Structure and function of -glucan debranching enzymes

Møller, Marie Sofie; Henriksen, Anette; Svensson, Birte

Published in:
Cellular and Molecular Life Sciences

Link to article, DOI:
[10.1007/s00018-016-2241-y](https://doi.org/10.1007/s00018-016-2241-y)

Publication date:
2016

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Møller, M. S., Henriksen, A., & Svensson, B. (2016). Structure and function of -glucan debranching enzymes. *Cellular and Molecular Life Sciences*, 73(14), 2619-2641. <https://doi.org/10.1007/s00018-016-2241-y>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Structure and function of α -glucan debranching enzymes

Marie Sofie MØLLER^{1,2}, Anette HENRIKSEN³, Birte SVENSSON¹

¹*Enzyme and Protein Chemistry, Department of Systems Biology, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.*

²*Center for Molecular Protein Science, Department of Chemistry, Lund University, 221 00 Lund, Sweden.*

³*Department of Large Protein Biophysics and Formulation, Global Research Unit, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark*

Corresponding author: Marie Sofie Møller, mariesofiemoller@gmail.com

Abstract

α -glucan debranching enzymes hydrolyse α -1,6-linkages in starch/glycogen, thereby playing a central role in energy metabolism in all living organisms. They belong to glycoside hydrolase families GH13 and GH57 and several of these enzymes are industrially important. Nine GH13 subfamilies include α -glucan debranching enzymes; isoamylase and glycogen debranching enzymes (GH13_11); pullulanase type I/limit dextrinase (GH13_12–14); pullulan hydrolase (GH13_20); bifunctional glycogen debranching enzyme (GH13_25); oligo- and glucan-1,6- α -glucosidases (GH13_31); pullulanase type II (GH13_39); and α -amylase domains (GH13_41) in two-domain amylase-pullulanases. GH57 harbours type II pullulanases. Specificity differences, domain organisation, carbohydrate binding modules, sequence motifs, three-dimensional structures and specificity determinants are discussed.

The phylogenetic analysis indicated that GH13_39 enzymes could represent a “missing link” between the strictly α -1,6-specific debranching enzymes and the enzymes with dual specificity and α -1,4-linkage preference.

Keywords: Substrate specificity; glycoside hydrolase family 13 subfamilies, domain architecture; multi-domain three-dimensional structure; sequence motifs and determinants; structure-function relationship; carbohydrate binding modules; phylogeny.

Abbreviations: CAZy, Carbohydrate-Active enZymes; CBM, carbohydrate binding module; CD, cyclodextrin; CDD, conserved domain database; GDE, glycogen debranching enzyme; GH, glycoside hydrolase; GHx, glycoside hydrolase family x; GH13_x, glycoside hydrolase family 13 subfamily x; ISA, isoamylase; PULI, type I pullulanase; PULII, type II pullulanase; PULII_I, type II pullulanase with one catalytic domain; PULII_II, type II pullulanase with two catalytic domains; SBS, starch binding site; SEX4, starch-excess protein-4.

Running title: α -glucan debranching enzymes

Introduction

α -glucan debranching enzymes are an important group of glycoside hydrolases playing a role in the energy metabolism of all living organisms by hydrolysis of α -1,6-glucosidic linkages in α -glucans. The term *α -glucans* here refers to polysaccharides consisting of glucose units connected by α -1,4-glucosidic linkages and which can contain α -1,6-branch points. Amylopectin and glycogen are the dominating branched α -glucans in living organisms, while the linear pullulan molecule produced by the fungus *Aureobasidium pullulans* resembles the branched α -glucans as it consists of α -1,4-linked maltotriose repeats connected by α -1,6-linkages (Fig. 1) [1]. Amylopectin, the major component of starch, is made up of hundreds of shorter α -1,4-glucan chains connected by α -1,6-branch points. Amylopectin is organised together with amylose, an essentially linear α -glucan, in the semi-crystalline starch granules (see [2] for a recent review on starch). Glycogen is the animal, fungal and bacterial counterpart to amylopectin, but is more extensively branched [3].

Starch and glycogen constitute the major energy source and storage in higher plants and microorganisms/animals, respectively. Generally, α -glucans are degraded in a series of reactions catalysed by several enzymes hydrolysing either α -1,4- and/or α -1,6-glucosidic linkages. α -1,6-linkages are hydrolysed by glycogen debranching enzymes (GDEs; EC 3.2.1.68 and 3.2.1.33), isoamylases (ISAs; EC 3.2.1.68), and type I pullulanases (PULIs; EC 3.2.1.41) (Table 1). Furthermore, type II pullulanases (PULII; also known as amylopullulanases) show α -glucan debranching activity along with endo- α -1,4-glucan hydrolase (α -amylase) activity (EC 3.2.1.1 and 3.2.1.41), i.e. they hydrolyse both α -1,4- and α -1,6-linkages in α -glucans (Table 1). In addition to these classical α -glucan debranching enzymes, we will briefly cover the so-called oligo- and glucan-1,6- α -glucosidases (EC 3.2.1.10), hydrolysing α -1,6-linkages in isomaltooligosaccharides and dextran – an essentially linear α -1,6-glucan, together with the pullulan hydrolases (EC 3.2.1.133 and 3.2.1.135), catalysing hydrolysis of α -1,6- and/or α -1,4-glucosidic linkages in pullulan and starch.

The present review has emphasis on structure-function relationships, protein sequences and structural features important for substrate specificity of α -glucan debranching enzymes. This includes an overview of the modular architecture and detailed reports on catalytic domains and carbohydrate binding modules (CBMs) of different enzymatically characterised and structure-determined α -glucan debranching enzymes. A bioinformatics analysis covers conserved regions shared among all the glycoside hydrolase family 13 (GH13) and GH57 enzymes, and signature sequence regions that distinguish specific classes of α -glucan debranching enzymes.

Classification of α -glucan debranching enzymes

α -glucan debranching enzymes belong to two glycoside hydrolase families, GH13 and GH57, according to the Carbohydrate Active Enzymes (CAZy) database classification system (<http://www.cazy.org/>) that assigns enzymes and CBMs into families sharing structural fold and stereochemistry of the catalytic mechanism. The classification is based on the protein sequence [4].

GH13, also known as the α -amylase family, is one of the largest GH-families in CAZy with more than 28,300 protein sequences. GH13 features 22 different substrate specificities involving α -glucosidic linkages including hydrolases, transglucosidases and isomerases [5]. Overall, GH13 members have very limited sequence similarity. Thus, only four short sequence regions are conserved (see below), and different substrate specificities can have different signature motifs [6]. All GH13 enzymes are retaining enzymes and have a catalytic TIM barrel ((β/α)₈-barrel) domain [7] with three invariant catalytic site residues: Asp (nucleophile/base), Glu (proton donor) and an Asp participating in distortion and stabilisation of the transition state. GH13 has been divided into subfamilies using a combination of cluster analysis, sequence similarity and phylogeny that reflect correlation of protein sequences and substrate specificities [5]. The α -glucan debranching catalytic domains occur in eight GH13 subfamilies (Table 1): GH13_11, ISAs and GDEs; GH13_12–14, PULIs; GH13_20, pullulan hydrolases; GH13_25, bifunctional GDEs from eukaryotes; GH13_31, oligo- and glucan-1,6- α -glucosidases; and GH13_39, PULIIs from extremophiles; while GH13_41 contains α -amylase domains of amylase-pullulanases (see below) having two separate catalytic domains of which the pullulanase domains belong to GH13_12 and 14 or are not yet assigned to a subfamily. Crystal structures are available for the mentioned subfamilies except GH13_25, GH13_39, and GH13_41 (Table 1).

The enzyme specificities of GH57 include α -amylase, amylopullulanase (PULII), branching enzyme, 4- α -glucanotransferase, and α -galactosidase [8]. Currently, GH57 includes 1345 members from archaea (1/4) and bacteria (3/4) (mostly extremophiles) [4]. Not all members are expected to be active enzymes, since some of the sequences lack one or both catalytic residues [9]. GH57 enzymes representing the different enzyme specificities have been biochemically characterised [8]. Among these are several PULIIs (see section below). Four GH57 enzymes have been structure determined (an α -amylase, a 4- α -glucanotransferease, and two branching enzymes) [4]. A fifth enzyme referred to as a GH57 maltose-forming α -amylase has been structure determined (PDB entry 4CMR) [10], however it is non-classified in CAZy [4]. The GH57 enzymes have a catalytic (β/α)₇-barrel domain and use a retaining mechanism with Glu and Asp as nucleophile/base and proton donor, respectively [11, 12]. The sequences show five conserved regions, and it has been suggested to subdivide GH57 to reflect enzyme specificities [11, 13, 14]. A search in the conserved domain database (CDD) [15] including all full-length GH57 protein sequences (1283) gave 383 with a PULII domain (domain classification in CDD: GH57N_APU, sequence cluster: cd10796 and domain: GH57N_APU_like_1, sequence cluster cd10797), therefore they most likely have PULII specificity.

Isoamylases and glycogen debranching enzymes

GH13_11 enzymes

A large diversity is found within the subfamilies of GH13. This is particularly true for GH13_11 that is formed by a very diverse group of α -glucan debranching enzymes and GH13_11 has been suggested to be further subdivided. The specificities in GH13_11 are ISAs and GDEs hydrolysing α -1,6-branch points in amylopectin, glycogen, and products thereof generated by the action of β -amylase (β -limit dextrins) [16]. Unlike

pullulanases, enzymes in GH13_11 do not hydrolyse α -1,6-glucosidic linkages in pullulan, but require an α -1,6-linked branch on an α -1,4-glucan main chain for activity. ISAs from plants, algae and the Gram-negative soil bacterium *Pseudomonas amyloclavata* often encode more than one isoform which may operate in multimeric complexes composed of different ISA isoforms [17, 18]. The GDEs are widespread among bacteria and an α -helix (helix 4) situated near the active site can serve as a useful signature to distinguish GDEs from ISAs and pullulanases [19]. Furthermore, a loop (loop 4) might define substrate preference; thus a short loop 4 is associated with preference for limit dextrans, while a longer loop 4 indicates specificity for larger branched α -glucans [17, 20]. This feature, however, might not adequately capture ISAs and GDEs, as variation in loop 4 length is observed among ISA isoforms from the same organism [17].

GH13_11 structures

Four GH13_11 enzymes are structure-determined (Table 1): ISAs from a bacterium; *Pseudomonas amyloclavata* (UniProt P10342) and a eukaryote; *Chlamydomonas reinhardtii* (GenBank AAP85534), and GDEs from *Escherichia coli* and *Sulfolobus solfataricus*. Five more GH13_11 members have been enzymatically characterised: GDEs from *Sulfolobus acidocaldarius* (BAA11864) [21], *Sulfolobus shibatae* (AAM81590) [22], *Corynebacterium glutamicum* (BAB99500) [23] and *Flavobacterium* sp. (AAB63356) [24], and an ISA from *Oryza sativa* (BAA29041) [25].

The GH13_11 crystal structures display the same overall multimodular organisation: an N-terminal carbohydrate-binding module family 48 (CBM48) (see section below), the catalytic domain, and a C-terminal β -sandwich domain (Fig. 2). Most GH13 enzymes contain this C-terminal β -sandwich domain. Its function in relation to debranching is unknown, but C-terminal domains in other GH13 enzymes *e.g.* GH13 branching enzymes, are shown to be involved in substrate binding [6, 26, 27] and/or shielding hydrophobic residues of the catalytic domain from solvent contacts [28]. In GH13_11 ISA from *Chlamydomonas reinhardtii* the C-terminal domain participates in dimerization [16].

The GH13_11 crystal structures illustrate structural and substrate specificity diversity within GH13_11 [19, 29, 30]. Noticeably, they show different oligomerization states: *Pseudomonas amyloclavata* and *Chlamydomonas reinhardtii* ISAs being a functional α -1,6-glucosidase monomer and dimer, respectively. Oligomerisation is also important for activity of *Sulfolobus solfataricus* GDE, that has both α -1,6-glucosidase and α -1,4-transferase activity, where the latter is related to the oligomerisation state, since the tetramer showed α -1,4-transferase activity, while the dimer did not [30].

The crystal structure of *Chlamydomonas reinhardtii* in complex with maltoheptaose (PDB entry 4OKD) revealed the presence of two surface binding sites (1 and 2), which are non-catalytic carbohydrate binding sites found on the surface of the catalytic domain that interact with sugar rings via stacking interactions with aromatic residues [31]. These sites, are located at the reducing end of the active site cleft and at the interface of the catalytic and C-terminal domains [16]. The two tryptophans forming surface binding site 1 (Trp757 and Trp 767) are conserved in all GH13_11 protein sequences based on a multiple sequence alignment of catalytic domains, while surface binding site 2 (His794, Trp652 and Trp856) is not conserved.

GH13_25 glycogen debranching enzymes

The GH13_25 GDEs are eukaryotic [4], bifunctional enzymes exhibiting both transferase (EC 2.4.1.25) and glucosidase (EC 3.2.1.33) activities [32] residing in two separate active sites. The reactions are thought to be channelled from one active site to the other: First, a maltosyl or maltotriosyl residue from a glycogen branch is transferred to the main chain by the transferase active site exposing an α -1,6-glucosyl stub that in turn is cleaved off at the glucosidase active site. The transferase reaction is retaining, while the glucosidase reaction is inverting [33, 34]. There is no published structure of a GH13_25 GDE, but the transferase part of the enzymes is predicted to belong to the GH13 (β/α)₈-barrel catalytic domain fold and have the three invariant GH13 catalytic site residues [34]. The glucosidase part has been classified into GH133, a subfamily of inverting α -1,6-glucosidases [4]. The enzymes from the other GH13 subfamilies included here and from GH57 are all retaining enzymes. Hence GH13_25 GDEs belong to a highly specialised and atypical group of α -glucan debranching enzymes. Due to the different characteristics mentioned above and lack of a 3D structure, this review will not cover GH13_25 in detail.

Pullulanases

Pullulanases are important industrial enzymes (see the section below on application), which motivated characterisation of a large number of pullulanases (see Tables S1 and S2 for tables with specific activity and kinetic parameters of pullulanases). An immense amount of published data includes enzymes characterised at the protein level to be pullulanases, but which have no published protein sequence. Enzymes without sequence information, however, have not been included in the present review.

Pullulanases are classified as type I (PULI) and type II (PULII) depending on the specificity. PULIs hydrolyse α -1,6-branch linkages in amylopectin, β -limit dextrin derived from amylopectin, and pullulan. Some PULIs also have low activity on glycogen (Tables S1 and S2). By contrast PULIIs hydrolyse both α -1,4- and α -1,6-glucosidic linkages in starch and pullulan, albeit some PULIIs only hydrolyse one of the linkage types in pullulan. PULIIs form two subgroups; one with one catalytic domain hydrolysing both bond types, and another having two catalytic domains, one being an α -amylase domain that hydrolyses α -1,4-linkages, and one PULI domain hydrolysing α -1,6-linkages. It has been suggested to formally divide PULIIs into these subgroups [35], but lack of general acceptance renders PULII literature confusing with regard to characteristic general and specialised PULII features. PULIIs are also called amylo- or amylase-pullulanases, which appears appropriate for the two-domain PULIIs, but amylase-pullulanase has been used for both PULII types. Here, we refer to PULIIs having one and two catalytic domains as PULII_I and PULII_II, respectively. In CAZy, the different pullulanases have EC number 3.2.1.41, but where dual-bond type specificity is shown the α -amylase EC 3.2.1.1 is also given. Thus, the PULII_IIs may get typecast in GH13 both as a pullulanase and an amylase subfamily member [4].

In summary, pullulanases are found in five different GH13 subfamilies (12, 13, 14, 39, and 41) and in GH57. The PULIs, *i.e.* enzymes with α -1,6-activity only, are in subfamilies GH13_12–14, while PULIIs cleaving both α -1,4- and α -1,6-glucosidic linkages are found in all five GH13 subfamilies and in GH57.

Type I pullulanases

Pullulanases from firmicutes, especially streptococci, generally belong to GH13_12, while GH13_13 contains pullulanases from plants (eukaryotes) and bacteria mainly found in soil and water, including photosynthetic bacteria. Plant pullulanases are historically called limit dextrinases, referring to their substrate preference for limit dextrans, the hydrolysis products of amylopectin, over amylopectin. GH13_13 appears as a branch between two GH13_14 branches of the phylogenetic tree of the GH13 subfamilies reflecting that these subfamilies are closely related, but distinct. The main difference between them is in the length of three loops [5]. GH13_14 contains pullulanases from a range of bacteria, including bacilli. Besides the variations in loop lengths and additional inserts (see below), which are mainly phylogenetic issues, major differences between the three pullulanase subfamilies appear in the auxiliary domains associated with the catalytic domains. These differences together with other small differences in the catalytic domains reflect individual substrate preferences (Tables S1 and S2).

Pullulanase type I structures

Only PULIs are represented by crystal structures (Table 1). Despite the overall high structural similarity among the multi-domain GH13_12–14 PULIs (Fig. 2), sequence identities and similarities of pullulanases are low even for the catalytic domains. Accordingly, the GH13_12–14 enzymes differ in substrate specificity and preferences. In addition to the catalytic (β/α)₈-barrel characteristic of GH13 [7], the typical pullulanase architecture has one or more N-terminal domains, including CBMs (see below) and a C-terminal domain common for most GH13 enzymes (see above). These auxiliary domains are associated with specificity differences between the three PULI GH13 subfamilies. Unfortunately, one or more N-terminal domains are missing from some of the GH13_12 and GH13_14 structures (Fig. 2). Recently, the first structure has been determined of a branched substrate in complex with a PULI, namely barley limit dextrinase (PDB entry 4J3W, Table 2) [36].

Type II pullulanases

PULIIs are a very diverse group of enzymes with regard to enzymatic properties (see [37] for a comprehensive review). The PULIIs are multi-modular GH13 and GH57 enzymes having one or more CBMs in addition to the catalytic domain(s). PULIIs are not straightforward to classify in the CAZy GH13 subfamily system. In some cases, a PULII_II is assigned both to a PULI subfamily (GH13_12–14) and an α -amylase subfamily. Some PULII_Is were assigned their own subfamily, GH13_39, and very recently, 44 PULII_IIs are classified in GH13_41 governed by their amylase domain together with 11 subfamily members, having only an amylase domain. Of these PULII_IIs the pullulanase domain in 34 sequences is classified in GH13_12, and in 2

sequences in GH13_14, while 8 were not assigned to a GH13 subfamily. The majority of the GH13_12+41 PULII_IIs are from *Streptococcus suis* strains.

Enzymatic properties are only reported for four PULIIs from GH13_12–14: one PULII_I from *Lactobacillus plantarum* (GH13_14; BAF93906) [38, 39], and PULII_IIs from *Streptococcus suis* (GH13_12; CAR47543; only the amylase domain was characterised), *Bifidobacterium breve* (GH13_14 and GH13_32; AAY89038) and *Bacillus* sp. KSM-1378 (GH13_12 and GH13_41; AAS36537) [40–42]. Very recently an α -glucan debranching enzyme from an uncultured bacterium originating from the gut of *Hermetia illucens* has been characterised [43]. It is partially sequenced (GenBank AGL50935), and proposed to be a PULII_II based on the enzymatic characterisation and CDD information [15, 43]. Its amylase domain is classified into the α -amylase subfamily GH13_28, while the pullulanase domain is not in a subfamily [4]. Finally, another characterised α -glucan debranching enzyme from *Thermococcus kodakarensis* (BAD85166) was not classified to a GH13 subfamily [4]. It was previously referred to as a PULII [44], but recently suggested to be a pullulan hydrolase type III (see below) [45].

GH13_39 is a small PULII subfamily of 62 full-length protein sequences from bacteria, mainly thermophiles. They have a single catalytic site as based on a CDD search for hydrolysing both α -1,4- and α -1,6-linkages [15]. GH13_39 enzymes, however, are not uniform with regard to number and types of domains additional to the GH13 catalytic domain (see section on CBMs below). Interestingly, very recently six protein sequences having both a GH13 and a GH77 catalytic domain (GH77 harbours to specificities: amylomaltase and 4- α -glucanotransferase) were added to GH13_39 [4]. This novel type of proteins has not yet been characterised. No GH13_39 PULIIs structure is available, but GH13_39 enzymes from *Alicyclobacillus*, *Bacillus*, *Geobacillus*, *Thermoanaerobacter* and *Thermoanaerobacterium* have been characterised [46–58]. For some of these the substrate bond specificity is not definite. Some are referred to as a “high-molecular amylase” in older literature, although they all hydrolyse both pullulan and starch. GH13_39 from *Alicyclobacillus* (ADZ99363) is described as an α -amylase [47]. PULII_I from *Geobacillus thermoleovorans* is one of the best-characterised GH13_39 members. It releases maltose, maltotriose and maltotetraose from soluble starch, glycogen, amylose and amylopectin, but only maltotriose from pullulan. Moreover, maltotriose and maltose were end-products of maltopentaose hydrolysis [51]. The PULII_Is from *Bacillus* sp. XAL601, *Thermoanaerobacterium saccharolyticum* and *Thermoanaerobacter pseudoethanolicus* released maltose, maltotriose, and maltotetraose from starch, but only maltotriose from pullulan [48, 53, 55]. Mutational analysis confirmed that the *Thermoanaerobacter pseudoethanolicus* enzyme has a single catalytic site responsible for both α -1,4- and α -1,6-linkage specificity [54].

Generally, PULII_Is are only able to hydrolyse pullulan α -1,6-linkages, while PULII_IIs can hydrolyse both α -1,4- and α -1,6-linkages in pullulan [38, 39, 48, 51, 53]. However, PULII_I from *Anoxybacillus* sp. SK3-4 (GenBank EPZ37192) is an exception, since the degradation products from pullulan is a mix of glucose, maltose, and maltotriose [59], and the enzyme has not been included in CAZy. It preferably cleaves α -1,6- rather than α -1,4-glucosidic bonds in pullulan [59].

GH57 type II pullulanases

GH57 PULII_Is have been characterised from several extreme thermophilic archaea: *Caldivirga* [60], *Pyrobaculum* [61], *Pyrococcus* [62], *Sulfolobus acidocaldarius* [63], *Staphylothermus* [64], and *Thermococcus* [45, 65-67]. None of these GH57 PULII_I members have been structure-determined, but they are predicted to have a single catalytic site with dual specificity for α -1,4- and α -1,6-linkages, and no known CBMs associated with the catalytic domain [4, 15]. However, a CDD-search with the characterised GH57 PULII_Is indicated the presence of several additional domains [15]. Remarkably, *Thermococcus siculi* PULII_I is suggested to possess two CBM9-like domains usually found at the C-terminus of xylanases [67].

GH13_20 – Maltogenic α -amylases, neopullulanases, and cyclomaltodextrinases

At least 29 GH13_20 members have been characterised. They cover different substrate and linkage specificities and include maltogenic α -amylase (EC 3.2.1.133), neopullulanase (EC 3.2.1.135), and cyclomaltodextrinase (EC 3.2.1.54) (see [68] for a comprehensive review). There seems not to be any consensus in the naming of GH13_20 enzymes which in the literature appears as being close to anarchistic. Lee et al. suggested that the above three groups of GH13_20 enzymes should be perceived as one, because they are not sufficiently different to keep individual names and enzyme codes [69].

Generally, the major substrates for GH13_20 are cyclodextrins (CD; cyclic α -1,4-linked glucose oligosaccharides), pullulan and starch. GH13_20 enzymes hydrolyse α -1,4- and α -1,6-glucosidic linkages, but not necessarily equally well. The maltogenic α -amylases prefer CDs and hydrolyse β -CDs (seven glucose units) ~100 times faster than starch or pullulan [70]. Their main product from hydrolysis is maltose. By contrast, GH13 α -amylases having strict endo-activity towards α -1,4-linkages cannot hydrolyse CDs or pullulan. GH13_20 maltogenic amylase moreover attacks α -1,4- much more efficiently than α -1,6-linkages [71]. Most of the characterised GH13_20 enzymes catalyse transglucosylation besides their glucosidase activity [68]. One of the outliers among the characterised GH13_20 enzymes is the structure-determined *Nostoc punctiforme* enzyme, which prefers longer malto-oligosaccharides (>G8) and performs hydrolysis without transglucosylation or CD-hydrolysing activities common for the GH13_20 enzymes [72]. Dimerisation has been shown for a number of GH13_20 enzymes to be indispensable for activity [69, 72-75] and some exist in a monomer/dimer equilibrium in solution [68].

GH13_20 enzymes are also called pullulan hydrolases and divided into groups depending on specificity: Pullulan hydrolases types I and II only hydrolyse α -1,4- and not α -1,6-linkages in pullulan. The final product is either panose (of pullulan hydrolase type I) or isopanose (of pullulan hydrolase type II). Pullulan hydrolase type III is a unique enzyme capable of hydrolysing both α -1,4 and α -1,6 glucosidic linkages in pullulan, and its final reaction products are a mixture of maltose, panose, and maltotriose [76].

Four GH13_20 have been structure-determined: a neopullulanase from *Geobacillus stearothermophilus*, α -amylase 2 from *Thermoactinomyces vulgaris* R-47 (TVAII), cyclomaltodextrinase from *Thermus* sp. IM6501 and debranching enzyme from *Nostoc punctiforme* (Table 1).

Oligo- and glucan 1,6- α -glucosidases

GH13_31 encompasses isomaltulose synthases (EC 5.4.99.11), α -glucosidases (EC 3.2.1.20), oligo- and glucan- α -1,6-glucosidases (EC 3.2.1.10 and 3.2.1.70). Oligo- and glucan- α -1,6-glucosidases hydrolyse α -1,6-linkages but differ in substrate size preference: i) glucan 1,6- α -glucosidases (G16G) favour isomaltooligosaccharides (glucose units connected by α -1,6-linkages) longer than the disaccharide isomaltose and are active on dextran (a linear α -glucan composed of mainly α -1,6-linked glucose units) and ii) oligo-1,6- α -glucosidases (O16G) prefer short isomaltooligosaccharides and have highest activity on isomaltose, but are inactive on dextran. Each type of enzyme is highly selective and shows little or no activity for other than α -1,6-linkages [77]. The GH13_31 members are unique in α -glucan debranching, since they primarily act on the linear α -glucan, dextran, but in addition can hydrolyse the α -1,6-linkage in panose (α -D-Glc-(1,6)- α -D-Glc-(1,4)-D-Glc) [78]. Pullulan, starch, or starch derived oligosaccharides are not hydrolysed by GH13_31 enzymes. Crystal structures are available of oligo- and glucan- α -1,6-glucosidases from *Bacillus cereus* (O16G), *Lactobacillus acidophilus* (G16G), and *Streptococcus mutans* (G16G) (Table 1).

Carbohydrate binding modules (CBMs) in relation to α -glucan debranching enzymes

A CBM is defined as a contiguous amino acid sequence within a carbohydrate-active enzyme with a discrete fold having carbohydrate-binding activity (www.cazy.org/Carbohydrate-Binding-Modules). The consensus function of such auxiliary domains is to bind polysaccharides and bring the catalytic domain in intimate contact with substrate, thus advancing carbohydrate hydrolysis. CBMs are grouped into 71 families according to CAZy [4] and possess a range of polysaccharide binding specificities. Several N-domains in α -glucan debranching enzymes have been identified as CBMs. Carbohydrate binding of most of the N-terminal domains has not been verified experimentally, but they share fold with functionally characterized CBMs [79]. The recognition of polysaccharides by CBMs plays a role in relation to metabolism, pathogen defence, polysaccharide biosynthesis, virulence, plant development etc. [80, 81]. In PULIs, CBMs are shown to be important for substrate specificity and catalytic efficiency [82].

