The crystal structure of a novel Carbohydrate Esterase 7 family esterase from a hot desert metagenome

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The carbohydrate esterase (CE) 7 enzymes are known for their specificity for a broad range of carboxyl ester substrates [1, 2]. Most studies investigating the basis for substrate specificity and thermal characteristics have been carried out on a highly thermostable member of this family [3, 4] and little is known about the thermolability determinants of homologous enzymes. Here we describe the crystal structure of a novel CE7 acetyl xylan esterase designated NaM1. NaM1 was encoded by a 966bp gene (*NaMet1*) obtained via *in silico* bio-mining of a Namib Desert hypolith metagenome, followed by chemical synthesis of the full length gene. Following cloning and expression, His_6-tagged NaM1 was purified by cobalt-affinity and fast-pressure liquid chromatography to >95% purity. Protein crystals obtained from sitting-drop crystallization experiments yielded a 1.7 Å X-ray diffraction dataset, allowing the NaM1 structure to be solved by molecular replacement. Functional analysis revealed NaM1 to be a thermolabile enzyme with optimal activity at 35^{o}C (pH 8), the lowest reported for the CE7 family. NaM1 degraded p-nitrophenol acetate, p-NP butyrate, 7-aminocephalosporanic acid and acetylated xylan. The crystal structure provides a basis for comparing substrate specificities and thermolability in CE7 deacetylases.

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