

Degradation of organic carbon of esters by microbial novel esterases in deep-sea sediments

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Microbial esterases play important roles in deep-sea organic carbon degradation and cycling, and many deep-sea esterases have evolved habitat-adapted characteristics, such as psychrophilic activity, halotolerance, and unique substrate specificity. Here, we isolated two novel esterases, H8 and Est22, from deep-sea sediments. H8 could effectively hydrolyze short-chain monoesters (C4-C10). H8 had a low optimal temperature (35°C) for activity and still remained 30% of the maximal activity at 0°C, suggesting that H8 was a cold-adapted enzyme. H8 had high salt tolerance, still stable in 4.5 M NaCl. Unlike reported halophilic/halotolerant enzymes with high acidic/basic residue ratios and low pI values, H8 contains a large number of basic residues, leading to its high basic/acidic residue ratio and high predicted pI (9.09). Mutational analysis suggested that basic residues play a role in the salt tolerance of H8. Est22 was a novel homoserine transacetylase (HTA)-like esterase. Est22 and its homologs could efficiently hydrolyze esters with acyl length of up to six carbons and had no transacetylase activity, different from HTAs and HTA-like acetyl esterases. By structural and mutational analysis of Est22 and its mutant L374D docked with *p*-nitrophenyl butyrate, key residues for substrate-binding and catalysis of Est22 are identified and found to be conserved in Est22 homologs. Comparative structural analysis indicated that differences in their catalytic tunnels lead to the different substrate specificities of Est22 and the other two HTA subfamilies. Phylogenetic analysis also showed that Est22 and its homologs form a separate branch in the HTA family. Altogether, due to their significant differences from the other two HTA subfamilies in sequence, substrate specificity and structure, Est22 and its homologs represent a new subfamily in the HTA family. Our study on deep-sea esterases sheds light on roles of these enzymes in deep-sea organic carbon degradation and cycling, and also on microbial adaptation to deep-sea environments.