Various CBMs occur in α -glucan debranching enzymes, especially PULIs and PULIIs contain several different CBMs. The most prevalent CBMs among PULIs and PULIIs of GH13_12–14 are CBM41 and CBM48. Recently, one of the N-domains associated with GH13_14 enzymes (previously known as X45) was classified to the new family, CBM68 (Fig. 2) [83]. GH13_12–14 members also have N-domains with unknown function in addition to known CBMs. An N-terminal domain of barley limit dextrinase (PULI) from GH13_13 resembles a CBM21 starch-binding domain based on structural homology (Fig. 2) [79]. The N-domain of a sorghum limit dextrinase has surprising impact on digestibility of sorghum starch [84]. GH13_11 enzymes have only one CBM, CBM48 also seen in the pullulanases (Fig. 2). According to CAZy, all GH13_39 have a CBM34 some have even two, other associated CBMs differ among GH13_39 members. CBM20 is thus found in characterised GH13_39 enzymes. The *Geobacillus stearothermophilus* GH13_39 even has a CBM48 [4],

and GH13_39 members have an N-domain (X25/N1) that resembles the N-domain in the structure of *Bacillus acidopullulyticus* GH13_14 PULI for which there is a structure (Fig. 2). N-terminal truncation of *Geobacillus thermoleovorans* GH13_39 PULII_I revealed this N-domain to be important in starch binding. Based on conserved amino acid residues at starch binding sites (SBSs) 1 and 2 of this domain, a relationship was suggested to CBM20, CBM48 and CBM69 [52]. Finally, some GH13_20s have CBM34 and GH13_31 enzymes lack CBMs. Generally, GH57 PULII_Is do not have CBMs, although two GH57 PULII_Is have two CBM20s (ACR80150 and AEX85885) [4, 85], and one GH57 PULII_I is annotated to have two CBM48s (ABR30664) [4].

CBM20

The CBM20s are known as classical C-terminal starch binding domains in microbial glucoamylases and amylases, and is one of the most thoroughly studied CBM families (see [85, 86] for reviews). CBM20 is mainly associated with starch-active proteins from GH families 13, 14, 15, and 77, but a few more GH families (14, 31, 57, 97, and 119) also contain CBM20. Furthermore, CBM20 is found in glucan phosphatases like the mammalian laforin and starch-excess protein-4 (SEX4) required for normal α -glucan breakdown, and the two starch phosphorylating enzymes glucan, water dikinase and phosphoglucan, water dikinase [4, 85, 87]. CBM20s have two sites capable of binding granular starch [85, 88, 89]. In a very recent study of GH13_39 PULII_I from *Thermoanaerobacter pseudoethanolicus* (ABY95795) the CBM20 lacked SBS2, showing that the presence of two starch binding sites is not a must for CBM20 function, since this specific domain still affect the *Thermoanaerobacter pseudoethanolicus* PULII_I activity on amylopectin and starch granules [90].

None of the CBM20s associated with α -glucan debranching enzymes have been structure-determined, but CBM20 structures are solved of enzymes from 14 organisms representing 6 different enzyme specificities: β -amylase (GH14; EC 3.2.1.2), β -cyclodextrin glucanotransferase (GH13_2; EC 2.4.1.19), maltogenic α -amylase (Novamyl; GH13_2; EC 3.2.1.133), glucoamylase (GH15; EC 3.2.1.3), laforin, and glycerophosphodiester phosphodiesterase [4]. These include the NMR structure of *Aspergillus niger* glucoamylase CBM20 in complex with β -CD (PDB entry 1AC0) having two structurally and functionally independent binding sites. It is proposed that the first is for initial starch recognition, whereas the second site has higher carbohydrate affinity and locks CBM20 into place. A model structure showing two α -glucan chains bound to CBM20 in perpendicular orientation, suggests that the CBM20 may force starch strands apart thus increasing the surface accessibility or localise the enzyme to non-crystalline areas of the starch granule [89]. Recently, CBM20 of human “starch-binding domain-containing protein 1” was found to be important for the protein stability and ability to interact with glycogen-associated proteins like glycogen synthase, glycogen debranching enzyme and laforin [90]. The “starch-binding domain-containing protein 1” is able to bind glycogen, and its CBM20 has been demonstrated to bind amylose [90].

CBM34

CBM34 has only been found associated with GH13 and GH77 proteins. Six CBM34 structures are solved: two in α -amylases from *Thermoactinomyces vulgaris* R-47 (TVAI, GH13_21; TVAII, GH13_20, Table 1), one in

amylase from *Pyrococcus furiosus* (no GH13 subfamily; PDB entry 4AEF) [91], and three in GH13_20 enzymes from *Bacillus*, *Geobacillus* and *Thermus* (Table 1). The CBM34 and CBM21 β -sheets share topology [92]. Raw starch-binding has been demonstrated in the case of the N-terminal domain of *Thermoactinomyces vulgaris* α -amylase TVAI. The crucial Trp65 [93] of TVAI has no obvious counterpart in TVAII despite pronounced sequence similarity with the TVAI N-domain [93]. However, the N-domain of TVAII has been shown to play an essential role in activity as well as dimer formation [93].

CBM41

CBM41 is only found with GH13 pullulanases. The tandem CBM41s of PULIs from streptococci are well-described and attach the bacterium to glycogen reserves in alveolar cells enabling polysaccharide degradation by the pathogen; hence these CBM41s are referred to as virulence factors [94]. Remarkably, one of the two CBM41s in the N-terminal region of *Streptococcus pneumoniae* pullulanase forms part of the active site, and has an important role in activity [82]. Both crystallography, small-angle X-ray scattering (SAXS) and truncation data support this CBM41-active site coupling. The second CBM41 and most distal to the catalytic domain (Fig. 2) is proposed to adhere to glycogen particles [82, 95]. Kinetic analysis of *Streptococcus pneumoniae* pullulanase lacking both the N-terminal domain and both CBM41s, showed this truncated pullulanase to be roughly half as effective as wild-type in releasing maltooligosaccharides from glycogen, which is its *in vivo* function [82]. Furthermore, *Streptococcus agalactiae* pullulanase without the two CBM41s was less adhesive to human cervical epithelial cells than the full-length enzyme, supporting that the carbohydrate binding motifs of the CBM41s mediate adhesion [96]. CBM41 in GH13_14 PULI from *Thermotoga maritima* (AAD36907) is also well-characterised member. It served as a model for CBM41 α -glucan binding as it was the first structure-determined CBM41 (PDB entry 2J72) [97]. It was shown to bind tightly to α -1,4-glucans, α -1,4-glucooligosaccharides, mixed α -1,4-1,6-glucooligosaccharides, and pullulan [82, 97, 98]. Two of the PULIs have been structure-determined with maltooligosaccharides bound to their CBM41(s) (PDB entries 2YA1, 2FHC, and 2FHF; Table 1).

CBM48

CBM48s and the starch-binding CBM20 (see above) are closely related, but CBM48s has only one SBS (corresponding to CBM20 SBS1), whereas CBM20 generally has two SBSs [85]. CBM48 is mostly seen in GH13 subfamilies acting on branched substrates: glycogen and starch branching enzymes (GH13_8–9), GDEs and ISAs (GH13_11), PULIs and PULIIs (GH13_12–14), and cyclomaltodextrinases (GH13_20) [99]. Furthermore, CBM48s are also present in GH10, 26, 43, 57 and 77, in the β -subunit (glycogen-binding) of AMP-activated protein kinases, and in the glucan phosphatase SEX4 [4]. The CBM48s from AMP-activated protein kinases are among the best studied [100–102]. Crystal structures of AMP-activated kinase (PDB entry 1Z0M) and starch phosphatase DSP4 (PDB entry 4PYH) showed glucan binding to CBM48 [100, 103]. A recently characterised CBM48 forms a part of Arabidopsis Protein Targeting to Starch (PTST) required for amylose biosynthesis. The CBM48 mediates interaction of PTST with starch granules, and also secures correct localisation of granule-bound starch synthase (GBSS) in Arabidopsis [104].

In most CBM48s from GH13_11–14, the residues predicted to constitute the canonical SBS1 are not unambiguously conserved. Indeed, carbohydrate binding to CBM48 from ISA, GDEs, and pullulanases has not been demonstrated experimentally, but CBM48s from these enzymes possess a conserved tryptophan corresponding to Trp563 of the CBM20 SBS2 site in *Aspergillus niger* glucoamylase, suggesting that these sequences may be intermediates between CBM20 and CBM48 rather than being CBM48 [85]. By contrast CBM48 of branching enzymes binds glucans in crystal structures, thus maltopentaose is accommodated by CBM48 of rice branching enzyme (PDB entry 3VU2). The occupied binding sites were shown to be conserved in other starch branching enzymes [105]. Likewise, the crystal structure of the *Escherichia coli* branching enzyme solved in complex with maltooligosaccharides (PDB entries 4LQ1 and 4LPC) had maltose bound to its conserved CBM48 SBS [106].

CBM68

Recently, GH13_14 *Anoxybacillus* PULI was realised to contain a new N-terminal CBM [83] classified as CBM68 in CAZy [4]. Its binding function is deduced from crystal structures; CBM deletion caused reduced thermostability and specific activity, and increased K_m [83]. Two *Anoxybacillus* structures (PDB entries 3WDI and 3WDJ) had parallel bound maltooligosaccharides accommodated close to each other between the N-terminal and the catalytic domains. These structures elicited assignment of the N-terminal domain as CBM68 with specificity for maltotriose and maltotetraose [83], and in *Bacillus acidopullulyticus* GH13_14 pullulanase the N-terminal domain previously known as X45 was classified as CBM68 [4]. CBM68 has so far only been seen in pullulanases, at present in 206 sequences [4].

α -glucan debranching enzymes similarities and differences at sequence and structure level

Phylogenetic analysis

Phylogenetic analysis of GH13 α -glucan debranching enzymes shows that PULIs in subfamilies GH13_12–14 are closely related with GH13_13 and 14 enzymes forming their own cluster (Fig. 3). In more detail, GH13_12 PULIs cluster together with GH13_12+41 PULII_IIs, and it seems plausible that GH13_12+41 and GH13_14+41 PULII_IIs evolved from a PULI by gene fusion, although there is currently no additional support for this hypothesis. The GH13_11 cluster is well-defined, adjacent to the PULI clusters, and slightly more distantly related to GH13_31. The GH13_39 PULII_Is cluster together with the GH13_20 sub-cluster. The analysis also shows that GH13_31 and GH13_20 enzymes are distantly related. Clearly, GH13_39 PULII_Is, however, are more closely related to GH13_20 enzymes, which, like GH13_39 enzymes, have one active site for hydrolysing both α -1,4- and α -1,6- linkages. GH13_20 enzymes, however, in general prefers α -1,4- over α -1,6-linkages, while enzymes of GH13_39 show the opposite trend. Based on this phylogenetic analysis we suggest that GH13_39 PULII_Is being intermediates of GH13_20 having dual bond-type specificity, and GH13_31 of strict α -1,6-specificity. GH13_39 PULII_Is evolutionary are also more evolutionary related to GH13_20 enzymes than to GH13_12–14 pullulanases in accordance with their associated CBMs, thus CBM34 are found with both GH13_20 and GH13_39 and is not found with GH13_12–14 enzymes. Finally, the

phylogenetic analysis showed GH57 PULII_Is as distantly related to GH13_13 and GH13_14 PULIs (Fig. 3). The different folds and the fact that there are no conserved sequence motifs shared between the GH57 and GH13 enzymes make joint phylogenetic analysis of these GH families futile.

Structure based comparison of α -glucan debranching enzymes

Comparison of overall structures of six α -glucan debranching GH13 subfamilies shows obvious differences with regard to number and types of non-catalytic domains (Fig. 2), but variation is also seen between the subfamilies in the catalytic domains around the active site (Fig. 5). GH13_11–14 and GH13_20 enzymes have an open cleft-shaped active site, as opposed to a closed pocket-shaped active site is seen for the exo-acting GH13_31 (Fig. 5). The architecture of the active sites defines size and structure of the preferred substrates; even small differences in the curvature of the cleft-shaped active sites can define optimal lengths of substrate main chains and branches. Such differences within and between subfamilies are discussed intensely in the literature on crystal structures of GH13_11–14 enzymes [16, 19, 30, 36, 107] for example in search for an explanation of GH13_11 enzymes having no or very low activity on pullulan. Superimposition of a GH13_13 structure in complex with a pullulan fragment with the GH13_11 structures shows it has an active site loop at the minus subsite region (glycon binding) that sterically hinders binding of the staircase-shaped pullulan (Fig. 5a). Closer inspection of all α -glucan debranching enzyme structures with substrates or products bound at the active site makes it clear that orientation and position of the ligands vary significantly among the subfamilies. Only the glucose unit bound at subsite –1 superimposes well across subfamilies (Fig. 6a). However, not all subfamilies are represented by structures where both the plus (aglycone binding) and minus subsites, i.e. both sides of the cleavage site, are occupied by ligand. PULI subfamilies 12–14 have superimposing glucose units at subsites +1 and +2 in addition to subsite –1, illustrating their close relationship. The lonely glucose unit bound in subsite +2 of the *Chlamydomonas reinhardtii* ISA (PDB entry 4OKD) and *Sulfolobus solfataricus* GDE (PDB entry 2VR5) of GH13_11 also superimposes with glucose units in GH13_12–14 structures. The plus subsites of the barley PULI have been proposed to be high-affinity subsites, while branch binding minus subsites are proposed to be of low-affinity [36].

Conserved regions

The α -glucan debranching enzymes are a relatively heterogeneous group when it comes to substrate specificity and preference. However, in spite of their mutual differences certain amino acid residues around the active site are conserved at positions defining the classical conserved sequences in GH13 (regions I–IV [6], Fig. 4). These motifs are to some extent useful to distinguishing the GH13 subfamilies. A great number of mutational studies are published on the importance of residues conserved among GH13 enzymes and within subfamilies. Here the focus is on residues important for modulating α -1,6-activity or for discriminating individual α -glucan debranching GH13 subfamilies where structure-determined enzymes serve as examples.

Region I is not interacting directly with ligands in the active site, but mutational analysis of the histidine has shown that it is essential for catalytic activity for a GH13_13 PULI from *Klebsiella* [108].

Region II harbours the catalytic nucleophile and even though the primary structure appears to differ considerably at certain positions, when comparing all structures of GH13 debranching subfamilies, a very strict conservation of the spatial position of not only the backbone, but also the rotamers of the varying residues make these superimpose perfectly (Fig. 6b). The valine (Val195) next to the catalytic nucleophile (Fig. 6b and c) was shown by mutational analysis to be important for the α -1,6-activity of the GH13_31 enzyme from *Streptococcus mutans* (PDB entry 2ZIC), since the corresponding alanine mutant changed specificity to α -1,4-activity [109]. Additionally, a lysine (Lys275) in region III and a glutamic acid (Glu371) outside the active site were shown to be central structural elements in the recognition of α -1,6-glucosidic linkages [109]. The valine of region II was also important for the preference for α -1,4- and α -1,6-glucosidic linkages of the GH13_20 *Thermoactinomyces vulgaris* TVAII (PDB entry 1BVZ). Mutation to alanine altered the linkage preference from α -1,6- to α -1,4-linkages [110]. Comparison of representative structures from α -glucan debranching GH13 subfamilies with that of a *Bacillus subtilis* α -amylase (GH13_28) with a maltopentaose molecule spanning the catalytic site (PDB entry 1BAG) clearly shows that the position equivalent to Val195 of *Streptococcus mutans* will constrain linkage type variability with respect to the subsite -1 and +1 linkage: A small amino acid like alanine, found in α -amylases, gives the spaciousness needed for the short and rigid α -1,4-linkage, whereas increased size of the amino acid in position 195 makes it harder to accommodate an α -1,4-linkage (Fig. 6c). The α -1,6-specific PULIs have large amino acids like methionine or leucine at this position, while GH13_20, GH13_31, and GH13_39 have the smaller valine (Fig. 4).

Region III forms subsite +2 and harbours the general acid/base catalyst. This region has been suggested to be important for substrate binding in PULIs, especially the tryptophan that stacks with the glucose unit in subsite +2 was proposed to be critical (Fig. 6d) [36]. This tryptophan is conserved in the different subfamilies (Fig. 4), but due to variation in the loop positions of GH13_20 and GH13_31 structures it clashes with ligands accommodated in GH13_13 structures where an α -1,6-linkage is spanning the catalytic site. Kuriki showed that mutation of an isoleucine adjacent to the conserved tryptophan in GH13_20 from *Bacillus stearothermophilus* (PDB entry 1J0H) modulated α -1,6-activity (Fig. 6d): the isoleucine to tryptophan mutant had reduced α -1,6-activity, while replacement with valine increased the α -1,6-activity [111]. The tryptophan of the GH13_31 enzyme from *Streptococcus mutans* (Trp238) was shown by mutational analysis to be very important for hydrolytic activity especially on long chain substrates [112]. Moreover, from a structure comparison of the four structure-determined GH13_11 members it is seen that GH13_11 structures vary in region III. Thus loop following the general acid/base of the GDE from *Sulfolobus solfataricus* has a completely different direction (Fig. 6d).

The backbone of region IV superimposes perfectly despite differences in amino acid residue types at some positions (Fig. 4 and Fig. 6e). Region IV harbours the aspartate that functions as transition state stabiliser, which shows rotamer variation among different GH13 structures (Fig. 6e) depending noticeably on the absence or presence of ligand [36]. By contrast, the invariant histidine in region IV is crucial for activity [108] forms part of subsite -1 and adopts the same rotamer in all the structures (Fig. 6e). Furthermore at region IV, the tyrosine in GH13_12-14 PULIs (Fig. 4) is on average 4.5 Å from the glucose unit at subsite +1.

Remarkably, this tyrosine ring superimposes with the aromatic ring of the equivalent histidine in GH13_11 (Fig. 6e).

In addition to the four classical conserved GH13 regions, region V is proposed to define subfamilies GH13_20 and GH13_31, GH13_20 having an MPKln and GH13_31 a corresponding QpDln motif in this region (Fig. 4). Enzymes with MPDLN are suggested to create an intermediary group having diverse enzyme specificity of α -amylase, cyclomaltodextrinase and neopullulanase [77, 113].

Regions VI and VII are suggested as signature regions of ISAs, PULIs and PULIIs [114-119]. Region VI was noted to distinguish ISAs and pullulanases: ISAs having VIYEVHVRG and PULIs IYELHIRDFS [114]. It is a part of the core of the $(\beta/\alpha)_8$ -barrel and not in the active site, but interacts directly with region VII that generally forms a part of subsite -2. Different signature motifs of region VII have been suggested: YNWGYDP (PULI) and NYWGY (ISA) [114]; -NWGY- (PULIs) and -NH(K/R)Y- (PULIIs) [118, 119]. Based on a multiple sequence alignment only including the catalytic domains of PULII_Is and PULII_IIs these motifs clearly should be further specified, as PULIs and PULII_IIs both have -NWGY- while -NH(K/R)Y- is signature motif for GH13_39 PULII_Is [43]. There are exceptions, however, thus PULII_IIs can thus possess the signature motif of a PULI. This correlates with the fact that the pullulanase domain of PULII_IIs is phylogenetically similar to PULI domains (Fig. 3). Comparison of region VII from PULI, ISA, and GDE structures shows the tryptophan and the second tyrosine to superimpose very well for these members of different subfamilies, and that the aromatic ring of the first tyrosine residues in the sequence motif VII motif superimpose despite a shift of one residue (Fig. 6f). The major difference between PULIs and GH13_11 enzymes in this region is the interplay of the asparagine and the aspartic acid of the PULIs, which interact with each other as well as with the glucose unit in subsite -2 and even a terminal glucose unit in the pullulan fragment (PDB entry 4J3X). In the GH13_11 enzymes, the asparagine makes contact with the glucose unit in subsite -2.

Biological function and industrial application of α -glucan debranching enzymes

Most α -glucan debranching enzymes serve in mobilization of energy in eukaryotes, archaea and bacteria by degrading the storage carbohydrates glycogen and amylopectin. α -glucan debranching enzymes, however, have also been shown to participate in biosynthesis of the α -glucans. Thus in plants debranching is required to modify the structure of the growing amylopectin molecule to a more ordered structure, which as a consequence promotes the formation of crystalline amylopectin regions essential to the integrity of the starch granule [120]. All plants characterised to date contain two conserved types of starch debranching enzymes; PULI (in plants called limit dextrinase) and ISA of which at least three genes (ISA1-3) are found [121]. ISA and PULI appear to be conserved separately during evolution of prokaryotes and higher plants, and these enzymes are more closely related to a bacterial counterpart than to the other type of plant debranching enzyme [121]. The physiological function of ISA in starch biosynthesis is well-established based on mutants deficient in one or more ISA isoforms in barley [122], Arabidopsis [123-125], maize [126], and rice [127-130]. Generally, ISA is assumed to catalyse editing of excessively branched chains or removal of improper branches

formed in biosynthesis of amylopectin by branching enzymes to achieve the cluster structure of amylopectin [131-133]. These studies show that lack of ISA results in production of the disordered water-soluble polysaccharide, phytoglycogen, that resembles glycogen and lacks the structural features of amylopectin that allow formation of crystalline regions in starch granules.

Compared with ISA, the role of the limit dextrinase (PULI) in starch biosynthesis is less well-established. However, besides during germination, substantial PULI activity has been detected in developing rice and maize endosperms [114, 134], and the presence of mRNA for barley limit dextrinase was found in this stage of the plant lifecycle [135]. PULI is thus proposed to participate in starch synthesis in rice, maize and Arabidopsis [121, 124, 129]. Mutational studies of the starch debranching enzymes in higher plants however display distinct and slightly different expression profiles of mutant phenotypes leading to contradictory interpretations. One explanation for these small, albeit significant differences could be some level of functional overlap of the various starch debranching enzymes. If the balance between the starch debranching enzymes or their degree of functional redundancy varies from species to species these small differences might explain the apparent contradictory observations [124].

Bacterial pullulanases are often extracellular being either secreted or attached to the cell surface [136]. Besides the important for degradation of α -glucans into maltooligosaccharides, to be transported into cells to enter the intracellular energy metabolism, the extracellular pullulanases can have another role: PULIs from *Streptococcus* were shown to be so-called virulence factors that facilitate binding of streptococci to host mucus e.g. in lungs. As already mentioned it is the CBM41s, which contribute to binding of cell surface attached pullulanases to the epithelial mucus layer [94, 96].

Industrial application

Industrial use of α -glucan debranching enzymes comprises two areas: degradation of starch into glucose, maltose, and maltodextrins and production of carbohydrates with new functions. Starch is a major raw material for the brewing industry and in the manufacture of various products such as bio-ethanol, coating agents in the paper industry, anti-staling agents, and sugar syrups. Debranching enzymes play a key role together with the amylases in these industrial processes. Starch saccharification at very high rate and at low temperature is attractive for commercial food, feed and fuel production. To this end, well-known high rate ethanol producers like *Saccharomyces cerevisiae* with high ethanol tolerance have been complemented with glucoamylases and α -glucan debranching enzymes to achieve the saccharification and debranching needed for complete hydrolysis of starch to ethanol by *S. cerevisiae* [137]. Furthermore, α -glucan degrading enzymes are prominent additives to detergents, where they function to remove starch stains from cloth. Due to the industrial importance of α -glucan debranching enzymes several review articles focus on this topic [37, 138-141]. Especially enzymes from thermophilic and mesophilic organisms received much attention in relation to industrial applications, due to their high stability under extreme temperature and pH conditions ensuring robust industrial processes [142].

GH13₂₀ enzymes play a role in relation to production of carbohydrates with new functions, e.g. of linear or cyclic oligosaccharides proposed to have prebiotic properties [143, 144]. An emerging biotechnology could

involve engineering of the repertoire of α -glucan debranching enzymes in planta for modification of starch structure and large-scale production for more or less easily digestible food and feed. This approach for utilisation of the industrial potential of α -glucan debranching enzymes is still in its infancy.

Conclusions

The present review emphasises that α -glucan debranching enzymes are a diverse group of enzymes, with remarkable differences and similarities currently found in GH13 subfamilies and GH57. The first PULI crystal structure solved a decade ago is today complemented by a very valuable knowledge-base of both free and carbohydrate complexed structures, including the first branched substrate complex structure, that is key to understanding the relationship between structure and function for α -glucan debranching enzymes. But important enzyme types remain to be structure-determined. The phylogenetic analysis presented here indicate that GH13_39 is a missing link between the strict α -1,6-acting PULIs and pullulan hydrolases with dual α -1,4- and α -1,6-linkage specificity, which draws special attention to the fact that novel structures of GH13_39 and GH57 PULII_Is are lacking.

The many new structures have made it possible to decipher α -1,6-bond type specificity versus α -1,4-bond type specificity and identify sub-structures and sequence motifs that functionally differentiates the various types of α -glucan debranching enzymes. These sub-structures and sequence motifs can serve as inspiration for enzyme design but novel structures of α -glucan debranching enzymes and/or complementary data are needed to add enough detail to the activity and specificity knowledge to allow enzymes of potential industrial relevance to be rationally engineered.

Acknowledgements

Bernard Henrissat is gratefully acknowledged for sharing information on new GH13 subfamilies of which GH13_41 is addressed in the present review. This work was supported by The Danish Council for Independent Research|Technology and Production Sciences (to MSM), and a Sapere Aude-Research Talent grant from the Danish Council for Independent Research|Technology and Production Sciences (to MSM).

