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REVIEW

Differences and similarities in enzymes from the neopullulanase subfamily isolated from thermophilic species

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Abstract: Six glycoside hydrolase (GH) family 13 members, classified under the polyspecific neopullulanase subfamily GH13_20 (also termed cyclomaltodextrinase) were analysed. They originate from thermophilic bacterial strains (*Anoxybacillus flavithermus*, *Laceyella sacchari*, and *Geobacillus thermoleovorans*) or from environmental DNA, collected after *in situ* enrichments in Icelandic hot springs. The genes were isolated following the CODEHOP consensus primer strategy, utilizing the first two of the four conserved sequence regions in GH13. The typical domain structure of GH13_20, including an N-terminal domain (classified as CBM34), the catalytic module composed of the A- and B-domains, and a C-terminal domain, was found in five of the encoded enzymes (abbreviated Amy1, 89, 92, 98 and 132). These five enzymes degraded cyclomaltodextrins (CDs) and starch, while only three, Amy92 *(L. sacchari)*, Amy98 *(A. flavithermus)* and Amy132 (environmental DNA), also harboured neopullulanase activity. The *L. sacchari* enzyme was monomeric, but with CD as the preferred substrate, which is an unusual combination. The sixth enzyme (Amy29 from environmental DNA), was composed of the ABC-domains only. Preferred substrate for Amy29 was pullulan, which was degraded to panose, and the enzyme had no detectable activity on CDs. In addition to its different activity profile and domain composition, Amy29 also displayed a different conservation (LPKF) in the fifth conserved region (MPKL) proposed to identify the subfamily. All enzymes had apparent temperature optima in the range 50–65 °C, while thermostability varied, and was highest for Amy29 with a half-life of 480 min at 80 °C. Calcium dependent activity or stability was monitored in four enzymes, but could not be detected for Amy29 or 98. Tightly bound calcium can, however, not be ruled out, and putative calcium ligands were conserved in Amy98.

Key words: cyclomaltodextrinase; maltogenic amylase; neopullulanase; starch; environmental DNA, GH13; calcium.

Abbreviations: CBM, carbohydrate-binding module; CD, cyclomaltodextrin; CDase, cyclomaltodextrinase; CODEHOP, consensus degenerate hybrid oligonucleotide primer; GH, glycoside hydrolase; GH13_20, glycoside hydrolase family 13 subfamily 20; MAase, maltogenic amylase; NPase, neopullulanase.

Introduction

Cyclomaltodextrinases (CDase, EC 3.2.1.54), maltogenic amylases (MAase, EC 3.2.1.33) and neopullulanases (NPase, EC 3.2.1.35) are all members of the glycoside hydrolase family 13 (GH13) and have recently been classified under a common subfamily, GH13_20 (subfamily 20 of glycoside hydrolase family 13), termed either the neopullulanase subfamily or cyclomaltodextrinase, a subfamily which in turn is part of the α-amylase family (Park et al. 2000; Lee et al. 2002; Oslancova & Janecek, 2002; Stam et al. 2006). This polyspecific group of enzymes hydrolyzes at least two of the three substrates cyclomaltodextrins (CDs), pullulan and starch, but has also been shown to catalyze transglycosylation of oligosaccharides (Park et al. 2000, 2007). The classification is based on both the amino acid sequence as well as the substrate specificities (Park et al. 2000; Lee et al. 2002). Based on results from sequence alignment, and comparison of primary structures, four highly conserved regions in the catalytic $(\beta/\alpha)_8$-barrel domain have been found in the GH13 family (MacGregor et al. 2001). These various enzymes in the GH13 family can be utilized in many different industrial applications. For example panose (from neopullulanase degradation) is used as a sweetener, and maltogenic amylases can be used as an antistaling agent in bread and for production of maltose syrup (Ooshima et al. 1988; van der Maarel et al. 2002).

Of special interest for the substrate specificity is

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