several atom percent during the incubation time of six months. Dinitrogen fixation could be observed only at lower levels. MCR subunits were the most prominent proteins in all incubations. In contrast, APR subunits were completely absent in incubations with nitrate as electron acceptor instead of sulfate. Using FPLC followed by elemental analysis, nitrogen incorporation into selected MCR-dominated fractions reached $\delta^{15}\text{N}$ values of 1758‰ for ANME-2 dominated black mats and 9‰ for ANME-1 dominated pink mats. The more active ANME-2 mats incorporated nitrogen substrates faster than the pink mats, where almost no incorporation was observed. RNA-based molecular analyses confirmed the presence of active nitrogen cycling microorganisms. The combination of different stable isotope labelled substrates thus allows us to identify key microbial processes and players in the complex mat systems, and to track the flow of carbon and nitrogen throughout the AOM community.