REFERENCES

- 1 Chi Z, Wang F, Chi Z, Yue L, Liu G and Zhang T (2009) Bioproducts from *Aureobasidium pullulans*, a biotechnologically important yeast. *Appl Microbiol Biotechnol* 82(5):793–804
- 2 Vamadevan V and Bertoft E (2015) Structure-function relationships of starch components. *Starch-Stärke* 67(1–2):55–68
- 3 Roach PJ, Depaoli-Roach AA, Hurley TD and Tagliabracci VS (2012) Glycogen and its metabolism: some new developments and old themes. *Biochem J* 441:763–787
- 4 Lombard V, Ramulu HG, Drula E, Coutinho PM and Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* 42(D1):D490–D495
- 5 Stam MR, Danchin EGJ, Rancurel C, Coutinho PM and Henrissat B (2006) Dividing the large glycoside hydrolase family 13 into subfamilies: towards improved functional annotations of α -amylase-related proteins. *Protein Eng Des Sel* 19(12):555–562
- 6 MacGregor EA, Janeček S and Svensson B (2001) Relationship of sequence and structure to specificity in the α -amylase family of enzymes. *Biochim Biophys Acta, Protein Struct Mol Enzymol* 1546(1):1–20
- 7 Kuriki T and Imanaka T (1999) The concept of the α -amylase family: structural similarity and common catalytic mechanism. *J Biosci Bioeng* 87(5):557–565
- 8 Janecek S, Svensson B and MacGregor EA (2014) α -amylase: an enzyme specificity found in various families of glycoside hydrolases. *Cell Mol Life Sci* 71(7):1149–1170
- 9 Janecek S and Blesak K (2011) Sequence-structural features and evolutionary relationships of family GH57 α -amylases and their putative α -amylase-like homologues. *Protein J* 30(6):429–435
- 10 Park K, Jung J, Park S, Lee M, Holden JF, Park C and Woo E (2014) Structural features underlying the selective cleavage of a novel exo-type maltose-forming amylase from *Pyrococcus* sp. ST04. *Acta Crystallogr Sect D-Biol Crystallogr* 70(6):1659–1668
- 11 Zona R, Chang-Pi-Hin F, O'Donohue M and Janecek S (2004) Bioinformatics of the glycoside hydrolase family 57 and identification of catalytic residues in amylopullulanase from *Thermococcus hydrothermalis*. *Eur J Biochem* 271(14):2863–2872
- 12 Kang S, Vieille C and Zeikus J (2005) Identification of *Pyrococcus furiosus* amylopullulanase catalytic residues. *Appl Microbiol Biotechnol* 66(4):408–413
- 13 Janeček S (2005) Amylolytic families of glycoside hydrolases: focus on the family GH-57. *Biologia* 60:177–184
- 14 Blesak K and Janecek S (2012) Sequence fingerprints of enzyme specificities from the glycoside hydrolase family GH57. *Extremophiles* 16(3):497–506
- 15 Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, Geer RC, He J, Gwadz M, Hurwitz DI, Lanczycki CJ, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C and Bryant SH (2015) CDD: NCBI's conserved domain database. *Nucleic Acids Res* 43(D1):D222–D226

- 16 Sim L, Beeren SR, Findinier J, Dauvillee D, Ball SG, Henriksen A and Palcic MM (2014) Crystal structure of the *Chlamydomonas* starch debranching enzyme isoamylase ISA1 reveals insights into the mechanism of branch trimming and complex assembly. *J Biol Chem* 289(33):22991–23003
- 17 Hussain H, Mant A, Seale R, Zeeman S, Hinchliffe E, Edwards A, Hylton C, Bornemann S, Smith A, Martin C and Bustos R (2003) Three isoforms of isoamylase contribute different catalytic properties for the debranching of potato glucans. *Plant Cell* 15(1):133–149
- 18 Tetlow IJ (2011) Starch biosynthesis in developing seeds. *Seed Sci Res* 21(1):5–32
- 19 Song H, Jung T, Park J, Park B, Myung PK, Boos W, Woo E and Park K (2010) Structural rationale for the short branched substrate specificity of the glycogen debranching enzyme GlgX. *Proteins Struct Funct Bioinf* 78(8):1847–1855
- 20 Abe J, Ushijima C and Hizukuri S (1999) Expression of the isoamylase gene of *Flavobacterium odoratum* KU in *Escherichia coli* and identification of essential residues of the enzyme by site-directed mutagenesis. *Appl Environ Microbiol* 65(9):4163–4170
- 21 Maruta K, Mitsuzumi H, Nakada T, Kubota M, Chaen H, Fukuda S, Sugimoto T and Kurimoto M (1996) Cloning and sequencing of a cluster of genes encoding novel enzymes of trehalose biosynthesis from thermophilic archaebacterium *Sulfolobus acidocaldarius*. *Biochim Biophys Acta* 1291(3):177–181
- 22 Van TTK, Ryu S, Lee K, Kim E and Lee S (2007) Cloning and characterization of glycogen-debranching enzyme from hyperthermophilic archaeon *Sulfolobus shibatae*. *J Microbiol Biotechnol* 17(5):792–799
- 23 Seibold GM and Eikmanns BJ (2007) The glgX gene product of *Corynebacterium glutamicum* is required for glycogen degradation and for fast adaptation to hyperosmotic stress. *Microbiol SGM* 1532212–2220
- 24 Krohn B, Barry G and Kishore G (1997) An isoamylase with neutral pH optimum from a *Flavobacterium* species: Cloning, characterization and expression of the *iam* gene. *Mol Gen Genet* 254(5):469–478
- 25 Fujita N, Kubo A, Francisco P, Nakakita M, Harada K, Minaka N and Nakamura Y (1999) Purification, characterization, and cDNA structure of isoamylase from developing endosperm of rice. *Planta* 208(2):283–293
- 26 Hong S and Preiss J (2000) Localization of C-terminal domains required for the maximal activity or for determination of substrate preference of maize branching enzymes. *Arch Biochem Biophys* 378(2):349–355
- 27 Ito H, Hamada S, Isono N, Yoshizaki T, Ueno H, Yoshimoto Y, Takeda Y and Matsui H (2004) Functional characteristics of C-terminal regions of starch-branching enzymes from developing seeds of kidney bean (*Phaseolus vulgaris* L.). *Plant Sci* 166(5):1149–1158
- 28 Palomo M, Kralj S, van der Maarel MJEC and Dijkhuizen L (2009) The unique branching patterns of *Deinococcus* glycogen branching enzymes are determined by their N-terminal domains. *Appl Environ Microbiol* 75(5):1355–1362
- 29 Katsuya Y, Mezaki Y, Kubota M and Matsuura Y (1998) Three-dimensional structure of *Pseudomonas* isoamylase at 2.2 angstrom resolution. *J Mol Biol* 281(5):885–897

- 30 Woo E, Lee S, Cha H, Park J, Yoon S, Song H and Park K (2008) Structural insight into the bifunctional mechanism of the glycogen-debranching enzyme TreX from the archaeon *Sulfolobus solfataricus*. *J Biol Chem* 283(42):28641–28648
- 31 Cuyvers S, Dornez E, Delcour JA and Courtin CM (2012) Occurrence and functional significance of secondary carbohydrate binding sites in glycoside hydrolases. *Crit Rev Biotechnol* 32(2):93–107
- 32 Min-ho L, Hyung-Nam S, Ji-Eun C, Lan TP, Sunghoon P, Jong-Tae P and Eui-Jeon W (2014) Association of bi-functional activity in the N-terminal domain of glycogen debranching enzyme. *Biochem Biophys Res Commun* 445(1):107–112
- 33 Liu W, Madsen N, Braun C and Withers S (1991) Reassessment of the catalytic mechanism of glycogen debranching enzyme. *Biochemistry* 30(5):1419–1424
- 34 Nakayama A, Yamamoto K and Tabata S (2001) Identification of the catalytic residues of bifunctional glycogen debranching enzyme. *J Biol Chem* 276(31):28824–28828
- 35 Ara K, Igarashi K, Saeki K and Ito S (1995) An alkaline amylopullulanase from alkalophilic *Bacillus* sp KSM-1378; kinetic evidence for 2 independent active-sites for the α -1,4 and α -1,6 hydrolytic reactions. *Biosci Biotechnol Biochem* 59(4):662–666
- 36 Møller MS, Windahl MS, Sim L, Bojstrup M, Abou Hachem M, Hindsgaul O, Palcic M, Svensson B and Henriksen A (2015) Oligosaccharide and substrate binding in the starch debranching enzyme barley limit dextrinase. *J Mol Biol* 427(6):1263–1277
- 37 Nisha M and Satyanarayana T (2013) Recombinant bacterial amylopullulanases. *Developments and perspectives*. *Bioengineered* 4(6):388–400
- 38 Kim J, Sunako M, Ono H, Murooka Y, Fukusaki E and Yamashita M (2008) Characterization of gene encoding amylopullulanase from plant-originated lactic acid bacterium, *Lactobacillus plantarum* L137. *J Biosci Bioeng* 106(5):449–459
- 39 Kim J, Sunako M, Ono H, Murooka Y, Fukusaki E and Yamashita M (2009) Characterization of the C-terminal truncated form of amylopullulanase from *Lactobacillus plantarum* L137. *J Biosci Bioeng* 107(2):124–129
- 40 Ferrando ML, Fuentes S, de Greeff A, Smith H and Wells JM (2010) ApuA, a multifunctional α -glucan-degrading enzyme of *Streptococcus suis*, mediates adhesion to porcine epithelium and mucus. *Microbiology* 156:2818–2828
- 41 Hatada Y, Igarashi K, Ozaki K, Ara K, Hitomi J, Kobayashi T, Kawai S, Watabe T and Ito S (1996) Amino acid sequence and molecular structure of an alkaline amylopullulanase from *Bacillus* that hydrolyzes α -1,4 and α -1,6 linkages in polysaccharides at different active sites. *J Biol Chem* 271(39):24075–24083
- 42 O'Connell Motherway M, Fitzgerald GF, Neirynck S, Ryan S, Steidler L and van Sinderen D (2008) Characterization of ApuB, an extracellular type II amylopullulanase from *Bifidobacterium breve* UCC2003. *Appl Environ Microbiol* 74(20):6271–6279
- 43 Lee YS, Seo SH, Yoon SH, Kim SY, Hahn BS, Sim JS, Koo BS and Lee CM (2016) Identification of a novel alkaline amylopullulanase from a gut metagenome of *Hermetia illucens*. *Int J Biol Macromolec* 82:514–522

- 44 Han T, Zeng F, Li Z, Liu L, Wei M, Guan Q, Liang X, Peng Z, Liu M, Qin J, Zhang S and Jia B (2013) Biochemical characterization of a recombinant pullulanase from *Thermococcus kodakarensis* KOD1. *Lett Appl Microbiol* 57(4):336–343
- 45 Guan Q, Guo X, Han T, Wei M, Jin M, Zeng F, Liu L, Li Z, Wang Y, Cheong G, Zhang S and Jia B (2013) Cloning, purification and biochemical characterisation of an organic solvent-, detergent-, and thermo-stable amylopullulanase from *Thermococcus kodakarensis* KOD1. *Process Biochem* 48(5–6):878–884
- 46 Koivula T, Hemila H, Pakkanen R, Sibakov M and Palva I (1993) Cloning and sequencing of a gene encoding acidophilic amylase from *Bacillus acidocaldarius*. *J Gen Microbiol* 139:2399–2407
- 47 Bai Y, Huang H, Meng K, Shi P, Yang P, Luo H, Luo C, Feng Y, Zhang W and Yao B (2012) Identification of an acidic α -amylase from *Alicyclobacillus* sp A4 and assessment of its application in the starch industry. *Food Chem* 131(4):1473–1478
- 48 Lee S, Morikawa M, Takagi M and Imanaka T (1994) Cloning of the aapT gene and characterization of its product, α -amylase-pullulanase (AapT), from thermophilic and alkaliphilic *Bacillus* sp. strain XAL601. *Appl Environ Microbiol* 60(10):3764–3773
- 49 Ferner-Ortner-Bleckmann J, Huber-Gries C, Pavkov T, Keller W, Mader C, Ilk N, Sleytr UB and Egelseer EM (2009) The high-molecular-mass amylase (HMMA) of *Geobacillus stearothermophilus* ATCC 12980 interacts with the cell wall components by virtue of three specific binding regions. *Mol Microbiol* 72(6):1448–1461
- 50 Chen J, Chen M, Chen L and Chu W (2001) Structure and expression of an amylopullulanase gene from *Bacillus stearothermophilus* TS-23. *Biotechnol Appl Biochem* 33:189–199
- 51 Nisha M and Satyanarayana T (2013) Characterization of recombinant amylopullulanase (gt-apu) and truncated amylopullulanase (gt-apuT) of the extreme thermophile *Geobacillus thermoleovorans* NP33 and their action in starch saccharification. *Appl Microbiol Biotechnol* 97(14):6279–6292
- 52 Nisha M and Satyanarayana T (2015) The role of N1 domain on the activity, stability, substrate specificity and raw starch binding of amylopullulanase of the extreme thermophile *Geobacillus thermoleovorans*. *Appl Microbiol Biotechnol* 99(13):5461–5474
- 53 Mathupala S, Saha B and Zeikus J (1990) Substrate competition and specificity at the active site of amylopullulanase from *Clostridium thermohydrosulfuricum*. *Biochem Biophys Res Commun* 166(1):126–132
- 54 Mathupala S, Lowe S, Podkovyrov S and Zeikus J (1993) Sequencing of the amylopullulanase (apu) gene of *Thermoanaerobacter ethanolicus* 39E, and identification of the active site by site-directed mutagenesis. *J Biol Chem* 268(22):16332–16344
- 55 Ramesh M, Podkovyrov S, Lowe S and Zeikus J (1994) Cloning and sequencing of the *Thermoanaerobacterium saccharolyticum* B6A-RI apu gene and purification and characterization of the amylopullulanase from *Escherichia coli*. *Appl Environ Microbiol* 60(1):94–101
- 56 Melasniemi H (1988) Purification and some properties of the extracellular α -amylase-pullulanase produced by *Clostridium thermohydrosulfuricum*. *Biochem J* 250(3):813–818

- 57 Spreinat A and Antranikian G (1992) Analysis of the amylolytic enzyme-system of *Clostridium thermosulfurogenes* EM1: Purification and synergistic action of pullulanases and maltohexaose forming α -amylase. *Starch-Stärke* 44(8):305–312
- 58 Matuschek M, Burchhardt G, Sahm K and Bahl H (1994) Pullulanase of *Thermoanaerobacterium thermosulfurigenes* EM1 (*Clostridium thermosulfurogenes*): Molecular analysis of the gene, composite structure of the enzyme, and a common model for its attachment to the cell surface. *J Bacteriol* 176(11):3295–3302
- 59 Kahar UM, Chan K, Salleh MM, Hii SM and Goh KM (2013) A high molecular-mass *Anoxybacillus* sp SK3-4 amylopullulanase: Characterization and its relationship in carbohydrate utilization. *Int J Mol Sci* 14(6):11302–11318
- 60 Li X and Li D (2015) Preparation of linear maltodextrins using a hyperthermophilic amylopullulanase with cyclodextrin- and starch-hydrolysing activities. *Carbohydr Polym* 119:134–141
- 61 Siddiqui MA, Habib-ur-Rehman and Rashid N (2014) Gene cloning and characterization of a type II pullulanase hydrolase from a hyperthermophilic archaeon, *Pyrobaculum calidifontis*. *Pak J Zool* 46(4):1077–1084
- 62 Dong G, Vieille C and Zeikus J (1997) Cloning, sequencing, and expression of the gene encoding amylopullulanase from *Pyrococcus furiosus* and biochemical characterization of the recombinant enzyme. *Appl Environ Microbiol* 63(9):3577–3584
- 63 Choi K and Cha J (2015) Membrane-bound amylopullulanase is essential for starch metabolism of *Sulfolobus acidocaldarius* DSM639. *Extremophiles* 19(5):909–920
- 64 Li X, Li D and Park K (2013) An extremely thermostable amylopullulanase from *Staphylothermus marinus* displays both pullulan- and cyclodextrin-degrading activities. *Appl Microbiol Biotechnol* 97(12):5359–5369
- 65 Erra-Pujada M, Debeire P, Duchiron F and O'Donohue M (1999) The type II pullulanase of *Thermococcus hydrothermalis*: Molecular characterization of the gene and expression of the catalytic domain. *J Bacteriol* 181(10):3284–3287
- 66 Brown S and Kelly R (1993) Characterization of amylolytic enzymes, having both α -1,4 and α -1,6 hydrolytic activity, from the thermophilic archaea *Pyrococcus furiosus* and *Thermococcus litoralis*. *Appl Environ Microbiol* 59(8):2614–2621
- 67 Jiao Y, Wang S, Lv M, Xu J, Fang Y and Liu S (2011) A GH57 family amylopullulanase from deep-sea *Thermococcus siculi*: Expression of the gene and characterization of the recombinant enzyme. *Curr Microbiol* 62(1):222–228
- 68 Park KH, Kim TJ, Cheong TK, Kim JW, Oh BH and Svensson B (2000) Structure, specificity and function of cyclomaltodextrinase, a multispecific enzyme of the α -amylase family. *Biochim Biophys Acta-Protein Struct Molec Enzym* 1478(2):165–185
- 69 Lee HS, Kim MS, Cho HS, Kim JI, Kim TJ, Choi JH, Park C, Lee HS, Oh BH and Park KH (2002) Cyclomaltodextrinase, neopullulanase, and maltogenic amylase are nearly indistinguishable from each other. *J Biol Chem* 277(24):21891–21897

- 70 Kim T, Kim M, Kim B, Kim J, Cheong T, Kim J and Park K (1999) Modes of action of acarbose hydrolysis and transglycosylation catalyzed by a thermostable maltogenic amylase, the gene for which was cloned from a *Thermus* strain. *Appl Environ Microbiol* 65(4):1644–1651
- 71 Kim J, Cha S, Kim H, Kim T, Ha N, Oh S, Cho H, Cho M, Kim M, Lee H, Kim J, Choi K, Park K and Oh B (1999) Crystal structure of a maltogenic amylase provides insights into a catalytic versatility. *J Biol Chem* 274(37):26279–26286
- 72 Dumbrepatil AB, Choi J, Park JT, Kim M, Kim TJ, Woo E and Park KH (2010) Structural features of the *Nostoc punctiforme* debranching enzyme reveal the basis of its mechanism and substrate specificity. *Proteins Struct Funct Bioinf* 78(2):348–356
- 73 Ohtaki A, Mizuno M, Yoshida H, Tonozuka T, Sakano Y and Kamitori S (2006) Structure of a complex of *Thermoactinomyces vulgaris* R-47 α -amylase 2 with maltohexaose demonstrates the important role of aromatic residues at the reducing end of the substrate binding cleft. *Carbohydr Res* 341(8):1041–1046
- 74 Cho H, Kim Y, Kim T, Lee H, Kim D, Kim J, Lee Y, Lee S and Park K (2000) Molecular characterization of a dimeric intracellular maltogenic amylase of *Bacillus subtilis* SUH4-2. *Biochim Biophys Acta* 1478(2):333–340
- 75 Mehta D and Satyanarayana T (2013) Dimerization mediates thermo-adaptation, substrate affinity and transglycosylation in a highly thermostable maltogenic amylase of *Geobacillus thermoleovorans*. *Plos One* 8(9):1–13
- 76 Ahmad N, Rashid N, Haider MS, Akram M and Akhtar M (2014) Novel maltotriose-hydrolyzing thermoacidophilic type III pullulan hydrolase from *Thermococcus kodakarensis*. *Appl Environ Microbiol* 80(3):1108–1115
- 77 Møller MS, Fredslund F, Majumder A, Nakai H, Poulsen JN, Lo Leggio L, Svensson B and Abou Hachem M (2012) Enzymology and structure of the GH13_31 glucan 1,6- α -glucosidase that confers isomaltooligosaccharide utilization in the probiotic *Lactobacillus acidophilus* NCFM. *J Bacteriol* 194(16):4249–4259
- 78 Makelainen H, Hasselwander O, Rautonen N and Ouwehand AC (2009) Panose, a new prebiotic candidate. *Lett Appl Microbiol* 49(6):666–672
- 79 Møller MS, Abou Hachem M, Svensson B and Henriksen A (2012) Structure of the starch-debranching enzyme barley limit dextrinase reveals homology of the N-terminal domain to CBM21. *Acta Crystallogr F-Struct Biol Cryst Commun* 68(Pt 9):1008–12
- 80 Boraston AB, Bolam DN, Gilbert HJ and Davies GJ (2004) Carbohydrate-binding modules: fine-tuning polysaccharide recognition. *Biochem J* 382:769–781
- 81 Guillén D, Sánchez S and Rodríguez-Sanoja R (2010) Carbohydrate-binding domains: multiplicity of biological roles. *Appl Microbiol Biotechnol* 85(5):1241–1249
- 82 Lammerts van Bueren A, Ficko-Blean E, Pluvinau B, Hehemann J, Higgins MA, Deng L, Ogunniyi AD, Stroeder UH, El Warry N, Burke RD, Czjzek M, Paton JC, Vocadlo DJ and Boraston AB (2011) The conformation and function of a multimodular glycogen-degrading pneumococcal virulence factor. *Structure* 19(5):640–651

- 83 Xu J, Ren F, Huang C, Zheng Y, Zhen J, Sun H, Ko T, He M, Chen C, Chan H, Guo R, Song H and Ma Y (2014) Functional and structural studies of pullulanase from *Anoxybacillus* sp. LM18-11. *Proteins Struct Funct Bioinf* 82(9):1685–1693
- 84 Gilding EK, Frère CH, Cruickshank A, Rada AK, Prentis PJ, Mudge AM, Mace ES, Jordan DR and Godwin ID (2013) Allelic variation at a single gene increases food value in a drought-tolerant staple cereal. *Nat Commun* 4:1483
- 85 Janeček S, Svensson B and MacGregor EA (2011) Structural and evolutionary aspects of two families of non-catalytic domains present in starch and glycogen binding proteins from microbes, plants and animals. *Enzyme Microb Technol* 49(5):429–440
- 86 Christiansen C, Abou Hachem M, Janecek S, Vikso-Nielsen A, Blennow A and Svensson B (2009) The carbohydrate-binding module family 20-diversity, structure, and function. *FEBS J* 276(18):5006–5029
- 87 Christiansen C, Abou Hachem M, Glaring MA, Vikso-Nielsen A, Sigurskjold BW, Svensson B and Blennow A (2009) A CBM20 low-affinity starch-binding domain from glucan, water dikinase. *FEBS Lett* 583(7):1159–1163
- 88 Penninga D, vanderVeen B, Knegt R, vanHijum S, Rozeboom H, Kalk K, Dijkstra B and Dijkhuizen L (1996) The raw starch binding domain of cyclodextrin glycosyltransferase from *Bacillus circulans* strain 251. *J Biol Chem* 271(51):32777–32784
- 89 Sorimachi K, Le Gal-Coëffet M, Williamson G, Archer D and Williamson M (1997) Solution structure of the granular starch binding domain of *Aspergillus niger* glucoamylase bound to β -cyclodextrin. *Structure* 5(5):647–661
- 90 Zhu Y, Zhang M, Kelly AR and Cheng A (2014) The carbohydrate-binding domain of overexpressed STBD1 is important for its stability and protein-protein interactions. *Biosci Rep* 34:311–320
- 91 Park J, Song H, Jung T, Lee M, Park S, Woo E and Park K (2013) A novel domain arrangement in a monomeric cyclodextrin-hydrolyzing enzyme from the hyperthermophile *Pyrococcus furiosus*. *Biochim Biophys Acta, Proteins Proteomics* 1834(1):380–386
- 92 Liu Y, Lai Y, Chou W, Chang MD and Lyu P (2007) Solution structure of family 21 carbohydrate-binding module from *Rhizopus oryzae* glucoamylase. *Biochem J* 40:321–330
- 93 Abe A, Yoshida H, Tono-zuka T, Sakano Y and Kamitori S (2005) Complexes of *Thermoactinomyces vulgaris* R-47 α -amylase 1 and pullulan model oligosaccharides provide new insight into the mechanism for recognizing substrates with α -(1,6) glycosidic linkages. *FEBS J* 272(23):6145–6153
- 94 van Bueren AL, Higgins M, Wang D, Burke RD and Boraston AB (2007) Identification and structural basis of binding to host lung glycogen by streptococcal virulence factors. *Nat Struct Mol Biol* 14(1):76–84
- 95 Ficko-Blean E and Boraston AB (2012) Insights into the recognition of the human glycome by microbial carbohydrate-binding modules. *Curr Opin Struct Biol* 22(5):570–577
- 96 Gourlay LJ, Santi I, Pezzicoli A, Grandi G, Soriani M and Bolognesi M (2009) Group B *Streptococcus* pullulanase crystal structures in the context of a novel strategy for vaccine development. *J Bacteriol* 191(11):3544–3552

- 97 Lammerts van Bueren A and Boraston AB (2007) The structural basis of α -glucan recognition by a family 41 carbohydrate-binding module from *Thermotoga maritima*. *J Mol Biol* 365(3):555–560
- 98 van Bueren A, Finn R, Ausio J and Boraston A (2004) α -glucan recognition by a new family of carbohydrate-binding modules found primarily in bacterial pathogens. *Biochemistry* 43(49):15633–15642
- 99 Machovic M and Janeček S (2008) Domain evolution in the GH13 pullulanase subfamily with focus on the carbohydrate-binding module family 48. *Biologia* 63(6):1057–1068
- 100 Polekhina G, Gupta A, van Denderen B, Fell S, Kemp B, Stapleton D and Parker M (2005) Structural basis for glycogen recognition by AMP-activated protein kinase. *Structure* 13(10):1453–1462
- 101 Koay A, Woodcroft B, Petrie EJ, Yue H, Emanuelle S, Bieri M, Bailey MF, Hargreaves M, Park J, Park K, Ralph S, Neumann D, Stapleton D and Gooley PR (2010) AMPK beta subunits display isoform specific affinities for carbohydrates. *FEBS Lett* 584(15):3499–3503
- 102 Amodeo GA, Momcilovic M, Carlson M and Tong L (2010) Biochemical and functional studies on the regulation of the *Saccharomyces cerevisiae* AMPK homolog SNF1. *Biochem Biophys Res Commun* 397(2):197–201
- 103 Meekins DA, Raththagala M, Husodo S, White CJ, Guo H, Koetting O, Vander Kooi CW and Gentry MS (2014) Phosphoglucan-bound structure of starch phosphatase Starch Excess4 reveals the mechanism for C6 specificity. *Proc Natl Acad Sci U S A* 111(20):7272–7277
- 104 Seung D, Soyk S, Coiro M, Maier BA, Eicke S and Zeeman SC (2015) PROTEIN TARGETING TO STARCH is required for localising GRANULE-BOUND STARCH SYNTHASE to starch granules and for normal amylose synthesis in Arabidopsis. *PLoS Biol* 13(2):1–29
- 105 Chaen K, Noguchi J, Omori T, Kakuta Y and Kimura M (2012) Crystal structure of the rice branching enzyme I (BEI) in complex with maltopentaose. *Biochem Biophys Res Commun* 424(3):508–511
- 106 Feng L, Fawaz R, Hovde S, Gilbert L, Chiou J and Geiger JH (2015) Crystal structures of *Escherichia coli* branching enzyme in complex with linear oligosaccharides. *Biochemistry* 54(40):6207–6218
- 107 Mikami B, Iwamoto H, Malle D, Yoon HJ, Demirkan-Sarikaya E, Mezaki Y and Katsuya Y (2006) Crystal structure of pullulanase: Evidence for parallel binding of oligosaccharides in the active site. *J Mol Biol* 359(3):690–707
- 108 Yamashita M, Matsumoto D and Murooka Y (1997) Amino acid residues specific for the catalytic action towards α -1,6-glucosidic linkages in *Klebsiella* pullulanase. *J Ferment Bioeng* 84(4):283–290
- 109 Saburi W, Rachi-Otsuka H, Hondoh H, Okuyama M, Mori H and Kimura A (2015) Structural elements responsible for the glucosidic linkage-selectivity of a glycoside hydrolase family 13 exo-glucosidase. *FEBS Lett* 589(7):865–869
- 110 Ito K, Ito S, Ishino K, Shimizu-Ibuka A and Sakai H (2007) Val326 of *Thermoactinomyces vulgaris* R-47 amylase II modulates the preference for α -(1,4)- and α -(1,6)-glycosidic linkages. *Biochim Biophys Acta* 1774(4):443–449

- 111 Kuriki T, Kaneko H, Yanase M, Takata H, Shimada J, Handa S, Takada T, Umeyama H and Okada S (1996) Controlling substrate preference and transglycosylation activity of neopullulanase by manipulating steric constraint and hydrophobicity in active center. *J Biol Chem* 271(29):17321–17329
- 112 Saburi W, Mori H, Saito S, Okuyama M and Kimura A (2006) Structural elements in dextran glucosidase responsible for high specificity to long chain substrate. *Biochim Biophys Acta* 1764(4):688–698
- 113 Oslancová A and Janecek S (2002) Oligo-1,6-glucosidase and neopullulanase enzyme subfamilies from the α -amylase family defined by the fifth conserved sequence region. *Cell Mol Life Sci* 59(11):1945–1959
- 114 Beatty MK, Rahman A, Cao HP, Woodman W, Lee M, Myers AM and James MG (1999) Purification and molecular genetic characterization of ZPU1, a pullulanase-type starch-debranching enzyme from maize. *Plant Physiol* 119(1):255–266
- 115 Tang K, Kobayashi RS, Champreda V, Eurwilaichitr L and Tanapongpipat S (2008) Isolation and characterization of a novel thermostable neopullulanase-like enzyme from a hot spring in Thailand. *Biosci Biotechnol Biochem* 72(6):1448–1456
- 116 Elleuche S, Qoura FM, Lorenz U, Rehn T, Brueck T and Antranikian G (2015) Cloning, expression and characterization of the recombinant cold-active type-I pullulanase from *Shewanella arctica*. *J Mol Catal B-Enzym* 11:670–677
- 117 Kang J, Park K, Choi K, Park C, Kim G, Kim D and Cha J (2011) Molecular cloning and biochemical characterization of a heat-stable type I pullulanase from *Thermotoga neapolitana*. *Enzyme Microb Technol* 48(3):260–266
- 118 Kelly A, Diderichsen B, Jorgensen S and McConnell D (1994) Molecular genetic analysis of the pullulanase B gene of *Bacillus acidopullulyticus*. *FEMS Microbial Lett* 115(1):97–105
- 119 Albertson G, McHale R, Gibbs M and Bergquist P (1997) Cloning and sequence of a type I pullulanase from an extremely thermophilic anaerobic bacterium, *Caldicellulosiruptor saccharolyticus*. *Biochim Biophys Acta* 1354(1):35–39
- 120 Zeeman SC, Kossmann J and Smith AM (2010) Starch: Its metabolism, evolution, and biotechnological modification in plants. *Annu Rev Plant Biol* 61:209–234
- 121 Dinges J, Colleoni C, James M and Myers A (2003) Mutational analysis of the pullulanase-type debranching enzyme of maize indicates multiple functions in starch metabolism. *Plant Cell* 15(3):666–680
- 122 Burton RA, Jenner H, Carrangis L, Fahy B, Fincher GB, Hylton C, Laurie DA, Parker M, Waite D, van Wegen S, Verhoeven T and Denyer K (2002) Starch granule initiation and growth are altered in barley mutants that lack isoamylase activity. *Plant J* 31(1):97–112
- 123 Wattebled F, Dong Y, Dumez S, Delvalle D, Planchot R, Berbezy P, Vyas D, Colonna P, Chatterjee M, Ball S and D'Hulst C (2005) Mutants of Arabidopsis lacking a chloroplastic isoamylase accumulate phytoglycogen and an abnormal form of amylopectin. *Plant Physiol* 138(1):184–195
- 124 Wattebled F, Planchot V, Dong Y, Szydlowski N, Pontoire B, Devin A, Ball S and D'Hulst C (2008) Further evidence for the mandatory nature of polysaccharide debranching for the aggregation of

- semicrystalline starch and for overlapping functions of debranching enzymes in Arabidopsis leaves. *Plant Physiol* 148(3):1309–1323
- 125 Streb S, Delatte T, Umhang M, Eicke S, Schorderet M, Reinhardt D and Zeeman SC (2008) Starch granule biosynthesis in arabidopsis is abolished by removal of all debranching enzymes but restored by the subsequent removal of an endoamylase. *Plant Cell* 20(12):3448–3466
 - 126 James M, Robertson D and Myers A (1995) Characterization of the maize gene *sugary1*, a determinant of starch composition in kernels. *Plant Cell* 7(4):417–429
 - 127 Nakamura Y, Yuki K, Park S and Ohya T (1989) Carbohydrate-metabolism in the developing endosperm of rice grains. *Plant Cell Physiol* 30(6):833–839
 - 128 Fujita N, Kubo A, Suh DS, Wong KS, Jane JL, Ozawa K, Takaiwa F, Inaba Y and Nakamura Y (2003) Antisense inhibition of isoamylase alters the structure of amylopectin and the physicochemical properties of starch in rice endosperm. *Plant Cell Physiol* 44(6):607–618
 - 129 Fujita N, Toyosawa Y, Utsumi Y, Higuchi T, Hanashiro I, Ikegami A, Akuzawa S, Yoshida M, Mori A, Inomata K, Itoh R, Miyao A, Hirochika H, Satoh H and Nakamura Y (2009) Characterization of pullulanase (PUL)-deficient mutants of rice (*Oryza sativa* L.) and the function of PUL on starch biosynthesis in the developing rice endosperm. *J Exp Bot* 60(3):1009–1023
 - 130 Li Q, Zhang G, Dong Z, Yu H, Gu M, Sun SSM and Liu Q (2009) Characterization of expression of the *OsPUL* gene encoding a pullulanase-type debranching enzyme during seed development and germination in rice. *Plant Physiol Biochem* 47(5):351–358
 - 131 Buléon A, Colonna P, Planchot V and Ball S (1998) Starch granules: structure and biosynthesis. *Int J Biol Macromol* 23(2):85–112
 - 132 Jeon J, Ryoo N, Hahn T, Walia H and Nakamura Y (2010) Starch biosynthesis in cereal endosperm. *Plant Physiol Biochem* 48(6):383–392
 - 133 Nakamura Y (2002) Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: Rice endosperm as a model tissue. *Plant cell physiol* 43(7):718–725
 - 134 Nakamura Y (1996) Some properties of starch debranching enzymes and their possible role in amylopectin biosynthesis. *Plant Sci* 121(1):1–18
 - 135 Burton RA, Zhang XQ, Hrmova M and Fincher GB (1999) A single limit dextrinase gene is expressed both in the developing endosperm and in germinated grains of barley. *Plant Physiol* 119(3):859–871
 - 136 Janulczyk R and Rasmussen M (2001) Improved pattern for genome-based screening identifies novel cell wall-attached proteins in gram-positive bacteria. *Infect Immun* 69(6):4019–4026
 - 137 Kim J, Kim H, Lim M, Ko H, Chin J, Lee HB, Kim I and Bai S (2010) Construction of a direct starch-fermenting industrial strain of *Saccharomyces cerevisiae* producing glucoamylase, α -amylase and debranching enzyme. *Biotechnol Lett* 32(5):713–719
 - 138 Doman-Pytka M and Bardowski J (2004) Pullulan degrading enzymes of bacterial origin. *Crit Rev Microbiol* 30(2):107–121
 - 139 Hii SL, Tan JS, Ling TC and Ariff AB (2012) Pullulanase: Role in starch hydrolysis and potential industrial applications. *Enzyme Research* 2012:1–14

- 140 Kelly RM, Dijkhuizen L and Leemhuis H (2009) Starch and α -glucan acting enzymes, modulating their properties by directed evolution. *J Biotechnol* 140(3–4):184–193
- 141 van der Maarel M, van der Veen B, Uitdehaag J, Leemhuis H and Dijkhuizen L (2002) Properties and applications of starch-converting enzymes of the α -amylase family. *J Biotechnol* 94(2):137–155
- 142 Haki G and Rakshit S (2003) Developments in industrially important thermostable enzymes: a review. *Bioresour Technol* 89(1):17–34
- 143 Goffin D, Delzenne N, Blecker C, Hanon E, Deroanne C and Paquot M (2011) Will isomalto-oligosaccharides, a well-established functional food in Asia, break through the European and American market? The status of knowledge on these prebiotics. *Crit Rev Food Sci Nutr* 51(5):394–409
- 144 Mussatto SI and Mancilha IM (2007) Non-digestible oligosaccharides: A review. *Carbohydr Polym* 68(3):587–597
- 145 Vester-Christensen MB, Abou Hachem M, Svensson B and Henriksen A (2010) Crystal structure of an essential enzyme in seed starch degradation: Barley limit dextrinase in complex with cyclodextrins. *J Mol Biol* 403(5):739–750
- 146 Møller MS, Vester-Christensen MB, Jensen JM, Abou Hachem M, Henriksen A and Svensson B (2015) Crystal structure of barley limit dextrinase-limit dextrinase inhibitor (LD-LDI) complex reveals insights into mechanism and diversity of cereal type inhibitors. *J Biol Chem* 290(20):12614–12629
- 147 Turkenburg JP, Brzozowski AM, Svendsen A, Borchert TV, Davies GJ and Wilson KS (2009) Structure of a pullulanase from *Bacillus acidopullulyticus*. *Proteins Struct Funct Bioinf* 76(2):516–519
- 148 Malle D, Itoh T, Hashimoto W, Murata K, Utsumi S and Mikami B (2006) Overexpression, purification and preliminary X-ray analysis of pullulanase from *Bacillus subtilis* strain 168. *Acta Crystallogr, Sect F: Struct Biol Cryst Commun* 62:381–384
- 149 Hondoh H, Kuriki T and Matsuura Y (2003) Three-dimensional structure and substrate binding of *Bacillus stearothermophilus* neopullulanase. *J Mol Biol* 326(1):177–188
- 150 Kamitori S, Kondo S, Okuyama K, Yokota T, Shimura Y, Tono-zuka T and Sakano Y (1999) Crystal structure of *Thermoactinomyces vulgaris* R-47 α -amylase II (TVAIL) hydrolyzing cyclodextrins and pullulan at 2.6 Å resolution. *J Mol Biol* 287(5):907–921
- 151 Kondo S, Ohtaki A, Tono-zuka T, Sakano Y and Kamitori S (2001) Studies on the hydrolyzing mechanism for cyclodextrins of *Thermoactinomyces vulgaris* R-47 α -amylase 2 (TVAIL). X-ray structure of the mutant E354A complexed with β -cyclodextrin, and kinetic analyses on cyclodextrins. *J Biochem* 129(3):423–428
- 152 Ohtaki A, Kondo S, Shimura Y, Tono-zuka T, Sakano Y and Kamitori S (2001) Role of Phe286 in the recognition mechanism of cyclomaltooligosaccharides (cyclodextrins) by *Thermoactinomyces vulgaris* R-47 α -amylase 2 (TVAIL). X-ray structures of the mutant TVAILs, F286A and F286Y, and kinetic analyses of the Phe286-replaced mutant TVAILs. *Carbohydr Res* 334(4):309–313
- 153 Kamitori S, Abe A, Ohtaki A, Kaji A, Tono-zuka T and Sakano Y (2002) Crystal structures and structural comparison of *Thermoactinomyces vulgaris* R-47 α -amylase 1 (TVAIL) at 1.6 Å resolution and α -amylase 2 (TVAIL) at 2.3 Å resolution. *J Mol Biol* 318(2):443–453

- 154 Yokota T, Tonozuka T, Shimura Y, Ichikawa K, Kamitori S and Sakano Y (2001) Structures of *Thermoactinomyces vulgaris* R-47 α -amylase II complexed with substrate analogues. *Biosci Biotechnol Biochem* 65(3):619–626
- 155 Mizuno M, Tonozuka T, Uechi A, Ohtaki A, Ichikawa K, Kamitori S, Nishikawa A and Sakano Y (2004) The crystal structure of *Thermoactinomyces vulgaris* R-47 α -amylase II (TVA II) complexed with transglycosylated product. *Eur J Biochem* 271(12):2530–2538
- 156 Ohtaki A, Mizuno M, Tonozuka T, Sakano Y and Kamitori S (2004) Complex structures of *Thermoactinomyces vulgaris* R-47 α -amylase 2 with acarbose and cyclodextrins demonstrate the multiple substrate recognition mechanism. *J Biol Chem* 279(30):31033–31040
- 157 Watanabe K, Hata Y, Kizaki H, Katsube Y and Suzuki Y (1997) The refined crystal structure of *Bacillus cereus* oligo-1,6-glucosidase at 2.0 Å resolution: Structural characterization of proline-substitution sites for protein thermostabilization. *J Mol Biol* 269(1):142–153
- 158 Hondoh H, Saburi W, Mori H, Okuyama M, Nakada T, Matsuura Y and Kimura A (2008) Substrate recognition mechanism of α -1,6-glucosidic linkage hydrolyzing enzyme, dextran glucosidase from *Streptococcus mutans*. *J Mol Biol* 378(4):913–922
- 159 Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729
- 160 Ilinkin I, Ye J and Janardan R (2010) Multiple structure alignment and consensus identification for proteins. *BMC Bioinformatics* 11:1–8
- 161 Pei J, Kim B and Grishin NV (2008) PROMALS3D: a tool for multiple protein sequence and structure alignments. *Nucleic Acids Res* 36(7):2295–2300
- 162 Robert X and Gouet P (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res* 42(W1):W320–W324

TABLES

Table 1. Overview of the α -glucan debranching enzymes covered in the present review. See Tables S1 and S2 for details on kinetic constants and relative specific activities of characterised GH13_11–14, GH13_39, and GH57.

GH family	Enzyme name	Glucosidic linkage specificity and substrate preference
13_11	Isoamylase (ISA)	α -1,6 (amylopectin and derived branched maltooligosaccharides)
	Glycogen debranching enzyme (GDE)	α -1,6 (glycogen and amylopectin)
13_12	Pullulanase type I (PULI)	α -1,6 (amylopectin and derived branched maltooligosaccharides and pullulan)
	Pullulanase type II with two catalytic domains (PULII_II)	α -1,4 and α -1,6 (starch, starch derivatives and pullulan)
13_13	Pullulanase type I/limit dextrinase (PULI)	α -1,6 (amylopectin derived branched maltooligosaccharides and pullulan)
13_14	Pullulanase type I (PULI)	α -1,6 (amylopectin and derived branched maltooligosaccharides and pullulan)
	Pullulanase type II with two catalytic domains (PULII_II)	α -1,4 and α -1,6 (starch, starch derivatives and pullulan)
13_20	Pullulan hydrolase/maltogenic α -amylase	α -1,4 and α -1,6 (starch, starch derivatives, pullulan and β -cyclodextrin)
13_31	Oligo- and glucan-1,6- α -glucosidase	α -1,6 (linear isomaltooligosaccharides, panose and dextran)
13_39	Pullulanase type II with one catalytic domain (PULII_I)	α -1,4 and α -1,6 (starch and derivatives and pullulan)
13_41	Pullulanase type II with two catalytic domains (PULII_II)	α -1,4 and α -1,6 (starch and derivatives and pullulan)
57	Pullulanase type II with one catalytic domain (PULII_I)	α -1,4 and α -1,6 (starch and derivatives and pullulan)

Table 2. Overview of the published crystal structures of GH13 α -glucan debranching enzymes.

GH subfamily	Organism	PDB entry	Ligand(s) in active site	Resolution (Å)	References
13_11 ISAs and GDEs	<i>Chlamydomonas reinhardtii</i> CC425	4J7R	None	2.3	[16]
		4OKD	Maltoheptaose	2.4	
	<i>Escherichia coli</i> str. K-12 substr. MG1655	2WSK	None	2.25	[19]
	<i>Pseudomonas amyloclavata</i> SB-15	1BF2	None	2.0	[29]
13_12 PULIs	<i>Streptococcus agalactiae</i>	2VNC	None	3.0	[30]
		2VR5	Acarbose	2.8	
		2VUY	None	3.0	
	<i>Streptococcus pneumoniae</i>	3FAW	None	2.1	[96]
		3FAX	Maltotetraose (only 3 glucose units solved)	2.4	
		2YA0	None	1.85	[82]
13_13 PULIs	<i>Hordeum vulgare</i> , barley	2YA1	2 x maltotetraose	2.25	
		2YA2	G-moranoline	2.37	
		4AIO	None	1.9	[36, 79, 145, 146]
		2Y4S	β -cyclodextrin	2.1	
		2Y5E	α -cyclodextrin	2.49	
		4CVW	Proteinaceous inhibitor	2.67	
		4J3S	Maltotriose + maltotetraose	1.75	
		4J3T	Maltotetraose	1.6	
		4J3U	Maltosyl-S- β -cyclodextrin	1.7	
		4J3V		1.45	

13_14 PULIs		4J3W	Branched thio-linked	1.67	
		4J3X	hexasaccharide	1.75	
			Amylopectin fragment		
			Pullulan fragment		
	<i>Klebsiella pneumoniae/aerogenes</i>	2FGZ	None	1.75	[107]
		2FH6	Glucose	1.8	
		2FH8	Isomaltose	1.9	
		2FHB	2 x maltose	1.8	
		2FHC	2 x maltotriose	1.85	
		2FHF	2 x maltotetraose	1.65	
	<i>Klebsiella oxytoca/pneumoniae</i> UNF 5023	2YOC	None	2.88	
	<i>Anoxybacillus</i> sp. LM18-11	3WDH	None	1.75	[83]
		3WDI	Maltotriose	2.2	
		3WDJ	Maltotetraose	2.22	
	<i>Bacillus acidopullulyticus</i>	2WAN	None	1.65	[147]
	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> str. 168	2E8Y	None	2.11	[148]
		2E8Z	α -cyclodextrin	2.2	
		2E9B	Maltotriose	2.3	
13_20 Maltogenic α - amylases/ cyclomaltodextrinase/ neopullulanase	<i>Bacillus</i> sp.	1EA9	None	3.2	[69]
	<i>Geobacillus stearothermophilus</i> TRS40	1J0H	None	1.9	[149]
		1J0I	Panose	2.4	
		1J0J	Maltotetraose	2.8	
		1J0K	Isopanose	3.2	
	<i>Thermoactinomyces vulgaris</i> R-47	1BVZ	None	2.6	[73, 150-156]
		1G1Y	β -cyclodextrin	3.0	
		1JF5	None	3.2	
		1JF6	None	3.2	
		1JI2	None	2.3	
		1JIB	Maltotetraose	3.3	
		1JL8	β -cyclodextrin	3.2	
		1VB9	4 ³ - α -panosylpanose	2.2	
		1VFK	Acarbose	2.8	
		1VFM	α -cyclodextrin	2.9	
		1VFO	β -cyclodextrin	2.81	
		1VFU	γ -cyclodextrin	3.1	
		1WZK	None	2.3	
		1WZL	None	2.0	
		1WZM	None	3.2	
		2D2O	Maltohexaose	2.1	
		3A6O	Acarbose	2.8	
	<i>Thermus</i> sp. IM6501	1GVI	β -cyclodextrin	3.3	[69, 71]
		1SMA	None	2.8	
	<i>Nostoc punctiforme</i> PCC73102 ATCC29133	2WC7	None	2.37	[72]
		2WCS	None	2.8	
		2WKG	None	3.0	
13_31 Oligo- and glucan-1,6- α -glucosidases	<i>Bacillus cereus</i> ATCC 7064	1UOK	None	2.0	[157]
	<i>Lactobacillus acidophilus</i> NCFM	4AIE	None	2.05	[77]
	<i>Streptococcus mutans</i> ATCC 25175	2ZIC	None	2.2	[158]
		2ZID	Isomaltotriose	2.2	

FIGURES

Fig. 1. Basic structural motifs of the α -glucan substrates amylopectin (a), glycogen (b), and pullulan (c).

Fig. 2. Domain organization (top) of the structure-determined α -glucan debranching enzymes and overall structure of selected GH13_11 and GH13_12–14 (bottom). The domain assignment and the residue numbering are according to the literature references for the structures (see Table 1). Some of domain assignments have been updated according to CAZy [4] and in the cases where there was no primary reference for the structure, the domains have been assigned based on superimposition with other related structures. An asterisk indicates domains lacking in the crystal structures. The colour code of the structures follows the domain organisation overview. Ligands (grey) are shown as stick representation, while catalytic residues (black) are shown as spheres.

Fig. 3. Phylogenetic tree including enzymatically characterised enzymes from GH13 α -glucan debranching subfamilies (see Figure S1 for associated multiple sequence alignment). The phylogenetic tree was made using the Maximum Likelihood method in MEGA6 [159], and it was based on a multiple protein sequence alignment including the catalytic domain of biochemically characterised enzymes from GH13_11 (9 sequences), GH13_12 (5, including two assigned as well to GH13_41), GH13_13 (8), GH13_14 (14), GH13_20 (12), GH13_31 (9), GH13_39 (10), and GH13_41 (44 – *i.e.* all available sequences assigned to this subfamily having a pullulanase domain), which was built using muscle in MEGA6 [159]. The catalytic domains were extracted based on CDD domain assignments (AmyAc_Glg_debranch, AmyAc_Pullulanase_LD-like, AmyAc_CMD, AmyAc_SI_OligoGlu_DGase) [15], while only the pullulanase catalytic domains were included in the case of PULII_IIs.

Fig. 4. Sections of a structure-guided multiple protein sequence alignment including all structures mentioned in Table 1 and the catalytic pullulanase domain from GH13_12 of selected characterised PULII_IIs from and GH13_39 PULII_Is (see Figure S2 for full alignment). The order of the protein sequences is as aligned by the applied Promals3D alignment algorithm. The consensus structure of the catalytic domain (grey ribbon; catalytic residues are shown as sticks) was generated based on the structures included in the alignment using MAPSCI [160]. Regions I–IV refer to the four conserved motifs of all GH13 proteins [6]. Regions V–VII are additional specificity motifs. The general motifs are given below regions I–IV: X, usually a hydrophobic residue; B, usually a hydrophilic residue; Z, a residue important for specificity [6]. The catalytic residues are indicated by asterisks, while green dashed boxes indicate residues of special interest and mentioned in the discussion. The structure guided alignment was generated using Promals3D [161] and visualised using ESPrpt 3.0 [162].

Fig. 5. Active site architecture of α -glucan debranching enzymes. **a** GH13_11 (PDB entry 4OKD); **b** GH13_13 (PDB entry 4J3W); **c** GH13_20 (PDB entry 1J0J); and **d** GH13_31 (PDB entry 2ZID). Ligands from the respective structures are shown as sticks.

Fig. 6. Differences and similarities of the structure determined α -glucan debranching GH13 enzymes. See text for description of specific residues labelled in the multipanel figure. **a** Superimposition of the catalytic residues and ligands at subsite –1 of representative structures of α -glucan debranching GH13 enzymes: GH13_11 (PDB entries 4J7R and 4OKD; maltoheptaose, pink), GH13_13 (PDB entries 4AIO, 4J3W, and 4J3X; 6²- α -D-maltotriosyl-maltotriose, blue; 6³- α -D-glucosyl-maltotriosyl-maltotriose, limon), GH13_20 (PDB entries 1BVZ and 1VB9; 4²- α -panosyl-panose, cyan), GH13_20 (PDB entries 1J0H and 1J0J; maltotetraose, purple), and GH13_31 (PDB entries 2ZIC and 2ZID; isomaltotriose, orange). **b** Superimposition of region II including the branched ligand from PDB entry 4J3W (blue sticks) representative structures from GH13 subfamilies 11 (PDB entries 4J7R and 2VNC; pink and green), 12 (PDB entry 2YA0, wheat), 13 (PDB entry 4AIO, blue), 14 (PDB entry 2E8Y, light blue), 20 (PDB entries 1J0H and 2WC7; purple and white), and 31 (PDB entry 2ZIC; orange). **c** Superimpositions of amino acid residues equal to Val195 of the *Streptococcus mutans* GH13_31 enzyme (colour code as for **b**). The maltopentaose from a *Bacillus subtilis* α -amylase (PDB entry 1BAG, teal sticks) and the branched ligand from the GH13_13 limit dextrinase from barley (PDB entry 4J3W, blue sticks) are included for labelling of subsites. **d** Superimposition of region III of the same structures as in **b** (only exception is that PDB entry 2E8Z is used instead of 2E8Y), with special focus on the tryptophan stacking with the glucose unit in subsite +2. **e** Superimposition of region IV including same structures as in **b**. **f** Superimposition of region VII of all GH13_11 structures (cyan sticks) and representative GH13_12–14 structures (PDB entries 2YA0, 4AIO, and 2E8Y; orange sticks) including the pullulan fragment from PDB entry 4J3X (limon sticks) and the maltoheptaose from PDB entry 4OKD. All structure visualisations were made using PyMOL (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC).

A Fig. 1

Fig. 2

Fig. 3

A
Fig. 4

B

C

D

A Fig. 5

B

C

D

E

F

Fig. 6

	V 165	I 100	VII 60	VI 10
31_4AIE	QPD LN	MDLVVNH	.DNGYDI	VVYQVYPKS
31_2ZIC	QPD LN	MDLVVNH	.DNGYDI	TVYQIYPKS
31_1UOK	QPD LN	MDLVVNH	.DNGYDI	VVYQIYPRS
20_2WCS	LPEFN	LDGVFNH	.NHR YHT	VFYQIFPDR
20_1SMA	MPK LN	LDAVFNH	.NHK YDT	VWYQIFPER
20_1J0H	MPK LN	LDAVFNH	.NHK YDT	VWYQIFPER
20_1EA9	MPK LN	LDAVFNH	.NHK YDT	IFYQIFPER
20_1BVZ	MPK LR	LDAVFNH	.HHK YDT	VIIYQIFPER
39_ADZ99363	LPQTD	LDGVFED	.NHK YDT	AIQALRGNG
39_AAA19800	EYNVT	LDGVFNH	.NHK YDT	VMYQIFPDR
39_AFI70750	LPEFK	LDGVFNH	.NHK YDA	DNPR LKGTSGY
14_2E8Y	GNDIA	LDVVFNH	YNWGYNP	VIIYETHLRD
14_3WDH	GNDLA	LDVVVNH	YNWGYNT	IIYEMHIRD
14_2WAN	GNEFA	MDVVVNH	YNWGYDP	VIIYEAHV RD
13_2YOC	CSDSA	MDVVVNH	YNWGYDP	TIHESHIRD
13_2FGZ	CSDSA	MDVVVNH	YNWGYDP	TIHESHIRD
13_4AIO	MNNTA	MDVVVNH	YNWGYNP	TIYELHIRD
12_2YA0	GGRLG	LDVVVNH	YNWGYDP	VIIYEAHV RD
12+41_CAR47543	GGRLG	LDVVVNH	YNWGYDP	IIYEAHV RD
12_3FAW	GGRLG	LDVVVNH	YNWGYDP	VIIYEAHV RD
12+41_AAS36537	GGRLG	LDVVFNH	YNWGYDP	IIYEVHV RD
11_2VNC	GNTLN	IDVVVNH	NYWGYDP	VIIYEVHVKG
11_2WSK	GNTLN	LDIVLNH	NYWGYNP	IIYEAHV KGLT
11_1BF2	GANFN	MDVVVNH	NYWGYMT	VIIYEVHV RG
11_4J7R	GNTLN	LDVVFNH	NFWGYST	VIIYEAHV RG
		XDXXXNH

	II 200	III 240	IV 310
31_4AIE	GFRMDVIEL	MTVGETWN	FWENHD
31_2ZIC	GFRMDVIDM	LTVGETNG	FWNNHD
31_1UOK	GFRMDVIN F	MTVGEMPG	YWNNHD
20_2WCS	GWRLDVPFE	YILGEVWG	LLASHD
20_1SMA	GWRLDVA NE	YILGEIWH	LLGSHD
20_1J0H	GWRLDVA NE	YILGEIWH	LLGSHD
20_1EA9	GWRLDVA NE	YILGEVWH	LLDSHD
20_1BVZ	GWRLDVA NE	LIVGEIWH	LLDSHD
39_ADZ99363	GWRLDSADN	AIVGEIWN	LVDSQD
39_AAA19800	GWRLDVA NE	PMIAENWG	LLGSHD
39_AFI70750	GWRLDVA NE	LILGEIWD	LIGSHD
14_2E8Y	GFRFDLLGI	LLFGE GWD	YVESHD
14_3WDH	GFRFDLMGI	IILGE GWD	YVECHD
14_2WAN	GFRFDLMAL	VLYGEPT	YVTSHD
13_2YOC	GFRFDLMGY	YFFGE GWD	YVSKHD
13_2FGZ	GFRFDLMLY	YFFGE GWD	YVSKHD
13_4AIO	GFRFDLMGH	YLYGE GWD	YVSAHD
12_2YA0	GFRFDMMGD	IMLGE GWR	YIAAHD
12+41_CAR47543	GFRFDMMGD	IMLGE GWR	YIAAHD
12_3FAW	GFRFDMMGD	IMIGE GWR	YIAAHD
12+41_AAS36537	GFRFDMMGD	VMIGE GVV	YIEAHD
11_2VNC	GFRFDLAAA	KLIAEPWD	YVTSHD
11_2WSK	GFRFDLAAV	KLIAEPWD	LVT AHD
11_1BF2	GFRFDLASV	D LFAEPWA	FIDVHD
11_4J7R	GFRFDLASI	KMIAEA WD	FVAAHD
	GXRDXDXXXZ	XXXG/AEZZZ	XXBBHD
	*	*	*

Figure S1
Full multiple sequence alignment of the catalytic domains from alpha-glucan debranching enzymes used for generating the phylogenetic tree in Fig. 3. The number after the GenBank accession number refers to the residue number at which the catalytic domain was defined to start from.

	1	10	20	30	40
11 BAA11864.1 150	FDWEDEHFFFRKIPFK	DSIIYETHIKGIT	KL.RQDLPENVRR		
11 AAB63356.1 198	...DATSVGSKPTRALK	DEVIIYEVHVRGL	TRN...DDSVPAAEER		
11 AAC76456.1 136	...YDWEDDAPPRTPWGS	STIIYEAHVGKLT	TYL.HPEIPVEIR		
11 BAA29041.1 152	...FDWQGDLPRLYPQK	DLVIYEMHVRGFT	KH...SSSNVEHP		
11 AAK42273.1 156	FEWDDEDFIKGKKVPLK	DTVIYEVHVGKFT	KL.RLDLPENIR		
11 BAB99500.1 150	...FDWGNDRAPRIPYN	ETVIYEAHVGKGMT	MTL.HPDVPEELR		
11 AAM81590.1 156	FEWDDEDFIKGKKVPLK	DTVIYEVHVGKFT	KL.RLDLPENLR		
11 AAP85534.1 244	...FDWEGDTPNLNPMES	LVIIYEAHVRGFT	AH...ASSGVAAP		
11 sp P10342 193	...STGTKPTRAQK	KDDVIYEVHVRGFT	EQ...DTSIPAQY		
12 AAK74446.1 586		EDAVIYEAHVRDFT	SD...PAIAKDLTK		R
12 AAN00098.1 611		EDAVIYEAHVRDFT	SD...RSLDGKLLKN		PF
12+41 AAS36537.1 1272		EDTIIYEVHVRDFT	SD...PNIGEDLKA		QF
12 ACT161883.1 510		EDAVIYEAHVRDFT	SD...KALEGKLTH		PF
12+41 CAR47543.1 1377		EDAVIYEAHVRDFT	AD...PAISDELTA		QF
13 CAA36431.1 431		...MTIHESHIRDL	SAW.DQTVPAELR		
13 AAA25124.1 443		...MTIHESHIRDL	SAW.DQTVPAELR		
13 AAD04189.1 273		...DITIIYELHIRD	FAH.DGTVDSDSR		
13 BAA28632.1 328		...DISIYELHIRD	FAH.DSTVDCNSR		
13 AAD11599.1 330		...DISIYELHIRD	FAH.DSTVDCPFR		
13 AAN26412.1 331		...DISIYELHIRD	FAH.DLTVHPDLR		
13 ABL84490.1 331		...DITIIYELHIRD	FAH.DGTVDSDSR		
13 CED95594.1 470		...EDAVIYEGHIRD	FSIR.DQSTSAAHR		
13 ACN58254.1 339		...EDAVIYETIHAD	ITGF...ENSGVKNR		
13 BAB62095.1 218		...TDAIYIELSIR	DFTSH...PDSGAVHK		
13 AAD30387.1 347		...EDAVIYETIHAD	MTGL...DNSGVKNK		
13 BAF93906.1 464		...TDAIVDELHIR	DFTSS...STSGVSAGN		R
13 AAY89038.1 1075		...TDAIITEANVR	DMTIN...PNSGVSEAN		R
13 AAB06264.1 330		...DAIYIEMHVR	DFTID...QSSGIGENLR		
13 AFL46505.1 460		...TDAIYIESHVR	DLSMF...DVTNSGYDS		GIKQK
13 AJG04842.1 460		...TDAIYIETHVR	DLSMF...DVTNSGYDS		KQK
13 AAD30387.1 347_2		...EDAVIYETIHAD	MTGL...DNSGVKNK		
13 CAC85704.1 218		...TDAIYIELSIR	DFTSH...PDSGAVHK		
13 AAS47565.1 372		...TDAIYIEMHVR	DFTSIY...PDSGNKYK		
13 AEW23439.1 217		...TDAIYIEMHVR	DFTIH...HESGVRQK		
13 CAC60156.1 431		...VDEVIYEAHVR	DFTSID...ANSGMKNK		
13 AAE10887.1 429		...FDEVIYEMDVR	DFTSID...PNSGMKNK		
13 AAC00283.1 213		...VDAVIYETHLR	DFTSIH...ENSGMINK		
13 CAC60157 429		...FDEVIYEMDVR	DFTSID...PNSGMKNK		
13 AAD36907 339		...EDAVIYETIHAD	ITGL...ENSGV...KN		K
20 BAA02473.1 130		...EAVIYQIFPER	FANG...DPSNDPPGT		EQW
20 AAA22622.1 134		...DTVWYQIFPER	FANG...NPSISPEGSR		PW
20 AAC46346.1 134		...DTIWIYQIFPER	FANG...NEALNPAGTL		PW
20 AAC15072.1 134		...DTVWYQIFPER	FANG...NPAISPKGAR		PW
20 AAF23874.1 137		...STVWYQIFPER	FANG...REDLSPKNAL		PW
20 AAK15003.1 134		...DTVWYQIFPER	FANG...NPSISPEGSR		PW
20 ABJ59624.1 136		...VWYQIFPER	FANG...DKSNDPVNV		KPW
20 ABS59068.1 41		...AVWYQIFPER	FANG...DRRNDPKAA		DAAVFNREWSISPWTSDWYKLPW
20 ACC84362.1 11		...HAFVYQIFPDR	FARS...KQPRKRLQE		ARW
20 BAI67607.1 9		...DAVFYQIFPER	FANA...DPSLDPPQNV		PW
20 AFM43699.1 134		...DTVWYQIFPER	FANG...NPAISPKGAR		PW
20 AHW51935.1 133		...NTVWYQIFPER	FANG...NPSRDPDTTL		PW
31 CAA37583.1 7		...KESVVYQIYPR	SFMDS...NGDGI		
31 BAA00534.1 6		...KEAVVYQIYPR	SFYDS...NGDGF		
31 BAA01368.1 7		...KEAVVYQIYPR	SFYDS...NGDGI		
31 BAA11354.1 6		...KKAVVYQIYPR	SFYDT...NGDGI		
31 AAK27723.1 24		...NAVVYQIYPR	SFODS...NGDGI		
31 AAV42157.1 6		...KNAVVYQVYPR	SFODS...NGDGI		
31 BAE79634.1 2		...HKAIVYQIYPR	SFMDT...NGDGI		
31 ACM89182.1 27		...NAVVYQIYPR	SFODT...NGDGF		
31 ACM89183.1 26		...NAVVYQIYPR	SFODT...NGDGL		
39 AAA23205.1 385		...GSVMYQIFPDR	FFNG...DSSNDHLKK		YSRGFPDVEYHSNWWYELPDNPNDKNKLGYTGDGIW
39 AAA19800.1 388		...GAVMYQIFPDR	FFNG...DTSNDHAKT		LSRGNDPIEFHNDNDNDLPDNPNNAGTPGYTGDGIW
39 BAA05832.1 475		...QAVVYQIFPDR	FFNG...NPNNDKVKE		YGRGNPIE.YREWSQLPDNPRMKDTPGYDGDGF
39 AAG44799.1 479		...QAVVYQIFPDR	FFNG...NPNNDKVKK		YARGNEPIE.HREWSQLPDNPRMKATPGYDGDGIW
39 ABR26448.1 397		...EAVVYQIFPDR	FFNG...NPSNDSKQ		QARGAQPIE.HRDWSDLPDNPRLKGTSGYDGDGEW
39 ABY95795.1 385		...GAVMYQIFPDR	FFNG...DPLNDRLEK		YSRGFPDVEYHDDWYDLPDNPNDKDKPGYTGDGIW
39 ACR15165.1 384		...GAVMYQIFPDR	FFNG...DTSNDHAKT		LSRGNDPIEFHNNNDNDLPDNPNNAGTPGYTGDGIW
39 ACV59878.1 541		...HAVIYIIMPDR	FFNG...NIATEENPNTQKGIYVGADGTESLGP		IQFHENWDSPPYPDPNIPPLSDPKIASLRGNGQW
39 ADZ99363.1 550		...HAVIYIIMPDR	FFNG...DIANDENPKTQKGIYTDVGGQETLGP		IQFHQDWNQSPYPDPNIPASSDPAIQALRGNGQW
39 AFI70750.1 397		...EAVVYQIFPDR	FFNG...NPSNDSKQ		QARGAQPIE.HRDWSDLPDNPRLKGTSGYDGDGEW
41 AHY15164.1 St_iniae_1419		...EDAVIYEAHVR	DFTSD...VAISSQLKN		QF
41 AGL48789.1 St_suis_1378		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 AHY17035.1 St_iniae_1419		...EDAVIYEAHVR	DFTSD...VAISSQLKN		QF
41 AGZ24199.1 St_suis_1377		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 ABP93225.1 St_suis_1252		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AER20299.1 St_suis_1389		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 AER22304.1 St_suis_1385		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 ADZ83524.1 Cl_lento_920		...DAIVYELHVR	DMSID...PNAPFINK		
41 AGW88268.1 St_suis_1385		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 AGG65362.1 St_suis_1389		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AIQ28595.1 Pa_FSL_723		...EDAVIYEAHVR	DFTSD...PAIQSSLGG		ERW
41 ADV71067.1 St_suis_1385		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AER16173.1 St_suis_1385		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AIQ51763.1 Pa_FSL_716		...EDAVIYEVHVR	DFTSD...VSISSLGG		ERW
41 CB113953.1 St_gallo_1441		...EDAVIYEAHVR	DFTSD...TAISDELQH		QF
41 CAZ56775.1 St_suis_1377		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AIQ34761.1 Pa_FSL_706		...EDAVIYEVHVR	DFTSD...VSIIVDSLGG		ERW
41 AEZ62583.1 St_infan_1443		...EDAVIYEAHVR	DFTSD...TAISEELRN		QF
41 CCF02755.1 St_maced_1180		...EDAVIYEAHVR	DFTSD...TAISDELQH		QF
41 CBZ48657.1 St_gallo_1441		...EDAVIYEAHVR	DFTSD...TAISDELQH		QF
41 AIQ57390.1 Pa_borea_723		...EDAVIYEAHVR	DFTSD...PAIQSSLGG		ERW
41 AGI39830.1 C_sterc_1095		...EDAVIWEVHVR	DFTSD...PSIESDLNA		RW
41 AEB82319.1 St_suis_1385		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 BAM47868.1 Am_xylan_1322		...EDAVIYEVHVR	DFTSD...PSIADQLTS		QF
41 AFR01291.1 St_suis_1385		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AGM98096.1 St_iniae_1419		...EDAVIYEAHVR	DFTSD...VAISSQLKN		QF
41 ADE32331.1 St_suis_1408		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 AHF60382.1 St_suis_1385		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 CAZ52647.1 St_suis_1377		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 ACX81318.1 St_gallo_1452		...EDAVIYEAHVR	DFTSD...TAISDELQH		QF
41 ABG83674.1 Cl_perfr_883		...TDSIIYEMHVR	DFTSIS...KDSGINNK		
41 AER45198.1 St_suis_1385		...EDAVIYEAHVR	DFTAD...PAISDELTA		QF
41 BAK28316.1 St_gallo_1441		...EDAVIYEAHVR	DFTSD...TAISDELQH		QF
41 CQR54568.1 Pa_riogr_717		...EDAVIYEVHVR	DFTSD...VSISSLGG		ERW
41 AGX06343.1 Ba_infan_1304		...EDAVIYEVHVR	DFTSD...PSIEADLNS		RW
41 AIQ22910.1 Pa_FSL_700		...EDAVIYEVHVR	DFTSD...VSIEDSLSG		ERW
41 AIQ67951.1 Pa_grami_717		...EDAVIYEVHVR	DFTSD...VSISSLGG		ERW
41 AIQ16995.1 Pa_FSL_716		...EDAVIYEVHVR	DFTSD...VSISSLGG		ERW
41 AER18240.1 St_suis_1385		...EDAVIYEAHVR	DFTSD...PAISDELTA		QF
41 AIQ46154.1 Pa_FSL_716		...EDAVIYEVHVR	DFTSD...VSISSLNG		ERW
41 AIQ40360.1 Pa_FSL_723		...EDAVIYEAHVR	DFTSD...PAIQSSLGG		ERW
41 AIY06268.1 Pl_PAMC_1296		...EDAVIYEVHVR	DFTSD...PSIEKELKS		RW
X AAG31033.1 152		...VGYQIFPDR	FFNG...DPSNDLKA		NLTDELWINEVSRGVPVETRW

80

11. BAA11864.1 | 150
11. AAB63356.1 | 198
11. AAC76456.1 | 136
11. BAA29041.1 | 152
11. AAK42273.1 | 156
11. BAB99500.1 | 150
11. AAM81590.1 | 156
11. AAP85534.1 | 244
11. sp|P10342|_193
12. AAK74446.1 | 586
12. AAN00098.1 | 611
12+41. AAS36537.1 | 1272
12. ACI61883.1 | 510
12+41. CAR47543.1 | 1377
13. CAA36431.1 | 431
13. AAA25124.1 | 443
13. AAD04189.1 | 273
13. BAA28632.1 | 328
13. AAD11599.1 | 330
13. AAN26412.1 | 331
13. ABL84490.1 | 331
13. CED95594.1 | 470
13. ACN58254.1 | 339
13. BAB62095.1 | 218
13. AAD30387.1 | 347
13. BAF93906.1 | 464
13. AAY89038.1 | 1075
13. AAB66264.1 | 330
13. AFL46505.1 | 460
13. AJG04842.1 | 460
13. AAD30387.1 | 347_2
13. CAC85704.1 | 218
13. AAS47565.1 | 372
13. AEW23439.1 | 217
13. CAC60156.1 | 431
13. AAE10887.1 | 429
13. AAC00283.1 | 213
13. CAC60157 | 429
13. AAD36907 | 339
20. BAA02473.1 | 130
20. AAA22622.1 | 134
20. AAC46346.1 | 134
20. AAC15072.1 | 134
20. AAF23874.1 | 137
20. AAK15003.1 | 134
20. ABJ59624.1 | 136
20. ABS59068.1 | 41
20. ACC84362.1 | 11
20. BAI67607.1 | 9
20. AFM43699.1 | 134
20. AHW51935.1 | 133
31. CAA37583.1 | 7
31. BAA00534.1 | 6
31. BAA01368.1 | 7
31. BAA11354.1 | 6
31. AAK27723.1 | 24
31. AAV42157.1 | 6
31. BAE79634.1 | 2
31. ACM89182.1 | 27
31. ACM89183.1 | 26
39. AAA23205.1 | 385
39. AAA19800.1 | 388
39. BAA05832.1 | 475
39. AAG44799.1 | 479
39. ABR26448.1 | 397
39. ABY95795.1 | 385
39. ACR15165.1 | 384
39. ACV59878.1 | 541
39. ADZ99363.1 | 550
39. AFI70750.1 | 397
41. AHY15164.1 | St_iniae_1419
41. AGL48789.1 | St_suis_1378
41. AHY17035.1 | St_iniae_1419
41. AGZ24199.1 | St_suis_1377
41. ABP93225.1 | St_suis_1252
41. AER20299.1 | St_suis_1389
41. AER22304.1 | St_suis_1385
41. AD283524.1 | Cl_lento_920
41. AGW88268.1 | St_suis_1385
41. AGG65362.1 | St_suis_1389
41. AIQ28595.1 | Pa_FSL_723
41. ADV71067.1 | St_suis_1385
41. AER16173.1 | St_suis_1385
41. AIQ51763.1 | Pa_FSL_716
41. CBI13953.1 | St_gallo_1441
41. CAZ56775.1 | St_suis_1377
41. AIQ34761.1 | Pa_FSL_706
41. AEZ62583.1 | St_infan_1443
41. CCF02755.1 | St_maced_1180
41. CBZ48657.1 | St_gallo_1441
41. AIQ57390.1 | Pa_borea_723
41. AGI39830.1 | C_sterc_1095
41. AEB82319.1 | St_suis_1385
41. BAM47868.1 | Am_xylan_1322
41. AFR01291.1 | St_suis_1385
41. AGM98096.1 | St_iniae_1419
41. ADE32331.1 | St_suis_1408
41. AHP60382.1 | St_suis_1385
41. CAZ52647.1 | St_suis_1377
41. ACX81318.1 | St_gallo_1452
41. ABG83674.1 | Cl_perfr_883
41. AER45198.1 | St_suis_1385
41. BAK28316.1 | St_gallo_1441
41. CQR54568.1 | Pa_riogr_717
41. AGX06343.1 | Ba_infan_1304
41. AIQ22910.1 | Pa_FSL_700
41. AIQ67951.1 | Pa_grami_717
41. AIQ16995.1 | Pa_FSL_716
41. AER18240.1 | St_suis_1385
41. AIQ46154.1 | Pa_FSL_716
41. AIQ40360.1 | Pa_FSL_723
41. AIY06268.1 | Pl_PAMC_1296
X | AG31033.1 | 152
DGPVTSLHCHCHQYFG.

[illegible]

	110	120	130	140	150
11 BAA11864.1 150	QVIE	FKKLVNSLHNA	GLEVIIDVYVNH	AEG.NHLG	PTLSFRGLD
11 AAB63356.1 198	PTRE	WKAMVKAFHDA	GKIVYIDVYVNH	EGG.GPWSG	TGDLGSVYNL
11 AAC76456.1 136	ALDE	FRDAIKALHKA	GIEVILDLVNLH	AEL.DLDG	PLFSLRGLD
11 BAA29041.1 152	AIN	FKTFVREAHKR	GIEVIMDVYVNH	AEG.NEKG	PLTSFRGLD
11 AAK42273.1 156	QVLS	FKKLVNLSHNA	GIEVIIDVYVNH	AEG.NHLG	PTLSFRGLD
11 BAB99500.1 150	AVA	FKGLVRSYHEA	GIEVILDLVNLH	AEG.NHMG	VSTIAFRGLD
11 AAM81590.1 156	QVIS	FKRMVNLHNA	GIEVIIDVYVNH	AEG.NHLG	PTLSFRGLD
11 AAP85534.1 244	SCDE	FKQLVKECHRR	GIEVILDLVNLH	AEG.NERG	PTLSFRGLD
11 sp P10342 193	PTAE	FOAMVQAFHNA	GIKVYMDVYVNH	AEG.GTWT	SSDPTTATISY
12 AAK74446.1 586	RIA	FKNLLINEIKKR	GMGAILDVYVNH	AKV.DLFE	DLFEN
12 AAN00098.1 611	RIA	LKQLIHDIHKK	GMGVILDVYVNH	AKT.YLFE	DLEFN
12+41 AAS36537.1 1272	RIK	FKNLLINEIKKR	DMGVVLDVYVNH	AQV.HIFE	DLVFN
12 ACI61883.1 510	RIA	LKNLVNEIKKR	GMGVIFDVYVNH	ART.YLFE	DLEFN
12+41 CAR47543.1 1377	RIE	FKNLVNEIKKO	GMGVILDVYVNH	SKT.FLFE	DLEFN
13 CAA36431.1 431	RIK	FRMTIAQIKQDL	GMNVIMDVYVNH	TNAAGPTDRTSVLD	KIVFW
13 AAA25124.1 443	RIK	FRMTIAQIKQDL	GMNVIMDVYVNH	TNAAGPTDRTSVLD	KIVFW
13 AAD04189.1 273	RIIE	YRQMVQALNRI	GLRVVMDVYVNH	DSS.GPCG	ISSVLDKIV
13 BAA28632.1 328	RIIE	YRQMVQALNRI	GLRVVMDVYVNH	DSS.GPGF	VSSVLDKIV
13 AAD11599.1 330	RIIE	YRLMVQALNRI	GLRVVMDVYVNH	YSS.GPFA	ITSVLDKIV
13 AAN26412.1 331	RIIE	FRKMVQALNRI	GLRVVLDVYVNH	LNSSGPGSDNSVLD	KIVFG
13 ABL84490.1 331	RIIE	YRQMVQALNRI	GLRVVMDVYVNH	LDSSGPGCIGISSVLD	KIVFG
13 CED95594.1 470	RVL	LEMMNQALHNI	GLRVVLDVYVNH	TSSASGVNAN	AVLD
13 ACN58254.1 339	RIR	VKEMVKALHRR	DIGVIMDVYVNH	YPTTYGI	GEL.SAFD
14 BAB62095.1 218	RIV	LKQAIHTLHEN	GLRVVMDVYVNH	REQ.SPLE	KLVPFG
14 AAD30387.1 347	RIIE	VKQMVKALHEN	GIRVILDVYVNH	YPTTYGWV	GVM.SPFD
14 BAF93906.1 464	RINE	MKQMVQGLHTN	GISVVMVDVYVNH	QSA.SAFE	KAEFG
14 AAY89038.1 1075	RVK	MKQMVSGLHKN	GLYVVMVDVYVNH	ASE.HAFN	KTVFG
14 AAB06264.1 330	ALK	LRTMIKKLHEN	GIGVVMVDVYVNH	TYHTKGKF	SIFD
14 AFL46505.1 460	RIT	MKQMIQGLHND	GLRAVMDVYVNH	MFD	AKA.SNLD
14 AJG04842.1 460	RIK	MKQMIQGLHND	GLRAVMDVYVNH	MFD	AKA.SNLD
14 AAD30387.1 347_2	RIIE	VKQMVKALHEN	GIRVILDVYVNH	YPTTYGWV	GVM.SPFD
14 CAC85704.1 218	RIV	LKQAIHTLHEN	GLRVVMDVYVNH	REQ.SPLE	KLVPFG
14 AAS47565.1 372	RIR	YKQMVMLNKA	GIRVILDVYVNH	TY	TVGDSPTD
14 AEW23439.1 217	RIIE	LKRAIRAFQOE	GIRVILDVYVNH	YV	RET.SSFE
14 CAC60156.1 431	RIT	LKQLIQSLHQO	RIGVNMVDVYVNH	TDF	VMV.SDFD
14 AAE10887.1 429	RIK	FKEMVLSLHRE	HIGVNMVDVYVNH	TFA	TQI.SDFD
14 AAC00283.1 213	RKT	LKQMINTLHQH	GLRVILDVYVNH	YVK	REN.SPFE
14 CAC60157 429	RIK	FKEMVLSLHRE	HIGVNMVDVYVNH	TFTAT	QI.SDFD
14 AAD36907 339	RIR	VKQMVKALHKK	GIGVIMDVYVNH	YPTTYGI	GEL.SAFD
20 BAA02473.1 130	..T	FRRLVDEAHR	GIKIILDVYVNH	A	GDDQFFAFR
20 AAA22622.1 134	..ET	LKTLIDRCHKEK	GIRVMLDVYVNH	C	GYEFAPFO
20 AAC46346.1 134	..QT	FKRLVELCHQK	AIRVMLDVYVNH	S	GYEFPFQO
20 AAC15072.1 134	..ET	LKTLVLRCHKEK	GIRVMLDVYVNH	C	GYEFAPFO
20 AAF23874.1 137	..ET	FRTLVSLQHR	GMRLMLDVYVNH	I	GSASFPQO
20 AAK15003.1 134	..ET	LKTLIDRCHKEK	GIRVMLDVYVNH	C	GYEFAPFO
20 ABJ59624.1 136	..DL	FAKVNEAHKR	GMKVMLDVYVNH	L	GQDSMQWO
20 ABS59668.1 41	IMARENPADPATWQWTSADKLF	FKELFLAEAKHL	GKQVILDVYVNH	V	SRDFWAFK
20 ACC84367.1 9	..EA	FKELFLDAHQR	NIKVILDVYVNH	S	GTGFFFFH
20 BAI67607 9	..ET	FKHALMAEAKR	GIRVILDVYVNH	C	GDGHWFAP
20 AFM43699.1 134	..ET	LKTLVLRCHKEK	GIRVMLDVYVNH	C	GYEFAPFO
20 AEW51935.1 133	..DT	LKTLIQESHKK	GIRVMLDVYVNH	S	GYEFAPFO
31 CAA37583.1 7	..DE	WDELHEHMER	NMKLMDVYVNH	T	SDE.HNWF
31 BAA00534.1 6	..DB	MFQLIDEVKKR	GMKILMDVYVNH	S	SDE.HAWF
31 BAA01368.1 7	..AB	WMTLDEAHKR	GKILVMDVYVNH	T	SDE.HPWF
31 BAA11354.1 6	..EB	WDELLEAAHKK	GKLVIMDVYVNH	T	

11	BAA11864.1	150
11	AB63356.1	198
11	AA676456.1	136
11	BAA29041.1	152
11	BAK2273.1	156
11	BAB99500.1	150
11	AM81590.1	156
11	AP85534.1	244
11	sp P10342	193
12	AAK74446.1	586
12	AAN00098	611
12+41	AAS36537.1	1272
12	ACI61883.1	510
12+41	CAR47543.1	1377
13	CAA36431.1	431
13	AAA25124.1	443
13	AD04189.1	273
13	BAA28632.1	328
13	AD11599.1	330
13	AA864412.1	331
13	ABL84490.1	331
13	CED95594.1	470
14	CNC58254.1	339
14	BAB62095.1	218
14	AAD30387.1	347
14	BAF93906.1	464
14	AA989038.1	1075
14	AAB06264.1	330
14	AFL46505.1	460
14	AG048442.1	460
14	AAD30387.1	347_2
14	CAC85704.1	218
14	AAS47565.1	372
14	AEW23439.1	217
14	CAC60156.1	431
14	AAE10887.1	429
14	AAC00283.1	213
14	CAC60157.1	429
14	AAD36907.1	339
20	BAA02473.1	130
20	AAA22622.1	134
20	AAC46346.1	134
20	AAC15072.1	134
20	AAF23874.1	137
20	AAK15003.1	134
20	ABJ59624.1	136
20	ABS95068.1	41
20	ACC84362.1	11
20	BAI67607.1	9
20	AFM43699.1	134
20	AHW51935.1	133
31	CAA37583.1	7
31	BAA00534.1	6
31	BAA01368.1	7
31	BAA11354.1	6
31	AAK27723.1	24
31	AAV42157.1	6
31	BAE79634.1	2
31	ACM89182.1	27
31	ACM89183.1	26
39	AAA23205.1	385
39	AAA19800.1	388
39	BAA05832.1	475
39	AA644799.1	479
39	ABR26448.1	397
39	ABY95795.1	385
39	ACR15165.1	384
39	ACV95878.1	541
39	ADZ99363.1	550
39	AFI7050.1	397
41	AHY15164.1	St.iniae 1419
41	AGL48789.1	St.suis 1378
41	AHY17035.1	St.iniae 1419
41	AG224199.1	St.suis 1377
41	ABP93225.1	St.suis 1252
41	AER20299.1	St.suis 1389
41	AER22234.1	St.suis 1385
41	AD283524.1	Cl.lento 920
41	AGW88268.1	St.suis 1385
41	AGG65362.1	St.suis 1389
41	AG265895.1	Pa.FSL 723
41	ADV71067.1	St.suis 1385
41	AER16173.1	St.suis 1385
41	AIQ517763.1	Pa.FSL 716
41	CB113953.1	St.gallo 1441
41	CA256775.1	St.suis 1377
41	AIQ347761.1	Pa.FSL 706
41	AE262583.1	St.infan 1443
41	CCF02753.1	St.maced 1180
41	CB248657.1	St.gallo 1441
41	AIQ57390.1	Pa.borea 723
41	AGT39830.1	C.sterc 1095
41	AE882319.1	St.suis 1385
41	BAM47868.1	Am.xylin 1322
41	AFR01291.1	St.suis 1385
41	AGM98096.1	St.iniae 1419
41	ADE32336.1	St.suis 1408
41	AHF60382.1	St.suis 1385
41	CA256247.1	St.suis 1377
41	ACX81318.1	St.gallo 1452
41	ABG83674.1	Cl.perfr 883
41	AER45198.1	St.suis 1385
41	BAK28316.1	St.gallo 1441
41	CQR54568.1	Pa.riogr 717
41	AGX06343.1	Ba.infan 1304
41	AIQ22910.1	Pa.FSL 700
41	AIQ67951.1	Pa.grami 717
41	AIQ16995.1	Pa.FSL 716
41	AER18240.1	St.suis 1385
41	AIQ46154.1	Pa.FSL 716
41	AIQ40360.1	Pa.FSL 723
41	AIY02668.1	Pl.PAMC 1296
X	AA631033.1	152

		180	190	200	210	
11	BAA11864.1	150	LNLSFRV	LQLVDS	LRVWVLEM	HVDGFRDLA
11	AAB63356.1	198	YNTRRPIA	QNLVDS	LAYWRDAL	GVDGFRDLA
11	AAC76456.1	136	LNLSHPAV	VDDYASAC	LRVWVETC	HVDGFRDLA
11	BAA29041.1	152	FNCNHPVV	REFIVDC	LRVWVTEM	HVDGFRDLA
11	AAK42273.1	156	LNLSHPRV	IQMVDS	LRVWVTEM	HVDGFRDLA
11	BAB99500.1	150	LNVRDPHS	LQLVDS	LRVWVTEM	HVDGFRDLA
11	AAM81590.1	156	LNLSHPRV	IQMVDS	LRVWVTEM	HVDGFRDLA
11	AAP85534.1	244	LNLCNQPVV	RQFILD	LKHVVTEY	HVDGFRDLA
11	sp P10342	193	FNTYNTVA	QNLVDS	LAYWANTM	GVDGFRDLA
12	AAK74446.1	586	LGTTTHMT	KRLVDS	IKYLVDTY	KVDGFRDMM
12	AAN00098.1	611	LGTTTHMS	RRVLDS	IKYLTSEF	KVDGFRDMM
12+41	AAS36537.1	1272	LGTTTHMS	RRVLDS	IKHVVDEY	KVDGFRDMM
12	ACI61883.1	510	LGTTTHMS	RRVLDS	ITYLTREF	KVDGFRDMM
12+41	CAR47543.1	1377	LGTTTHMS	RRVLDS	IKYLVDEY	KVDGFRDMM
13	CAA36431.1	431	SAPHRMF	AKLIADS	LAVWTTDY	KIDGFRDLM
13	AAA25124.1	443	SAPHRMF	AKLIADS	LAVWTTDY	KIDGFRDLM
13	AAD04189.1	273	TASEHFMV	DRLLVDD	LLNWAYNY	KVDGFRDLM
13	BAA28632.1	328	TASEHFMV	DRLLVDD	LLNWAYNY	KVDGFRDLM
13	AAD11599.1	330	TASEHFMV	DRLLVDD	LLNWAYNY	KVDGFRDLM
13	AAN26412.1	331	TASEHFMV	ERLLVDD	LKNWAYNY	KVDGFRDLM
13	ABL84490.1	331	TASEHFMV	DRLLVDD	LLNWAYNY	KVDGFRDLM
13	CED95594.1	470	TATEHRMM	GKLVDS	LVLLAKEY	GVDGFRDVM
14	ACN58254.1	339	JASERPMM	RKFIVDT	VTVVWKEY	HVDGFRDQM
14	BAB62095.1	218	JASERPMM	RRWLVD	VVFWAKEY	GVDGFRDLM
14	AAD30387.1	347	JASERPMM	RKFIVDT	LKWWVTEY	KVDGFRDQM
14	BAF93906.1	464	TASNHEMF	GKYIDS	VTVWAKNY	IDGFRDEM
14	AAY89038.1	1075	VASERAMA	RKYVDS	VKYWATEY	NVDGFRDLM
14	AAB06264.1	330	LATEKPMV	RKFILD	ITYWTEEF	HVDGFRDLM
14	AFL46505.1	460	TASERQMM	HKLIVDS	VSYYWKEY	HVDGFRDLM
14	AJG04842.1	460	TASERQMM	HKLIVDS	VDYYWKEY	HVDGFRDLM
14	AAD30387.1	347_2	JASERPMM	RKFIVDT	LKWWVTEY	KVDGFRDQM
14	CAC85704.1	218	JASERPMM	RRWLVD	VVFWAKEY	GVDGFRDLM
14	AAS47565.1	372	JASERPMM	RKFIVDS	VKYWATEY	KVDGFRDLM
14	AEW23439.1	217	JASERPMM	RKFIVDS	VTVWAKNY	IDGFRDEM
14	CAC60156.1	431	FATEHPMA	QKFVDS	VNYVWKEY	HVDGFRDLM
14	AAE10887.1	429	LXAEAPMV	QKFIDS	LKYVWKEY	HVDGFRDLM
14	AAC00283.1	213	JASERPMM	RKFIVDT	VTVWAKNY	IDGFRDEM
14	CAC60157	429	JASERPMM	RKFIVDT	VTVWAKNY	IDGFRDEM
14	AAD36907	339	JASERPMM	RKFIVDT	VTVWAKNY	IDGFRDEM
20	BAA02473.1	130	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AAA22622.1	134	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AAC46346.1	134	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AAC15072.1	134	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AAF23874.1	137	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AAK15003.1	134	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	ABJ59624.1	136	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	ABS59068.1	41	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	ACC84362.1	11	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	BAI67607.1	9	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AFM43699.1	134	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
20	AHW51935.1	133	LNTEHPMA	QKFVDS	LKYVWKEY	HVDGFRDLM
31	CAA37583.1	7	LHLFSKKQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	BAA00534.1	6	YLHYFSKKQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	BAA01368.1	7	LHLFSKKQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	BAA11354.1	6	YLHYFSKKQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	AAK27723.1	24	FHQYFSKKQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	AAV42157.1	6	LHFFADQQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	BAE79634.1	2	LHFFADQQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	ACM89182.1	27	LHFFADQQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
31	ACM89183.1	26	LHFFADQQPD	LNWVNEKV	RRVYVDM	LNWVNEKV
39	AAA23205.1	385	LNWVNEKV	RRVYVDM	LNWVNEKV	RRVYVDM
39	AAA19800.1	388	LNWVNEKV	RRVYVDM	LNWVNEKV	RRVYVDM
39	BAA05832.1	475	VPYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	AAE44799.1	479	VPYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	ABR26448.1	397	VSYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	ABY95795.1	385	VSYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	ACR15165.1	384	VSYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	ACV59878.1	541	VSYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	ADZ99363.1	550	VSYP	LNWVNEKV	RRVYVDM	LNWVNEKV
39	AFI70750.1	397	VSYP	LNWVNEKV	RRVYVDM	LNWVNEKV
41	AHY15164.1	1419	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AGL48789.1	1378	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AHY17035.1	1419	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AGZ24199.1	1377	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	ABP93225.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AER20299.1	1389	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AER22304.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	ADZ83524.1	920	VASEPMMV	RKFIVDS	IKYLVDEF	KVDGFRDMM
41	AGW88268.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AGG65362.1	1389	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AIQ28595.1	723	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	ADV71067.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AER16173.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AIQ51763.1	716	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	CB113953.1	1441	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	CAZ56775.1	1377	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AIQ34761.1	706	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AEZ62583.1	1443	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	CCF02755.1	1180	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	CBZ48657.1	1441	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AIQ57390.1	723	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AGI39830.1	1095	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AEB82319.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	BAM47868.1	1322	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AFR01291.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AGM98096.1	1419	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	ADE32331.1	1408	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AHF60382.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	CAZ52647.1	1377	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	ACX81318.1	1452	VATERPMMV	RKFIVDS	IKYLVDEF	KVDGFRDMM
41	ABG83674.1	883	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AER45198.1	1385	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	BAK28316.1	1441	PGTTTHMT	RRVLDS	IKYLVDEF	KVDGFRDMM
41	CQR54568.1	717	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AGX06343.1	1304	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AIQ22910.1	700	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AIQ67951.1	717	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AIQ16995.1	716	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AER18240.1	1385	LGTTTHMS	RRVLDS	IKYLVDEF	KVDGFRDMM
41	AIQ46154.1	716	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AIQ40360.1	723	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
41	AIY06268.1	1296	LATS HKMA	EKLIVDS	VKYWFSEY	KIDGMWDM
X	AAG31033.1	152	LNVLNTEV	ROYLINV	AIYWL SI	GFDGLRDTTP

11	BAA11864.1	150	.RQL	.YSVNM	.ST
11	AAB63356.1	198	.NSC	.DAP	.OHG
11	AAC76456.1	136	.RT	.PEFRQ	.DAP
11	BAA29041.1	152	.RGC	.SLWDP	.VNVYGSPVEGDMTTTGTPLA
11	AAK42273.1	156	.REL	.YSVNM	.LNT
11	BAB99500.1	150	.REF	.DDVDR	.LAT
11	AAM81590.1	156	.REL	.YSVNM	.LNT
11	AAP85534.1	244	.RAH	.SAWHPQQYDQETGQVRAMSSGGAIVTAEIMTDGAGVPTGYPLA	.PP
11	sp P10342	193	.NSCLNGAYTASAPNCPNGGYNFDA	.ADSNVA	.NRR
12	AAK74446.1	586	.AAS	.TEE	.TEL
12	AAN00098.1	611	.AAA	.TEL	.TEL
12+41	AAS36537.1	1272	.AES	.EQI	.EQI
12	ACI61883.1	510	.AAA	.EQI	.EQI
12+41	CAR47543.1	1377	.AEA	.TEL	.TEL
13	CAA36431.1	431	.KAO	.LIL	.LIL
13	AAA25124.1	443	.KAO	.LIL	.LIL
13	AAD04189.1	273	.KSA	.LQS	.LQS
13	BAA28632.1	328	.KST	.MIR	.MIR
13	AAD11599.1	330	.KKT	.MIR	.MIR
13	AAN26412.1	331	.KHT	.MVK	.MVK
13	ABL84490.1	331	.KHT	.MVR	.MVR
13	CED95594.1	470	.KOG	.JLD	.JLD
14	ACN58254.1	339	.KKT	.MLE	.MLE
14	BAB62095.1	218	.IET	.MKA	.MKA
14	AAD30387.1	347	.KVT	.MLA	.MLA
14	BAF93906.1	464	.STT	.MKN	.MKN
14	AAV89038.1	1075	.QTT	.MNO	.MNO
14	AAB06264.1	330	.VKQ	.SRQ	.SRQ
14	AFL46505.1	460	.VKT	.MNA	.MNA
14	AJG04842.1	460	.IKT	.MNA	.MNA
14	AAD30387.1	347_2	.KVT	.MLA	.MLA
14	CAC85704.1	218	.IET	.MKA	.MKA
14	AAS47565.1	372	.VET	.MLA	.MLA
14	AEW23439.1	217	.IDT	.MND	.MND
14	CAC60156.1	431	.KDT	.MAK	.MAK
14	AAE10887.1	429	.KDT	.MSK	.MSK
14	AAE00283.1	213	.IDT	.VLY	.VLY
14	CAC60157	429	.KDT	.MSK	.MSK
14	AAD36907	339	.KKT	.MLE	.MLE
20	BAA02473.1	130	.HA	.FWR	.FWR
20	AAA22622.1	134	.HE	.FWR	.FWR
20	AAC46346.1	134	.HQ	.FWR	.FWR
20	AAC15072.1	134	.HQ	.FWR	.FWR
20	AAF23874.1	137	.HV	.FWK	.FWK
20	AAK15003.1	134	.HE	.FWR	.FWR
20	ABJ59624.1	136	.HH	.FWK	.FWK
20	ABS59068.1	41	.HV	.FWK	.FWK
20	ACC84362.1	11	.TPG	.FWQ	.FWQ
20	BAI67607.1	9	.HT	.FWR	.FWR
20	AFM43699.1	134	.HE	.FWR	.FWR
20	AHW51935.1	133	.HD	.FWR	.FWR
31	CAA37583.1	7	.EER	.LPT	.LPT
31	BAA00534.1	6	.YET	.DDSRPYV	.VGR
31	BAA01368.1	7	.ELPDGEPQSGKKYASGSRYMMNGPRVHEF	.LQE	.LQE
31	BAI11354.1	6	.LPE	.GQKYG	.IGK
31	AAK27723.1	24	.SEI	.ADN	.ADN
31	AAV42157.1	6	.KDP	.DKN	.DKN
31	BAE79634.1	2	.KIP	.AQH	.AQH
31	ACM89182.1	27	.SEL	.DDL	.DDL
31	ACM89183.1	26	.SEL	.QDL	.QDL
39	AAA23205.1	385	.HD	.FWV	.FWV
39	AAA19800.1	388	.HD	.FWT	.FWT
39	BAA05832.1	475	.TE	.FWR	.FWR
39	AAG44799.1	479	.TE	.FWR	.FWR
39	ABR26448.1	397	.PA	.FWR	.FWR
39	ABY95795.1	385	.HD	.FWV	.FWV
39	ACR15165.1	384	.HD	.FWT	.FWT
39	ACV59878.1	541	.NFNTA	.WWG	.WWG
39	ADZ99363.1	550	.YNV	.TWS	.TWS
39	AFI70750.1	397	.PA	.FWR	.FWR
41	AHY15164.1	St iniae 1419	.AAS	.TEL	.TEL
41	AGL48789.1	St suis 1378	.AEA	.TEL	.TEL
41	AHY17035.1	St iniae 1419	.AAS	.TEL	.TEL
41	AGZ24199.1	St suis 1377	.AEA	.TEL	.TEL
41	ABP93225.1	St suis 1252	.AEA	.TEL	.TEL
41	AER20299.1	St suis 1389	.AEA	.TEL	.TEL
41	AER22304.1	St suis 1385	.AEA	.TEL	.TEL
41	ADZ83524.1	Cl lento 920	.IET	.MKQ	.MKQ
41	AGW88268.1	St suis 1385	.AEA	.TEL	.TEL
41	AGG65362.1	St suis 1389	.AEA	.TEL	.TEL
41	AIQ28595.1	Pa FSL 723	.ADA	.VOA	.VOA
41	ADV71067.1	St suis 1385	.AEA	.TEL	.TEL
41	AER16173.1	St suis 1385	.AEA	.TEL	.TEL
41	AIQ51763.1	Pa FSL 716	.QDA	.VOA	.VOA
41	CB113953.1	St gallo 1441	.AET	.VOM	.VOM
41	CAZ56775.1	St suis 1377	.AEA	.TEL	.TEL
41	AIQ34761.1	Pa FSL 706	.AEA	.VOH	.VOH
41	AEZ62583.1	St infan 1443	.AET	.VOM	.VOM
41	CCF02755.1	St maced 1180	.AET	.VOM	.VOM
41	CBZ48657.1	St gallo 1441	.AET	.VOM	.VOM
41	AIQ57390.1	Pa borea 723	.ADA	.VOA	.VOA
41	AGI39830.1	C sterc 1095	.YEA	.IQN	.IQN
41	AEB82319.1	St suis 1385	.AEA	.TEL	.TEL
41	BAM47868.1	Am xylan 1322	.AES	.IQL	.IQL
41	AFR01291.1	St suis 1385	.AEA	.TEL	.TEL
41	AGM98096.1	St iniae 1419	.AAS	.TEL	.TEL
41	ADE32331.1	St suis 1408	.AEA	.TEL	.TEL
41	AHF60382.1	St suis 1385	.AEA	.TEL	.TEL
41	CAZ52647.1	St suis 1377	.AEA	.TEL	.TEL
41	ACX81318.1	St gallo 1452	.AET	.VOM	.VOM
41	ABG83674.1	Cl perfr 883	.TKT	.MSE	.MSE
41	AER45198.1	St suis 1385	.AEA	.TEL	.TEL
41	BAK28316.1	St gallo 1441	.AET	.VOM	.VOM
41	CQR54568.1	Pa riogr 717	.YDA	.VOA	.VOA
41	AGX06343.1	Ba infan 1304	.YPS	.IQN	.IQN
41	AIQ22910.1	Pa FSL 700	.AEA	.VON	.VON
41	AIQ67951.1	Pa gram 717	.SDA	.VOA	.VOA
41	AIQ16995.1	Pa FSL 716	.ADA	.VOA	.VOA
41	AER18240.1	St suis 1385	.AEA	.TEL	.TEL
41	AIQ46154.1	Pa FSL 716	.QDA	.VOA	.VOA
41	AIQ40360.1	Pa FSL 723	.ADA	.VOA	.VOA
41	AIY06268.1	Pl PAMC 1296	.YPS	.IQK	.IQK
X	AAG31033.1	152	.SES	.EFR	.EFR

239

240

250

260

270

11

BAA11864.1

150

FFVA

..I

QDD

..PILS

..OV

..

KLIA

EP

DVGGGGYQVGNFPY

..

LW

..

AE

WN

G

KYR

LD

TRRF

11

AAB63356.1

198

CFNF

DKND

SGNALNRIVAE

LP

PR

PAAGGAA

..

DLIA

EP

DAIGGNSYQVGGPPA

..

GW

..

AE

WN

G

KYR

LD

TRRF

11

AACT76456.1

136

LFTA

..I

QNC

..PVLIS

..OV

..

KLIA

EP

DIAPGGGYQVGNFPY

..

LF

..

AE

WN

G

KYR

LD

TRRF

11

BAA29041.1

152

LIDM

..I

SND

..PILIG

..DV

..

KLIA

EP

DAAGGYQVGGPPH

..

W

..

KI

WSE

WN

G

KYR

LD

TRRF

11

AAK42273.1

156

FFTA

..I

QDD

..PILIS

..OV

..

KLIA

EP

DVGGGGYQVGNFPY

..

QW

..

AE

WN

G

KYR

LD

TRRF

11

BAB99500.1

150

FFDL

..LV

QDD

..PVVS

..OV

..

KLIA

EP

DVGGGGYQVGNFPY

..

LW

..

TE

WN

G

KYR

LD

TRRF

11

AAM81590.1

156

FFTA

..I

QDD

..PILIS

..OV

..

KLIA

EP

DVGGGGYQVGNFPY

..

QW

..

AE

WN

G

KYR

LD

TRRF

11

AAP85534.1

244

LVES

..DC

SED

..PILR

..NT

..

KMAI

EP

DCDGLNQVGAFPHY

..

GRW

..

SE

WN

G

KYR

LD

TRRF

11

sp|P10342|

193

LIRE

..F

TVR

..P

AAGSGS

..

DLFA

EP

DAIGGNSYQVGGPPH

..

QGW

..

SE

WN

G

KYR

LD

TRRF

12

AAK74446.1

586

AYKA

..

RAIN

..

NL

..

IMLG

EP

WRTYAGDENMPTKAA

..

DDQW

..

MK

HT

DT

AV

FSD

DIRNN

LKSG

12

AAN00098.1

611

AYKE

..

KAIN

..

NM

..

IMIG

EP

WRTFAGDQGGQPVKPA

..

DDQW

..

MS

KT

DT

VG

FSD

DIRNN

LKSG

12

124-1|AAS36537.1|

1272

AFDE

..

KKIN

..

NI

..

VMIG

EP

WTFAGDEGEVPVQAA

..

DDQW

..

MQ

YT

E

AV

FSD

EFNNEL

LKSG

12

ACI61883.1

510

AFKA

..

KAIN

..

NI

..

IMIG

EP

WRTYQGGDEGKKEIAA

..

DDQW

..

MK

AT

NT

VG

FSD

DIRNN

LKSG

12

124-1|CAR47543.1|

1377

AFTE

..

QKIN

..

NI

..

IMLG

EP

WRTFTGDANQPVQPA

..

DDQW

..

MS

KT

DT

VG

FSD

EFNNEL

LKSG

13

CAA36431.1

431

AWER

..

KALN

..

DI

..

YFFG

EP

DSNQSDRFEIA

..

SQIN

..

LK

GT

G

IG

FSD

RLRDS

VRRG

13

AAA25124.1

443

AWER

..

KALN

..

DI

..

YFFG

EP

DSNQSDRFEIA

..

SQIN

..

LK

GT

G

IG

FSD

RLRDS

VRRG

13

AAD04189.1

273

LITD

..

HGVD

GS

KI

..

YLYG

EP

DFAEVARNQRGNG

..

SQIN

..

MS

GT

G

IG

FSD

RLRDS

VRRG

13

BAA28632.1

328

AKSA

..

RSLT

..R

DV

HGVYGS

KI

LYG

EP

DFGEVAQNKRGINA

..

SQIN

..

MS

GT

G

IG

FSD

RLRDS

VRRG

13

AAD11599.1

330

AKSA

..

QSLT

..I

DE

HGVYGS

KI

LYG

EP

WNFGEVARNQRGNG

..

SQIN

..

MS

GT

G

IG

FSD

RLRDS

VRRG

13

AAN26412.1

331

ATNM

..

QGLS

..K

NI

DGVEGSS

KI

LYG

EP

DFGEVANNRGNVA

..

SQIN

..

LG

GT

G

IG

FSD

RLRDS

VRRG

13

ABL84490.1

331

AKAA

..

QSLT

..R

DA

HGVYGS

KI

LYG

EP

DFAEVARNQRGNG

..

SQIN

..

MS

GT

G

IG

FSD

RLRDS

VRRG

13

CED95594.1

470

ARTA

..

QAVD

..

DI

..

YFYG

EP

WNFGEVADNRLEFQA

..

TQAN

..

MAG

S

E

VG

FSD

RI

REAV

VRRG

14

ACN58254.1

339

VEKA

..

HKID

..

TI

..

ILYG

EP

..

GGWGAPIRFG

..

KND

..

VAG

T

HVAA

FSD

EP

RD

LRGS

14

BAB62095.1

218

VRDA

..

DAID

..

SI

..

LVYG

EP

..

DLPTPLPPEQKA

..

TMAN

..

AQ

QL

PR

FAY

FSD

EP

RD

LRGS

14

AAD30387.1

347

IKSE

..

SKIE

..

SV

..

VLYG

EP

..

GGWGAPIRFG

..

KAD

..

VGT

T

GIAA

FSD

EP

RD

LRGS

14

BAF93906.1

464

LRAA

..

TALD

..

HI

..

IMYG

EP

..

GDSNANNIPE

..

TSIN

NY

KNV

P

GIG

FNP

GB

DA

..

14

AAV89038.1

1075

VRAA

..

DEID

..

KA

..

LVIG

EP

DMTDAIGNQET

..

TOPN

..

ASK

V

KG

VAF

FSD

SL

RD

LKGS

14

AAB06264.1

330

VANE

..

RKRN

..

SA

..

LVYG

EP

VMGNSSTCLVEEMATILSSCHQY

..

..

SIG

L

FND

RI

RD

LRGD

14

AFL46505.1

460

VKQA

..T

KRID

..

SA

..

LVFG

EP

..

SLGTPLPDEKA

..

NOTN

..

TK

QM

P

GIG

H

FND

NL

RD

LKGS

14

AJG04842.1

460

VKRT

..T

TKID

..

SA

..

LVFG

EP

..

SLGTPLPEAKA

..

NOTN

..

AK

QM

P

GIAH

FND

NL

RD

LKGS

14

AAD30387.1

347_2

IKSE

..

SKIE

..

SV

..

VLYG

EP

..

GGWGAPIRFG

..

KAD

..

VGT

T

GIAA

FSD

EP

RD

LRGS

14

CAC85704.1

218

VRDA

..

DAID

..

SI

..

LVYG

EP

..

DLPTPLPPEQKA

..

TMAN

..

AQ

QL

PR

FAY

FSD

EP

RD

LRGS

14

AAV47565.1

372

VQEA

..

HQID

..

SI

..

IIFYG

EP

QAGGSPPLPANLOFTKG

..

KQRG

..

..

TRIA

V

FND

H

RD

LRKGS

14

AEW23439.1

217

VRAA

..

DEID

..

TV

..

ILYG

EP

..

DLATPLPSEKKT

..

TIAN

..

AK

HT

P

RIAY

FSD

EP

RD

VYKGS

14

CAC60156.1

431

ISNE

..

HAIN

..

GI

..

VLYG

EP

WTGGTSGLSDDQLVTKG

..

QQKG

..

..

LIG

V

FND

N

RD

L

DGN

14

AAE01887.1

429

AASE

..

HAIN

..

GI

..

ALYG

EP

WTGGTSALPDDQLLTKG

..

AQKG

..

..

MGV

V

FND

N

RD

L

VGN

14

CAC00283.1

213

MKEK

..

TKAK

..

GI

..

LFYG

EP

..

DLATPLPHEQKA

..

ALAN

..

AP

RM

P

GIG

V

FND

MF

RD

VKGN

14

CAC60157.1

429

AASE

..

HAIN

..

GI

..

ALYG

EP

WTGGTSALPDDQLLTKG

..

AQKG

..

..

MGV

V

FND

N

RD

L

VGN

14

VAA36907.1

339

VERA

..

HKID

..

TI

..

ILYG

EP

..

GGWGAPIRFG

..

KSD

..

..

HVAA

FSD

EP

RD

LRGS

20

BAA02473.1

130

EFRK

..

KSLN

..

DA

..

LIVG

EP

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

..

		280	290	300
11	BAA11864.1	150	W.....RGD..PVPY.....	EEETAN.....RLGSPDLYAG.....
11	AAB63356.1	198	Q.....NKIGHV.....	PGTILAT.....RFAGSNDLYGD.....
11	AAC76456.1	136	W.....LHY..DLPL..TG	..GAFAG.....RFASSDVFKR.....
11	BAA29041.1	152	I.....KGT..DGF.....	..AGGFAE.....CLCGSPHYLQA.....
11	AAK42273.1	156	W.....RGE.....	..SEIAN.....RLCGSPDIYLG.....
11	BAB99500.1	150	W.....RGE..PATL..	..GEFAS.....RLTGSSDIYAN.....
11	AAM81590.1	156	W.....RGE.....	..ALP..YSEIAN.....RLCGSPDIYLG.....
11	APR85534.1	244	I.....KGTDGPWAGDFA.....	..SAICGSFNIIYAN.....NTPHETDWWANNGGRQWK.....
11	sp P10342	193	Q.....NELGS..MTIY.....	..VTQDAN.....DFSGSSNLFSQ.....
12	AAK74446.1	586	Y.....PNEGQ..PAFI..TG	..GKRD..VNTIIFK.....NLIAOPTNF.....
12	AAN00098.1	611	F.....PNEGQ..PAFI..TG	..GKRD..VNTIIFK.....NLIAOPTNF.....
12	AAS36537.1	1272	F.....PNEGQ..PAFI..TG	..GKRD..VNTIIFK.....NLIAOPTNF.....
12	ACI61883.1	510	F.....PNEGQ..PAFI..TG	..GKRD..VNTIIFK.....NLIAOPTNF.....
12	CAR47543.1	1377	F.....PNEGQ..PAFI..TG	..GKRD..VNTIIFK.....NLIAOPTNF.....
13	CAA36431.1	431	G.....PFDG..DALRQNGGIGSGAGVLPNELASLSDQVRHLADLTRLGM	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	AAA25124.1	443	G.....PFDG..DALRQNGGIGSGAGVLPNELASLSDQVRHLADLTRLGM	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	AAD04189.1	273	N.....PFCNPLQOGFN..TGLFLEPNNGFYQGNEDTRRLATYADHIQIGL	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	BAA28632.1	328	N.....PFCNPLQOGFN..TGLFLEPNNGFYQGNEDTRRLATYADHIQIGL	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	AAD11599.1	330	S.....PFCNPLQOGFN..TGLFLEPNNGFYQGNEDTRRLATYADHIQIGL	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	AAN26412.1	331	G.....PFCNPLQOGFN..TGLFLEPNNGFYQGNEDTRRLATYADHIQIGL	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	ABL84490.1	331	N.....PFCNPLQOGFN..TGLFLEPNNGFYQGNEDTRRLATYADHIQIGL	..AGNLADPVMIDKDGAAKKGSEIDYNGAPGGY.....
13	CED95594.1	470	A.....LFATESKDDYLRDQ.....	..TLRLSLAGNLQNYVLKDFNGNSAKGSSFSWNSQPSAY.....
13	ACN58251.1	339	V.....FNPVS..KGFA..MG	..GYKK..ETKIKR.....GVVGSINYDGKLLKS.....
13	BAB62095.1	218	T.....FHLPD..RGFALGNP	..GGREGVKLAIAG.....SLRALGGFL.....
13	AAD30387.1	347	V.....FNATV..KGFL..MG	..ALAK..ETGVKRR.....GVAGSIEYDEVIRS.....
13	BAF93906.1	464	SNNGSGAGGFA..AG	..NTAS..TTTVAG.....ALLASGSWNGNGTVQ.....
13	AAY89038.1	1075	V.....FSDED..TGFI..AG	..KADK..ENLIAT.....NVLGCNNKREGIDENGHCN.....
13	AAB06264.1	330	L.....DGY..KTGYV..HG	..NLSD..VGRILKQ.....GKKAIDDF.....
13	AFL46505.1	460	V.....FEDTD..AGFI..MG	..KAGL..ETRIKRR.....GIVGEIAYDKDIOG.....
13	AJG04842.1	460	V.....FEDTE..AGFV..MG	..KTGL..ETRIKRR.....GIVGEIAYNKETIG.....
13	AAD30387.1	347_2	V.....FNATV..KGFL..MG	..ALAK..ETGVKRR.....GVAGSIEYDEVIRS.....
13	CAC85704.1	218	T.....FHLPD..RGFALGNP	..GGREGVKLAIAG.....SLRALGGFL.....
13	AAS47565.1	372	DNDGT..IKGYV..QG	..MVNQ..RDNVIR.....GIMGAIDDF.....
13	AEW23439.1	217	T.....FDIHE..RGFA..LG	..DCSY..KEAVIG.....AIRGSIHFL.....
13	CAW60156.1	431	V.....FDKTA..QGFA..TG	..DPNQ..VDVKNK.....GVIGSIQDF.....
13	AAE10887.1	429	V.....FDSSA..QGFA..TG	..ATGL..TDAIKN.....GVEGSIQDF.....
13	AAC00283.1	213	T.....FHKA..TGFA..LG	..NGES..AQAVMH.....GVEGSIQDF.....
13	CAC60157	429	V.....FDSSA..QGFA..TG	..ATGL..TDAIKN.....GVEGSIQDF.....
13	AAD36907	339	V.....FNPVS..KGFA..MG	..GYKK..ETKIKR.....GVVGSINYDGKLLKS.....
20	BAA02473.1	130	F.....AKEEI.....AT	..GEIH..AERFDA.....ELTRARMLY.....
20	AAA22622.1	134	F.....AQHEIRASQFV.....	..SARQ.....QMMHVLHSY.....
20	AAC46346.1	134	F.....AKEEI.....AK	..GMGT.....HVLHSY.....
20	AAC15072.1	134	F.....AKEEI.....AD	..EDMS..ASEFAD.....RLMHVLSY.....
20	AAF23874.1	137	F.....AKEEI.....AD	..QTIS..ASRMAH.....RVNAHLMNG.....
20	AAK15003.1	134	F.....AKEEI.....FK	..GARQ.....QMVHVLHSY.....
20	ABJ59624.1	136	F.....INKKE.....RI	..HKLD..GKELSE.....HLTDQLMMY.....
20	ABS59068.1	41	A.....AGDRVVLEQVQ.....SR	..SPTQ..FDSILA.....HVRRSY.....
20	ACC84362.1	11	T.....INKE.....RI	..DYQP..YPPFLA.....AEYATKIQEVLQLY.....
20	BAI67607.1	9	F.....INKE.....RI	..GGID..ASALAA.....GLNALHAEI.....
20	AFM43699.1	134	F.....AKEEI.....SH	..SARQ..FADIMV.....RLHLSY.....
20	AHW51935.1	133	A.....LHQQS.....SH	..DAQV.....ALTKHLSY.....
31	CAA37583.1	7	W.....GKWMKPPF.....	..DVKPCS..LLTLKE.....NLTKWQKA.....
31	BAA00534.1	6	N.....SGPGG..KWDI..TG	..DPIA..LKKTMT.....RWQTA.....
31	BAA01368.1	7	S.....PAGKWKLPF.....	..RPWS..LADLKK.....TMKWKQKE.....
31	BAA11354.1	6	I.....DQSGS..KWNT..TG	..DLVE..LKQILS.....RWQYE.....
31	AAK27723.1	24	P.....GKEKW.....	..VPFE..VKNLRD.....RMTEQOEA.....
31	AAV42157.1	6	Y.....VKEL.....	..DLKPLD..LGECLKK.....VLVWQTKI.....
31	BAE79634.1	2	K.....PLPLDLPG.....	..NVPA..LKTIFN.....KWQTEL.....
31	ACM89182.1	27	W.....DDKPPWTPADL.....	..ETKL..AEQQDA.....RIMAGYQTA.....
31	AAA23205.1	385	ILDKSFDDGNGVHNPI.....	..D.....AAKLDO.....RLMSIYERY.....
31	AAA19800.1	388	LIGQSFDDGNGVHNPI.....	..NPID..AAKLDO.....RLMSIYERY.....
31	BAA05832.1	475	L.....RNGK.....	..AEEIDA.....RLTAIREDY.....
31	AAG44799.1	479	L.....RNGK.....	..AEEIDA.....RLTAIREDY.....
31	ABR26448.1	397	L.....KNGN.....	..AEEADK.....RLTAIREDY.....
31	ABY95795.1	385	ILDKQFDDGNGVHNPI.....	..NPID..AAKLDO.....RLMSIYERY.....
31	ACR15165.1	384	LIGQSFDDGNGVHNPI.....	..NPID..AAKLDO.....RLMSIYERY.....
31	ACV59878.1	541	F.....RGTYNDGNSV..QH	..HADV..AAGFNQ.....ELMRLYSEY.....
31	ADZ99363.1	550	F.....RGTYNDGNSV..QH	..HADV..AAGFNQ.....ELMRLYSEY.....
31	AFI70750.1	397	L.....KNGN.....	..AEEADK.....RLTAIREDY.....
41	AHY15164.1	1St iniae_1419	F.....GNEGE..PAFL..TG	..GSRS..INGLFS.....NIKAOPTNF.....
41	AGL48789.1	1St suis_1378	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AHY17035.1	1St iniae_1419	F.....GNEGE..PAFL..TG	..GSRS..INGLFS.....NIKAOPTNF.....
41	AGZ24199.1	1St suis_1377	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	ABP93225.1	1St suis_1252	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AER20299.1	1St suis_1389	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AER22304.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AD283524.1	1Cl lento_920	S.....DGAGK..GFA..TG	..ATGQ..ESSIVE.....GVKGAINTF.....
41	AGW88268.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AGG65362.1	1St suis_1389	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AIQ28595.1	1Pa FSL_723	Y.....GSEGD..PRFI..TG	..GARP..LTTILN.....NIKAOPGNF.....
41	ADV71067.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AER16173.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AIQ51763.1	1Pa FSL_716	F.....GSEGE..PRFI..TG	..GARS..IATILN.....NIKAOPGNF.....
41	CB113953.1	1St gallo_1441	Y.....PSEGA..AAFI..TG	..GAKD..LOTILN.....NIKAOPGNF.....
41	CAZ56775.1	1St suis_1377	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AIQ34761.1	1Pa FSL_706	Y.....GSEGD..PRFI..TG	..GARP..LTTILN.....NIKAOPGNF.....
41	AEZ62583.1	1St infan_1443	Y.....PSEGA..PCFI..TG	..GVKN..LOELFN.....SIKAOPNNF.....
41	CCF02755.1	1St maced_1180	Y.....PSEGA..AAFI..TG	..GAKD..LOTILN.....NIKAOPGNF.....
41	CBZ48657.1	1St gallo_1441	Y.....PSEGA..AAFI..TG	..GAKD..LOTILN.....NIKAOPGNF.....
41	AIQ57390.1	1Pa borea_723	Y.....GSEGD..PRFI..TG	..GARP..LTTILN.....NIKAOPGNF.....
41	AGI39830.1	1C sterc_1095	F.....GHEGE..PRFI..TG	..GARD..IQTIFN.....NIKAOPGNF.....
41	AEB82319.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	BAM47868.1	1Am xylan_1322	F.....GSEGD..PRFI..TG	..GARN..IQTIFN.....NIKAOPGNF.....
41	AFR01291.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AGM98096.1	1St iniae_1419	F.....GNEGE..PAFL..TG	..GSRS..INGLFS.....NIKAOPTNF.....
41	ADE32331.1	1St suis_1408	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AHF60382.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	CAZ52647.1	1St suis_1377	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	ACX81318.1	1St gallo_1452	Y.....PSEGA..AAFI..TG	..GAKD..LOTILN.....NIKAOPGNF.....
41	ABG83674.1	1Cl perfr_883	S.....DGAGK..GFA..TG	..ASGK..EGAIYA.....GVKGSVDDF.....
41	AER45198.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	BAK28316.1	1St gallo_1441	Y.....PSEGA..AAFI..TG	..GAKD..LOTILN.....NIKAOPGNF.....
41	CQR54568.1	1Pa riogr_717	Y.....GSEGE..PRFI..TG	..GARS..IAAIFN.....NITARPSNI.....
41	AGX06343.1	1Ba infan_1304	F.....GSEGE..PRFI..TG	..GARD..LGVIFN.....NIKAOPGNF.....
41	AIQ22910.1	1Pa FSL_700	Y.....GSEGD..PRFI..TG	..GARP..IATIFN.....NIKAOPGNF.....
41	AIQ67951.1	1Pa gram_717	Y.....GSEGE..PRFI..TG	..GARS..IATIFN.....NITARPSNI.....
41	AIQ16995.1	1Pa FSL_716	Y.....GSEGE..PRFI..TG	..GARS..IETILD.....NIKAOPGNF.....
41	AER18240.1	1St suis_1385	Y.....PNEGQ..PAFI..TG	..GAKS..VESVFN.....NIKAOPGNF.....
41	AIQ46154.1	1Pa FSL_716	F.....GSEGE..PRFI..TG	..GARS..IATILN.....NIKAOPGNF.....
41	AIQ40360.1	1Pa FSL_723	Y.....GSEGD..PRFI..TG	..GARP..LTTILN.....NIKAOPGNF.....
41	AIY06268.1	1Pl_PAMC_1296	F.....GSEGE..PRFI..TG	..GARN..VATIFN.....NIKAOPGNF.....
X	AAG31033.1	152	A.....RGA..LNGYT.....	..ASMK..LAEYIA.....GIL.....

11 |BAA11864.1| 150SNKT|FAS|IN|YIT|S|D|G|F|T|I|Q|DLV|SYN|QKHNEAN.....KLNNEDGMNENYSWNCGVEGETNDSNLYCREKQR|NF|FV
11 |AAB63356.1| 198DGRK|P|WHS|IN|FV|V|A|D|G|F|T|I|N|D|V|AYND|KQNNQ.....GGIVAE|QRRKAA|T|G|L
11 |AAC76456.1| 136NGRL|P|SAA|IN|LV|T|A|D|G|F|T|I|R|D|C|V|CNH|KHNEAN.....GEENRD|GTNNNYS|NNHKK|G|G|G
11 |BAA29041.1| 152GGRK|P|WHS|IN|FV|C|A|D|G|F|T|I|A|D|V|T|YN|KKY|NSSN.....GEDNR|DGNH|NLS|WNC|G|E|E|G|E|F|A|G|S|L|RR|K|K|Q|N|F|F
11 |AAK42273.1| 156NNKT|P|FAS|IN|YV|T|S|D|G|F|T|I|E|D|V|T|YN|QKHNEAN.....GFNNQ|DGMNENYS|WNC|G|A|E|G|P|T|D|N|Q|V|V|I|C|R|K|Q|N|F|F
11 |BAB99500.1| 150NGRR|P|FAS|IN|FV|T|A|D|G|F|T|I|N|D|V|S|YN|E|K|H|N|N|A.....GEDGR|DGE|SHNR|S|WNC|G|V|E|G|P|T|D|D|E|I|M|Q|L|R|A|Q|O|R|N|F|L
11 |AAM81590.1| 156NNKT|P|FAS|IN|YV|T|S|D|G|F|T|I|E|D|V|T|YN|QKHNEAN.....GFNNQ|DGMNENYS|WNC|G|T|E|G|T|T|D|N|Q|V|I|M|C|R|K|Q|N|F|F
11 |AAP85534.1| 244GGRG|P|HAS|IN|FV|V|A|D|G|F|T|I|A|D|V|A|YNN|KHN|EAN.....GENNR|DGE|QHNN|S|WNC|G|E|E|G|P|T|T|K|W|E|V|N|L|R|Q|R|Q|M|N|L|T
11 |sp|P10342| 193SGRS|P|WNS|IN|F|D|V|D|G|M|T|I|K|D|V|Y|SCN|G|A|N|S|Q|A.....WPY|G|P|S|D|G|G|T|S|T|N|Y|S|W|D|Q|G|S|A|G.....TGA|A|V|D|Q|R|R|A|R|T|G|M
12 |AAK74446.1| 586EADS|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|K.....AENYAE|I|H|R|R|L|R|I|G|N
12 |AAN00098.1| 611EADS|P|GDV|V|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|K|S|I|K|K|D|P|K|V.....AEEI|H|R|R|L|R|I|G|N
12+41 |AAS36537.1| 1272MADQ|P|GDV|V|Q|Y|I|A|A|D|N|L|T|I|Y|D|V|I|A|Q|S|I|K|K|D|P|E|I.....AEND|E|I|H|R|R|I|V|G|N
12 |ACI61883.1| 510EADA|P|GDV|V|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|K|S|I|K|K|D|P|K|V.....AEEI|H|R|R|L|R|I|G|N
12+41 |CAR47543.1| 1377LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENY|E|I|H|Q|R|I|G|N
13 |CAA36431.1| 431AAD|P|TEV|V|N|Y|V|S|K|D|N|L|T|I|W|D|M|I|S|Y|K|A|Q|E|A|D|L.....ATRV|R|M|Q|A|V|S|L
13 |AAA25124.1| 443AAD|P|TEV|V|N|Y|V|S|K|D|N|L|T|I|W|D|M|I|S|Y|K|A|Q|E|A|D|L.....DTRV|R|M|Q|A|V|S|L
13 |AAD04189.1| 273DGLP|V|G|Y|T|A|S|P|I|E|T|I|N|Y|V|S|A|D|N|E|T|I|F|D|V|I|S|V|K|T|P|M|L|S|V.....DTRV|R|M|Q|A|V|S|L
13 |BAA28632.1| 328DGSP|V|G|Y|T|S|S|P|V|E|T|I|N|Y|V|S|A|D|N|E|T|I|F|D|I|V|S|I|K|T|P|I|G|L|S|I.....DECR|I|N|H|I|A|S
13 |AAD11599.1| 338DGSP|V|G|Y|A|S|S|P|I|E|T|I|N|Y|A|S|A|D|N|E|T|I|F|D|I|I|S|L|K|T|P|M|D|L|S|I.....DECR|I|N|H|I|A|S
13 |AAN26412.1| 331GGTP|V|G|Y|A|Q|P|I|E|T|I|N|Y|V|S|A|D|N|E|T|I|F|D|I|V|S|L|K|T|P|T|Y|I|T|V.....DECR|V|N|H|I|A|T
13 |ABL84490.1| 331DGLP|V|G|Y|T|S|S|P|I|E|T|I|N|Y|V|S|A|D|N|E|T|I|F|D|V|I|S|V|K|T|P|M|N|L|S|V.....DECR|I|N|H|I|A|S
13 |CED95594.1| 470ALD|P|ADS|I|N|Y|V|S|K|D|N|E|T|I|W|D|I|I|Q|Y|K|H|A|I|G|L|S|I.....ENRV|R|V|Q|N|A|A
13 |ACN58254.1| 339FASD|P|EET|I|N|Y|V|A|C|D|N|H|T|F|W|D|K|N|Y|L|A|A|K|D|R|K|K.....KWT|E|E|L|K|N|Q|I|S|G
13 |BAB62095.1| 218CHP|ROS|I|N|Y|V|E|C|D|N|H|T|F|W|D|K|M|E|A|A|N|H|D|E|P|E|W.....LRR|K|R|Q|K|I|A|T
13 |AAD30387.1| 347FAKD|P|QET|I|N|Y|V|E|V|D|N|H|T|F|W|D|K|N|Y|L|A|A|Q|A|D|T|T|V.....KWT|E|E|L|K|D|A|Q|I|A|G
13 |BAF93906.1| 464AFLT|P|SOS|I|N|Y|V|E|C|D|S|F|T|I|N|D|S|L|W|S|A|D|P|N|D|S|V|A.....THQ|A|R|V|T|I|A|N
13 |AAY89038.1| 1075NGTAD|T|N|Y|G|G|A|D|Q|V|V|Q|V|E|I|D|N|L|T|I|Y|D|K|L|V|K|S|A|P|N|D|S|E|E.....T|R|A|R|A|K|I|A|D
13 |AAB06264.1| 330AKE|P|DEC|V|N|Y|V|S|C|D|N|L|T|I|F|D|K|L|Q|K|M|V|G|E|D|I|F.....WID|R|A|T|R|I|A|N
13 |AFL46505.1| 460FTQE|P|DQA|I|T|Y|V|E|A|D|N|H|T|F|W|D|K|L|N|L|T|N|P|Q|D|N|D|A.....T|R|A|R|A|K|I|A|D
13 |AJG04842.1| 460FTAEP|DQA|I|T|Y|V|E|A|D|N|H|T|F|W|D|K|L|N|L|T|N|P|Q|D|T|D|A.....T|R|A|R|A|K|I|A|D
13 |AAD30387.1| 347_2FAKD|P|QET|I|N|Y|V|E|V|D|N|H|T|F|W|D|K|N|Y|L|A|A|Q|A|D|T|T|V.....KWT|E|E|L|K|D|A|Q|I|A|G
13 |CAC85704.1| 218CHP|ROS|I|N|Y|V|E|C|D|N|H|T|F|W|D|K|M|E|A|A|N|H|D|E|P|E|W.....LRR|K|R|Q|K|I|A|T
13 |AAS47565.1| 372AQEP|TES|I|V|V|S|C|D|N|L|T|I|F|D|K|I|E|K|S|N|P|D|S|E|E.....D|R|I|R|M|N|L|I|A|N
13 |AEW23439.1| 217FSP|ROS|V|N|Y|V|E|C|D|N|H|T|F|W|D|K|M|A|V|A|N|A|H|E|S|E|Y.....I|R|R|K|R|Q|K|I|A|T
13 |CAC60156.1| 431TSA|P|SET|I|N|Y|V|T|S|D|N|H|T|F|W|D|K|I|L|A|S|N|P|S|D|T|E|A.....D|R|I|K|M|D|E|L|A|H
13 |AAE10887.1| 429TSSP|GET|I|N|Y|V|T|S|D|N|H|T|F|W|D|K|I|A|L|S|N|P|D|S|E|A.....D|R|I|K|M|D|E|L|A|Q
13 |AAC00283.1| 213IVPE|P|SOS|I|N|Y|V|E|S|D|N|H|T|F|W|D|K|M|S|F|A|L|P|Q|E|N|D|S.....R|R|K|R|S|R|Q|I|A|V
13 |CAC60157| 429TSSP|GET|I|N|Y|V|T|S|D|N|H|T|F|W|D|K|I|A|L|S|N|P|D|S|E|A.....D|R|I|K|M|D|E|L|A|Q
13 |AAD36907| 339FALD|P|EET|I|N|Y|A|A|C|D|N|H|T|F|W|D|K|N|Y|L|A|A|K|A|D|K|K|K.....E|W|T|E|E|L|K|N|Q|I|A|G
20 |BAA02473.1| 130PEQAA|Q|G|L|W|N|L|L|D|S|D|T|E|R|F|L|T|S|C|G|G|N.....E|B|A|K|F|I|A|V
20 |AAA22622.1| 134PNNV|N|E|A|F|N|L|L|G|S|D|T|S|R|I|L|T|V|C|G|G|D|I.....R|K|V|K|I|L|F
20 |AAC46346.1| 134PTNV|N|E|A|F|N|L|L|G|S|D|T|P|R|I|L|T|L|C|K|E|D|V.....R|K|A|K|I|S|F
20 |AAC15072.1| 134PKQV|N|E|A|F|N|L|L|G|S|D|T|P|R|I|L|T|V|C|G|G|D|V.....R|K|V|K|I|L|F
20 |AAF23874.1| 137MKQA|N|E|V|M|F|N|L|L|D|S|D|T|K|R|L|T|R|C|R|N|D|E|K|K|R|A|L.....L
20 |AAK15003.1| 134PNNV|N|E|A|F|N|L|L|G|S|D|T|S|R|I|L|T|V|C|G|G|D|I.....R|K|V|K|I|L|F
20 |ABJ59624.1| 136PDI|V|N|Q|A|M|M|N|L|D|S|D|T|A|R|I|L|T|I|A|N|G|N|Q|D|L|A|Q|A.....I
20 |ABS59068.1| 41PAEV|N|Y|G|L|Q|N|L|D|S|D|T|D|R|I|P|S|M|I|N|P|D|R|R|Y|D|A|E.....N|S|P|R|W|N|P|N|Y|D|V|R|K|P|T|A|E|E|I|R|R|Q|I|V
20 |ACC84362.1| 11PWEI|Q|I|T|Q|L|N|L|L|A|S|D|T|A|R|I|M|T|I|A|G|G|D|I|A|S.....V|E|L|S|T
20 |BAI67607.1| 9PAGF|H|G|M|L|N|L|L|G|S|D|T|E|R|V|L.....T|R|H|A|G|D|V.....E|A|A|L|S|Y
20 |AFM43699.1| 134PKHV|N|E|A|F|N|L|L|G|S|D|T|P|R|I|L|T|I|C|G|G|D|V.....R|K|A|K|I|L|F
20 |AHW51935.1| 133PASV|N|A|V|Q|F|N|L|L|D|S|D|T|A|R|I|L.....T|T|S|K|E|N|K|A|L.....V|K|I|Q|Y
31 |CAA37583.1| 7LEHT|G|W|N|S|L|Y|W|N|N|D|O|P|R|V|V|S|R|F|G|N|D|G|M|Y|R|I|E|S.....A|K|M|L|A
31 |BAA00534.1| 6LMNV|G|W|N|T|L|Y|F|E|N|D|O|P|R|V|I.....S|A|G|A|M|T|R|E|L|R|K.....Q|S|R|O|S|I|S|N
31 |BAA01368.1| 7LEGK|G|W|N|S|L|Y|L|N|N|D|O|P|R|A|V|S|R|F|G|D|D|G|K|Y|R|V|E|S.....A|K|M|L|A
31 |BAA11354.1| 6LADT|G|W|N|A|L|Y|F|E|N|D|O|A|R|V|V|S|R|W|G|N|D|T|T|Y|R|A|E|C.....A|K|A|F|A
31 |AAK27723.1| 24VRKA|G|W|A|S|L|F|F|C|N|D|O|P|R|V|V|S|R|W|G|N|D|S|D|R|D|R|S|R|E.....L|S|A|K|A|F|G
31 |AAV42157.1| 6DFDH|A|W|N|S|L|F|W|E|N|D|I|P|R|V|I|S|R|W|G|N|Q|E|Y|R|V|Q|C.....A|K|M|F|A
31 |BAE79634.1| 2ELGQ|G|W|N|S|L|F|W|N|N|D|L|P|R|V|L|S|I|W|G|N|T|G|K|Y|R|E|K|S.....A|K|A|L|A
31 |ACM89182.1| 27VENV|G|W|A|S|L|F|T|G|N|D|O|P|R|V|V|S|R|W|G|D|D|S|S|E|E|R|V.....R|S|A|K|A|L|G
31 |ACM89183.1| 26IARR|G|W|A|S|L|F|L|D|N|D|O|P|R|V|V|S|R|W|G|D|D|T|S|K|T|G|R|I.....R|S|A|K|A|L|A
39 |AAA23205.1| 385PLPV|F|Y|S|T|M|N|L|L|G|S|D|T|M|R|I|L|T|V|E|G|Y|N|S|A|D|E|N|Q|N.....S|Q|A|A|K|D|L|A|V|R|K|R|L|K|I|A|A
39 |AAA19800.1| 388PLPA|F|Y|S|T|M|N|L|L|G|S|D|T|M|S|I|L|T|V|E|G|Y|N|S|A|D|E|N|E|N.....S|D|A|A|R|K|L|A|E|Q|K|L|K|I|A|T
39 |BAA05832.1| 475PEEA|F|Y|A|L|M|N|L|I|G|S|D|T|A|R|A|I|F|L|G|N|G|T|D|S|Y|R|A.....E|L|D|P|N|Y|N|E|Q|L|G|K|R|L|K|I|A|A
39 |AAG44799.1| 479PEEA|F|Y|A|L|M|N|L|I|G|S|D|T|A|R|A|V|F|L|G|N|G|T|D|S|Y|R|A.....E|L|D|P|N|Y|N|E|Q|L|G|K|R|L|K|I|A|A
39 |ABR26448.1| 397PSEA|F|Y|A|L|M|N|L|I|G|S|D|T|A|R|A|V|F|L|G|N|G|T|D|S|Y|R|A.....E|L|D|P|N|Y|N|E|E|L|G|K|R|L|K|I|A|A
39 |ABY95795.1| 385PLPV|F|Y|S|T|M|N|L|L|G|S|D|T|M|R|I|L|T|V|E|G|Y|N|S|A|N|E|N|Q|N.....S|Q|E|A|K|D|L|A|V|R|K|R|L|K|I|A|A
39 |ACR15165.1| 384PLPA|F|Y|S|T|M|N|L|L|G|S|D|T|M|R|I|L|T|V|E|G|Y|N|S|A|D|E|N|E|N.....S|D|A|A|Q|L|A|E|Q|K|L|K|I|A|T
39 |ACV59878.1| 541PLQS|F|Y|S|M|M|N|L|V|D|S|D|T|M|R|I|L|T|I|E|N|A|P|Q|P|G|D|L|S.....A|L|Q|Q|D|E|Y|R|P|S|P|A|A|Q|L|G|R|E|I|L|V|S
39 |ADZ299363.1| 550PLQS|F|Y|A|M|M|N|L|V|D|S|D|T|M|R|I|L|T|I|E|N|A|P|E|P|G|S|M|S.....A|L|Q|Q|A|T|Y|Q|P|T|A|D|Q|L|G|R|E|I|L|V|S
39 |AFI70750.1| 397PSEA|F|Y|A|L|M|N|L|I|G|S|D|T|A|R|A|V|F|L|G|N|G|T|D|S|R|E|R|A.....E|L|D|P|N|Y|N|E|E|L|G|K|R|L|K|I|A|V
41 |AHY15164.1| 1St iniae_1419TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|A|R.....AENYAE|I|H|R|R|L|R|I|G|N
41 |AGI48789.1| 1St suis_1378LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....AENYAE|I|H|R|R|L|R|I|G|N
41 |AHY17035.1| 1St iniae_1419TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|A|R.....AENYAE|I|H|R|R|L|R|I|G|N
41 |AG224199.1| 1St suis_1377LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....AENYAE|I|H|R|R|L|R|I|G|N
41 |ABP93225.1| 1St suis_1252LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AER20299.1| 1St suis_1389LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|I.....AENYTE|I|H|Q|R|I|G|N
41 |AER22304.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|I.....AENYTE|I|H|Q|R|I|G|N
41 |AD283524.1| 1Cl lento_920ATNP|TEV|V|S|V|V|T|A|D|N|L|N|L|W|D|K|V|L|A|M|G|L|A|D|A|K|T.....P|Y|A|S|I|T|G|N|V|L|E|N|E|A|V|R|K|S|I|A|N
41 |AGW82668.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|I.....AENYTE|I|H|Q|R|I|G|N
41 |AGG65362.1| 1St suis_1389LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AIQ28595.1| 1Pa FSL_723PADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|L|A|L|H|K|N|P|Q|N.....AENYAE|I|Q|R|I|G|N
41 |ADV71067.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AER16173.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AIQ51763.1| 1Pa FSL_716PADD|P|GDV|V|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|S|I|K|K|D|P|A|I.....P|A|N|E|L|I|Q|R|I|G|N
41 |CB113953.1| 1St gallo_1441TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|K|S|I|Q|K|D|P|A|V.....AENAEAE|I|Q|R|R|L|R|I|G|N
41 |CAZ56775.1| 1St suis_1377LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AIQ34761.1| 1Pa FSL_706PADD|P|GDV|I|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|T|I|K|K|D|P|T|I.....T|E|N|E|L|I|Q|R|I|G|N
41 |AEZ62583.1| 1St infan_1443TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|H|D|I|I|A|K|S|I|H|K|D|P|S|I.....AENAEAE|I|L|R|R|O|I|G|N
41 |CCF02755.1| 1St maced_1180TADD|T|G|D|V|I|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|K|S|I|Q|K|D|P|A|V.....AENAEAE|I|Q|R|R|L|R|I|G|N
41 |CBZ48657.1| 1St gallo_1441TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|K|S|I|Q|K|D|P|A|V.....AENAEAE|I|Q|R|R|L|R|I|G|N
41 |AIQ57390.1| 1Pa borea_723PADD|P|GDV|I|Q|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|L|A|L|H|K|N|P|Q|N.....A|G|N|E|L|I|Q|R|I|G|N
41 |AGI39830.1| 1C sterc_1095KADDN|P|GDV|V|Q|Y|I|E|C|D|N|L|T|I|Y|D|V|I|A|L|S|I|K|K|D|P|S|K.....P|E|N|D|E|I|H|R|R|I|G|N
41 |AEB82319.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|I.....AENYTE|I|H|Q|R|I|G|N
41 |BAM47868.1| 1Am xylan_1322KADDN|P|GDV|V|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|S|I|K|K|D|P|K|D.....H|A|E|E|I|H|Q|R|I|G|N
41 |AFR01291.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AGM98096.1| 1St iniae_1419TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|A|R.....AENYAE|I|H|R|R|L|R|I|G|N
41 |ADE32331.1| 1St suis_1408LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |AHP60382.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|I.....AENYTE|I|H|Q|R|I|G|N
41 |CAZ52647.1| 1St suis_1377LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |ACX81318.1| 1St gallo_1452TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|H|D|V|I|A|K|S|I|Q|K|D|P|A|V.....AENAEAE|I|Q|R|R|L|R|I|G|N
41 |ABG383674.1| 1Cl perfr_883TDS|P|SET|I|N|Y|V|T|A|D|N|L|N|L|W|D|K|I|I|K|A|G|K|E|K|A|A|G|F|L|E|M|R|D|G|V|L|T|G|E|D|A|N|K|Y|S|S|V|Q|E|A|V|N|K|K|A|K|P|Y|S|V|I|T|E|N|P|L|D|N|E|Y|V|R|R|S|I|T|T
41 |AER45198.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|V.....TENYTE|I|H|Q|R|I|G|N
41 |BAK28316.1| 1St gallo_1441TADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|A|V.....AENAEAE|I|Q|R|R|L|R|I|G|N
41 |CQR54568.1| 1Pa riogr_717PADD|P|GDV|V|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|T|I|K|K|D|P|S|I.....P|A|N|E|L|I|Q|R|I|G|N
41 |AGX06343.1| 1Ba infan_1304ADDN|P|GDM|V|Q|Y|I|E|A|D|N|L|P|I|Y|D|V|I|A|Q|S|I|K|K|D|P|A|I.....P|E|N|N|L|E|I|H|R|R|I|G|N
41 |AIQ22910.1| 1Pa FSL_700PADD|P|GDV|I|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|T|I|K|K|D|P|A|I.....E|K|N|E|L|I|Q|R|I|G|N
41 |AIQ67951.1| 1Pa gram1_717PADD|P|GDV|V|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|T|I|K|K|D|P|S|I.....P|A|N|E|L|I|Q|R|I|G|N
41 |AIQ16995.1| 1Pa FSL_716PADD|P|GDV|I|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|S|I|K|K|D|P|A|I.....P|G|N|E|L|I|Q|R|I|G|N
41 |AER18240.1| 1St suis_1385LADD|P|GDV|I|Q|Y|I|A|A|D|N|L|T|I|F|D|I|I|A|Q|S|I|K|K|D|P|S|I.....AENYTE|I|H|Q|R|I|G|N
41 |AIQ46154.1| 1Pa FSL_716PADD|P|GDV|V|P|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|Q|S|I|K|K|D|P|A|I.....P|A|N|E|L|I|Q|R|I|G|N
41 |AIQ40360.1| 1Pa FSL_723PADD|P|GDV|I|Q|Y|I|E|A|D|N|L|T|I|H|D|V|I|A|L|A|L|H|K|N|P|Q|N.....P|A|N|E|L|I|Q|R|I|G|N
41 |AIY06268.1| 1Pl PAMC_1296AADD|P|GDM|V|Q|Y|I|E|A|D|N|L|P|I|Y|D|V|I|A|Q|S|I|K|K|D|P|A|I.....P|E|N|N|L|E|I|H|R|R|I|G|N
X |AAG31033.1| 152G|N|V|N|A|G|M|G|F|N|I|G|S|D|T|S|R|V|L|T|D|V|I|G|G|L|N|D|S|P|N.....E|S|T|A|R|L|K|I|S

[illegible]

	400	410	420
11	BAA11864.1	150	NAFC...QDNETSW...YDW...NLDENR...VRFDH...FVRR
11	AAB63356.1	198	NTYN...LDSSAANN...LYW...SR.SALEAD...HETYY...TKRR
11	AAC76456.1	136	NAYC...QDNQLTWW...LDW...SQASSG...LTAF...TAA
11	BAA29041.1	152	NTYC...HDHYVYVY...FRW...DK.KEESSD...LQR...CSL
11	AAK42273.1	156	NAFC...QDNEITW...FDW...NLDERK...SKFLE...FVKK
11	BAB99500.1	150	NVYC...QDNEILAW...VNW...DQ.AEENAD...LVSV...TRR
11	AAM81590.1	156	NAFC...QDNEITW...FDW...NLDERK...SKFLE...FVKK
11	AAP85534.1	244	NTYC...HDSEILNY...LRW...DQLAEDPHG...FNR...VRL
11	sp P10342	193	NAYC...LDSSANNWLTYSW...FYT...AQR
12	AAK74446.1	586	DSYD...SSDAVNNK...FDW...TK.ATDGKAYPENVKSRDY...MKMG
12	AAN00098.1	611	DSYD...SSDAINNH...FDW...AA.ATDNKKHPISTKTQAY...TAG
12+41	AAS36537.1	1272	DSYD...SSDIINNR...FDW...EK.ATDAEKYPVNNVTRDY...TAG
12	ACI61883.1	510	DSYD...SSDAVNNH...FDW...AK.ATDSIAHPISNQTAKY...TQG
12+41	CAR47543.1	1377	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
13	CAA36431.1	431	DSYD...SGDWNNR...VDY...SLQDNNYVNV...
13	AAA25124.1	443	DSYD...SGDWNNR...VDY...SLQDNNYVNV...
13	AAD04189.1	273	DSYN...SGDWNNR...LDF...TYETNN...
13	BAA28632.1	328	DSYN...SGDWNNK...LDF...TYETNN...
13	AAD11599.1	330	DSYD...SGDWNNK...LDF...TYETNN...
13	AAN26412.1	331	DSYN...SGDWNNR...LDF...SYNSNN...
13	ABL84490.1	331	DSYN...SGDWNNK...LDF...TYETNN...
13	CED95594.1	470	NSYD...SGDWNNY...VDF...TYNTNN...
13	ACN58254.1	339	NSYD...APISING...FGY...ER.KRQFID...VFNY...YKG
13	BAB62095.1	218	NSYK...SPDAVNNQ...LDW...ER.KSRYED...DVR...YQG
13	AAD30387.1	347	NSYK...SPISING...LDY...AR.KAEFID...VFNY...YKG
13	BAF93906.1	464	NSYNGLEFLDSNGTYHGNYWPGSNLYTPVVG...DGVVNA...MNV...
13	AAV89038.1	1075	NSYN...AGDAVNA...IDW...DN.VKDNQN...AVNF...IGN
13	AAB06264.1	330	NTYN...AGDNIINNK...IDW...NR.TVEQAG...SVNY...YKG
13	AFL46505.1	460	NSYK...SPDSVNNQ...LDW...SLKEKFFD...TFK...YCD
13	AJG04842.1	460	NSYK...SPDSVNNQ...LDW...KR.KMDRQQ...DVDY...MTG
13	AAD30387.1	347_2	NSYK...SPDSVNNQ...LDW...KL.KMDRQA...DVDY...MKMG
13	CAC85704.1	218	NSYK...SPISING...LDY...AR.KAEFID...VFNY...YKG
13	AAS47565.1	372	NSYK...SPDAVNNQ...LDW...ER.KSRYED...DVR...YQG
13	AEW23439.1	217	NSYN...SPDHINQ...IEW...SRKHTYEE...FLY...HQG
13	CAC60156.1	431	NSYN...SPDEVNNQ...VDW...NEKSRWEEDVRE...IMK
13	AAE10887.1	429	NSYN...AGDSVNNQ...FDW...SR.KAQPKD...VFDY...FSS
13	AAC00283.1	213	NSYQ...SSDSINQ...LDW...SR.KAQYPD...VFNY...YSG
13	CAC60157	429	NSYN...AGDAVNE...FDW...DR.RETFKE...DVHY...IRR
13	AAD36907	339	NSYN...AGDAVNE...FDW...SR.KAQYPD...VFNY...YSG
20	BAA02473.1	130	...DPDCRRP...MIW...ER.KLOFID...VFNY...HKG
20	AAA22622.1	134	...DPECCKK...MVW...EKEQN...RGLFE...FYKE
20	AAC46346.1	134	...DPEGCKK...MIW...DPMQONKELHGHVKQIALRQ...
20	AAC15072.1	134	...DPECCKK...MVW...DEHQONRELFRRHVKQ...
20	AAF23874.1	137	...DPECCKK...MIW...DPEKONKELYEHVKQIALRQ...
20	AAK15003.1	134	...DPLCKK...MVW...EKEQN...QDMLQ...MKR
20	ABJ59624.1	136	...DPECCKK...MVW...DPMQONKELHGHVKQIALRQ...
20	ABS59068.1	41	...DPECCKK...MDW...GK...
20	ACC84362.1	11	...PDRKKP...MLW...EDLSYEDEVSCPVPNCNRPADK.NVFNRD...LFD...YRK
20	BAI67607.1	9	...DPPSRRG...FPL...EA.NWNQEIFNTHROLITIRQT...
20	AFM43699.1	134	...DPPGCRGA...MPW...NEESWNTRLLDGIRTFAA...RAH
20	AHW51935.1	133	...DPECCKK...MVW...DPEKONKELYEHVKQIALRQ...
31	CAA37583.1	7	...DPPGCRAC...MIW...DE.AKQDKD...MLA...FLTQ
31	BAA00534.1	6	...RDNARTP...MQW...DDQNHAGFTTGEPWITVNPNYKEINVQAIQNKDSIFY...YKK
31	BAA01368.1	7	KKGR...DHARTP...MQWDDGKYAGFT...DGE.AWLAVNPRYQEIINVKES.LADEDS...IFY...YQK
31	BAA11354.1	6	...RDNARTP...MQW...DDSENAGFTTAGTPWIPVNPNYKEINVKAALDDPNSVFHY...YKK
31	AAK27723.1	24	AGFT...DNARTP...MQW...DGSFNAGFTTGTPTWLKVNSRYSE.INVAKALQEPDSIFY...YQS
31	AAV42157.1	6	...PADAAAE...PW...ISVNPNNHVEINAAEEFDDPDPSVYT...YKK
31	BAE79634.1	2	NVKG...RDNARRP...MQW...NDEKNAGFSEVDPWLSVNPNYKDINVENALADPNISIFYT...YQK
31	ACM89182.1	27	RMIG...RDNARTP...MQW...DASQNAAGFSTADKTWLPVNPNYKDINVQAALKNSNSIFYT...YQK
31	ACM89183.1	26	AGFT...APDAATE...PW...ISVNPNNHAEINAAEEFDDPDPSVYA...YKK
39	AAA23205.1	385	AGFT...MPDAPVE...PW...IAVNPNNHTEINAADETDDPDPSVYS...FHKR
39	AAA19800.1	388	...DPPDRRT...FPW...GREDT...LQT...FKK
39	BAA05832.1	475	...DPPDRRT...FPW...GNEDTA...LQD...FKN
39	AAG44799.1	479	...DPPDRRT...YPW...GSEDK...LIAHY...YQK
39	ABR26448.1	397	...DPPDRRT...YPW...GSEDT...LIAHY...YQK
39	ABY95795.1	385	...DPPDRRT...YPW...GKEDQ...LLSHY...YQK
39	ACR15165.1	384	...DPPDRRT...FSW...GREDK...LQD...FKK
39	ACV59878.1	541	...DPPDRRT...FPW...GNEDT...LQD...FKN
39	ADZ99363.1	550	...DPLNRRT...YPW...DNQNL...LLNH...YRL
39	AFI70750.1	397	...DPLSRDT...YPW...GHENEA...LLNH...YRL
41	AHY15164.1	St iniae_1419	DSYD...DPPNRRT...YPW...GKEDQ...LLSHY...YQK
41	AGL48789.1	St suis_1378	DSYD...ATDAINNH...FDW...SK.ATDSKVFFESTKSOAY...MKG
41	AHY17035.1	St iniae_1419	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	AGZ24199.1	St suis_1377	DSYD...ATDAINNH...FDW...SK.ATDSKVFFESTKSOAY...MKG
41	ABP93225.1	St suis_1252	DSYD...SSDAVNNK...FDW...SK.ATDSKVFFESTKSOAY...MKG
41	AER20299.1	St suis_1389	DSYD...SSDAVNNK...FDW...TK.ATDETLYPENTRTQAF...TKG
41	AER22304.1	St suis_1385	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	ADZ83524.1	Cl lento_920	NSYK...SSDAVNNK...FDW...TK.ATDETLYPENTRTQAF...TKG
41	AGW88268.1	St suis_1385	DSYD...SPDSINNM...ISW...TK.ATDEALYPENTRTQAF...TKG
41	AGG65362.1	St suis_1389	DSYD...SSDAVNNK...FDW...ANRNSY...KEVTE...YEG
41	AIQ28595.1	Pa FSL_723	DSYD...SSDAVNA...FDW...TK.ATDEALYPENTRTQAF...TKG
41	ADV71067.1	St suis_1385	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	AER16173.1	St suis_1385	DSYD...SSDAVNNK...FDW...AA.ATDEVNFPVQNTATREY...TAG
41	AIQ51763.1	Pa FSL_716	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	CB113953.1	St gallo_1441	DSYD...SSDAVNNK...FDW...SK.VTDEVKYPVQNTTKDY...TAG
41	CAZ56775.1	St suis_1377	DSYD...STDAINNH...FDW...AK.ATDETYPENTRTQAF...TKG
41	AIQ34761.1	Pa FSL_706	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	AEZ62583.1	St infan_1443	DSYD...SSDAVNNK...FDW...TK.ATDESQFPVQNTTRKY...TSG
41	CCF02755.1	St maced_1180	DSYD...STDAINNH...FDW...AK.ATDGKVPENAKTQAY...TKG
41	CBZ48657.1	St gallo_1441	DSYD...STDAINNH...FDW...AK.ATSEAYPENTRTQAF...TKG
41	AIQ57390.1	Pa borea_723	DSYD...STDAINNH...FDW...AK.ATDETYPENTRTQAF...TKG
41	AGI39830.1	C sterc_1095	DSYN...SSDAVNA...FDW...AA.ATDEVNFPVQNTATREY...TAG
41	AEB82319.1	St suis_1385	DSYD...SSDAVNNK...FDW...AK.ATDAEKYPVNSVTRKY...TAG
41	BAM47868.1	Am xylan_1322	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	AFR01291.1	St suis_1385	DSYD...STDAVNNK...FDW...AK.VTDNDAYPINVATKDY...TAG
41	AGM98096.1	St iniae_1419	DSYD...ATDAINNH...FDW...SK.ATDSKVFFESTKSOAY...MKG
41	ADE32331.1	St suis_1408	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	AHF60382.1	St suis_1385	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	CAZ52647.1	St suis_1377	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	ACX81318.1	St gallo_1452	DSYD...STDAINNH...FDW...AK.ATDEALYPENTRTQAF...TKG
41	ABG83674.1	Cl perfr_883	NSYK...SPDAINNK...INW...SGVFDY...YQG
41	AER45198.1	St suis_1385	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	BAK28316.1	St gallo_1441	DSYD...STDAINNH...FDW...AK.ATDETYPENTRTQAF...TKG
41	CQR54568.1	Pa riogr_717	DSYD...SSDAVNNK...FDW...AK.ATDEVNFPVQNTTRAY...TSG
41	AGX06343.1	Ba infan_1304	DSYD...SSDAINNK...FDW...QK.ATNASAYKVNTMTREY...TEG
41	AIQ22910.1	Pa FSL_700	DSYD...SSDAVNNK...FDW...TK.ATDESQFPVQNTTRKY...TSG
41	AIQ67951.1	Pa gram_717	DSYD...SSDAVNNR...FDW...AK.ATDEVNFPVQNTTRAY...TSG
41	AIQ16995.1	Pa FSL_716	DSYD...SSDAVNNK...FDW...SK.VTDEVNFPVQNTTKDY...TSG
41	AER18240.1	St suis_1385	DSYD...SSDAVNNK...FDW...TK.ATDEALYPENTRTQAF...TKG
41	AIQ46154.1	Pa FSL_716	DSYD...SSDAVNNK...FDW...SK.VTDEVNFPVQNTTKDY...TAG
41	AIQ40360.1	Pa FSL_723	DSYD...SSDAVNA...FDW...SA.ATDEVNFPVQNTATREY...TAG
41	AIY06268.1	Pl PAMC_1296	DSYD...SSDAINNO...FDW...QK.ATNKKEFAVNNTRTY...TEG
X	AAG31033.1	152	...HDEQRY...IQW...DR...LNVEVYE...TKR

11 |BAA11864.1| 150 |LTN|FYKAH.....
 11 |AAB63356.1| 198 |LIA|FRKAH.....
 11 |AAC76456.1| 136 |LIH|LRKRI.....
 11 |BAA29041.1| 152 |MTK|FRKQ.....
 11 |AAK42273.1| 156 |MIQ|FYRAH.....
 11 |BAB99500.1| 150 |LLR|IRANH.....
 11 |AAM81590.1| 156 |MIQ|FYKAH.....
 11 |AAP85534.1| 244 |LIH|FRRA.....
 11 |sp|P10342| 193 |LIA|FRKAH.....
 12 |AAK74446.1| 586 |LIA|LR.....
 12 |AAN00098.1| 611 |LIT|LR.....
 12+41 |AAS36537.1| 1272 |LIE|LR.....
 12 |ACI61883.1| 510 |LIA|LR.....
 12+41 |CAR47543.1| 1377 |LIA|LR.....
 13 |CAA36431.1| 431IHSD.....
 13 |AAA25124.1| 443
 13 |AAD04189.1| 273
 13 |BAA28632.1| 328
 13 |AAD11599.1| 330
 13 |AAN26412.1| 331
 13 |ABL84490.1| 331
 13 |CED95594.1| 470
 14 |ACN58254.1| 339 |LIK|LR.....
 14 |BAB62095.1| 218 |LIA|LR.....
 14 |AAD30387.1| 347 |LIE|LR.....
 14 |BAF93906.1| 464 |LMK|FK.....
 14 |AAY89038.1| 1075 |LIA|LR.....
 14 |AAB06264.1| 330 |LIN|LR.....
 14 |AFL46505.1| 460 |LIE|LR.....
 14 |AJG04842.1| 460 |LIQ|VR.....
 14 |AAD30387.1| 347_2 |LIE|LR.....
 14 |CAC85704.1| 218 |LIA|LR.....
 14 |AAS47565.1| 372 |LIR|LR.....
 14 |AEW23439.1| 217 |LIE|LR.....
 14 |CAC60156.1| 431 |MIH|LR.....
 14 |AAE10887.1| 429 |LIH|LR.....
 14 |AAC00283.1| 213 |LIS|LR.....
 14 |CAC60157| 429 |LIH|LR.....
 14 |AAD36907| 339 |LIK|LR.....
 20 |BAA02473.1| 130 |LIR|LRHRLASLTRGN..
 20 |AAA22622.1| 134 |YRS|LRGEI.....
 20 |AAC46346.1| 134 |LIA|LRKAYKAF.....
 20 |AAC15072.1| 134 |YRA|LRGD.....
 20 |AAF23874.1| 137 |LIA|LRKQENTLLT.....
 20 |AAK15003.1| 134 |YRS|LRGEI.....
 20 |ABJ59624.1| 136
 20 |ABS59068.1| 41 |LIR|ERIHS.D.ALTLGEF
 20 |ACC84362.1| 11 |YPA|LRTG DY.....
 20 |BAI67607.1| 9 |QPA|MRRG.....
 20 |AFM43699.1| 134 |YRA|LRGD.....
 20 |AHW51935.1| 133 |LIA|LRKHHPAF.....
 31 |CAA37583.1| 7 |LIE|LRKN.....
 31 |BAA00534.1| 6 |LIG|LRKQ.....
 31 |BAA01368.1| 7 |LIQ|LRKQ.....
 31 |BAA11354.1| 6 |LIK|LRHS.....
 31 |AAK27723.1| 24 |LIA|MRHN.....
 31 |AAV42157.1| 6 |LIK|LRHE.....
 31 |BAE79634.1| 2 |LIQ|LRKE.....
 31 |ACM89182.1| 27 |LIA|LRHN.....
 31 |ACM89183.1| 26 |LIA|LRH.....
 39 |AAA23205.1| 385 |VVN|IR.NENQVLKTGDL
 39 |AAA19800.1| 388 |VSS|IR.NNNQVLKTGDL
 39 |BAA05832.1| 475 |VGK|IR.TKHQDVLAKGTI
 39 |AAG44799.1| 479 |VGT|VR.TEHQDVLAKGTI
 39 |ABR26448.1| 397 |VGH|IR.QRHQSLLAHGDI
 39 |ABY95795.1| 385 |VVN|IR.NENQVLKTGDL
 39 |ACR15165.1| 384 |ISS|IR.NNNQVLKTGDL
 39 |ACV59878.1| 541 |LGA|IR.NANPVLQTGDF
 39 |ADZ99363.1| 550 |LGA|IR.AANPVLQTGTG
 39 |AFI70750.1| 397 |VGH|IR.QHHQSLLAHGDI
 41 |AHY15164.1|St_iniae_1419 |LIA|LR.....
 41 |AGL48789.1|St_suis_1378 |LIA|LR.....
 41 |AHY17035.1|St_iniae_1419 |LIA|LR.....
 41 |AGZ24199.1|St_suis_1377 |LIA|LR.....
 41 |ABP93225.1|St_suis_1252 |LIA|LR.....
 41 |AER20299.1|St_suis_1389 |LIA|LR.....
 41 |AER22304.1|St_suis_1385 |LIA|LR.....
 41 |ADZ83524.1|Cl_lento_920 |LIA|LR.....
 41 |AGW88268.1|St_suis_1385 |LIA|LR.....
 41 |AGG65362.1|St_suis_1389 |LIA|LR.....
 41 |AIQ28595.1|Pa_FSL_723 |LIK|LR.....
 41 |ADV71067.1|St_suis_1385 |LIA|LR.....
 41 |AER16173.1|St_suis_1385 |LIA|LR.....
 41 |AIQ51763.1|Pa_FSL_716 |LIQ|LR.....
 41 |CBI13953.1|St_gallo_1441 |LIA|IR.....
 41 |CAZ56775.1|St_suis_1377 |LIA|LR.....
 41 |AIQ34761.1|Pa_FSL_706 |LME|LR.....
 41 |AEZ62583.1|St_infan_1443 |LIA|LR.....
 41 |CCF02755.1|St_maced_1180 |LIA|IR.....
 41 |CBZ48657.1|St_gallo_1441 |LIA|IR.....
 41 |AIQ57390.1|Pa_borea_723 |LIK|LR.....
 41 |AGI39830.1|C_sterc_1095 |LIA|LR.....
 41 |AEB82319.1|St_suis_1385 |LIA|LR.....
 41 |BAM47868.1|Am_xylan_1322 |LIK|LR.....
 41 |AFR01291.1|St_suis_1385 |LIA|LR.....
 41 |AGM98096.1|St_iniae_1419 |LIA|LR.....
 41 |ADE32331.1|St_suis_1408 |LIA|LR.....
 41 |AHF60382.1|St_suis_1385 |LIA|LR.....
 41 |CAZ52647.1|St_suis_1377 |LIA|LR.....
 41 |ACX81318.1|St_gallo_1452 |LIA|IR.....
 41 |ABG83674.1|Cl_perfr_883 |LIS|LR.....
 41 |AER45198.1|St_suis_1385 |LIA|LR.....
 41 |BAK28316.1|St_gallo_1441 |LIA|IR.....
 41 |CQR54568.1|Pa_riogr_717 |LIQ|LR.....
 41 |AGX06343.1|Ba_infan_1304 |LIE|LR.....
 41 |AIQ22910.1|Pa_FSL_700 |LME|LR.....
 41 |AIQ67951.1|Pa_grami_717 |LIQ|LR.....
 41 |AIQ16995.1|Pa_FSL_716 |LIK|LR.....
 41 |AER18240.1|St_suis_1385 |LIA|LR.....
 41 |AIQ46154.1|Pa_FSL_716 |LIQ|LR.....
 41 |AIQ40360.1|Pa_FSL_723 |LIK|LR.....
 41 |AIY06268.1|Pl_PAMC_1296 |LIE|LR.....
 X |AAG31033.1| 152 |LGE|LRKNTIPALST....

Full structure based multiple sequence alignment generated using Promals3D. The intercepts included in Fig. 4 are indicated by red lines and labelled region I–VII. The numbering above the sequences follows the residue numbers of PDB entry 4AIO. In the case of the GH13_12+41 and GH13_39 sequences only the catalytic domains were included.

[illegible]

31_4A1E
31_221C
31_1U0K
20_2WS
20_1SMA
20_1J0H
20_1B2
20_1B2Z
39_AD299363
39_AA19800
39_AFI70750
14_2E8Y
14_3WDH
14_2WAN
13_2Y0C
13_2FG2
13_4A1O
12_2YA0
12+41_CAR47543
12_3FAW
12+41_AAS36537
11_2VNC
11_2WSK
11_1B2
11_4J7R

VI

31_4A1E
31_221C
31_1U0K
20_2WS
20_1SMA
20_1J0H
20_1B2
20_1B2Z
39_AD299363
39_AA19800
39_AFI70750
14_2E8Y
14_3WDH
14_2WAN
13_2Y0C
13_2FG2
13_4A1O
12_2YA0
12+41_CAR47543
12_3FAW
12+41_AAS36537
11_2VNC
11_2WSK
11_1B2
11_4J7R

[illegible]

VII

I

[illegible]

IV

31_4A1E	FDHA.WNSIF	WENH	310	320	330	340	350	360	370	380	390
31_221C		PRVLSRWGN		DOYRVSACAL	ALL	HMHN	CT	TV	TC	EE	TCMT
31_1UOK		PRVLSRWGN		TKYRRKSA	AL	LL	HMHN	CT	TV	TC	EE
20_2WCS		PRVLSRWGN		DOMYRRSA	AL	LL	HMHN	CT	TV	TC	EE
20_1SMA		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1J0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1E9		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AA19800		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AFI70750		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2E8Y		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2W0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2YOC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2F6Z		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_4A1O		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_2YA0		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_CAR47543		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_3FAW		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_AAS36537		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2VNC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2WSK		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_4J7R		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT

31_4A1E		PRVLSRWGN		DOYRVSACAL	ALL	HMHN	CT	TV	TC	EE	TCMT
31_221C		PRVLSRWGN		TKYRRKSA	AL	LL	HMHN	CT	TV	TC	EE
31_1UOK		PRVLSRWGN		DOMYRRSA	AL	LL	HMHN	CT	TV	TC	EE
20_2WCS		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1SMA		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1J0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1E9		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AA19800		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AFI70750		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2E8Y		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2W0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2YOC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2F6Z		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_4A1O		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_2YA0		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_CAR47543		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_3FAW		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_AAS36537		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2VNC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2WSK		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_4J7R		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT

31_4A1E		PRVLSRWGN		DOYRVSACAL	ALL	HMHN	CT	TV	TC	EE	TCMT
31_221C		PRVLSRWGN		TKYRRKSA	AL	LL	HMHN	CT	TV	TC	EE
31_1UOK		PRVLSRWGN		DOMYRRSA	AL	LL	HMHN	CT	TV	TC	EE
20_2WCS		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1SMA		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1J0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1E9		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AA19800		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AFI70750		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2E8Y		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2W0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2YOC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2F6Z		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_4A1O		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_2YA0		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_CAR47543		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_3FAW		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_AAS36537		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2VNC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2WSK		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_4J7R		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT

31_4A1E		PRVLSRWGN		DOYRVSACAL	ALL	HMHN	CT	TV	TC	EE	TCMT
31_221C		PRVLSRWGN		TKYRRKSA	AL	LL	HMHN	CT	TV	TC	EE
31_1UOK		PRVLSRWGN		DOMYRRSA	AL	LL	HMHN	CT	TV	TC	EE
20_2WCS		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1SMA		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1J0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1E9		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AA19800		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AFI70750		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2E8Y		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2W0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2YOC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2F6Z		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_4A1O		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_2YA0		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_CAR47543		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_3FAW		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_AAS36537		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2VNC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2WSK		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_4J7R		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT

31_4A1E		PRVLSRWGN		DOYRVSACAL	ALL	HMHN	CT	TV	TC	EE	TCMT
31_221C		PRVLSRWGN		TKYRRKSA	AL	LL	HMHN	CT	TV	TC	EE
31_1UOK		PRVLSRWGN		DOMYRRSA	AL	LL	HMHN	CT	TV	TC	EE
20_2WCS		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1SMA		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1J0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1E9		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AA19800		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AFI70750		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2E8Y		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2W0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2YOC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2F6Z		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_4A1O		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_2YA0		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_CAR47543		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_3FAW		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_AAS36537		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2VNC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2WSK		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_4J7R		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT

31_4A1E		PRVLSRWGN		DOYRVSACAL	ALL	HMHN	CT	TV	TC	EE	TCMT
31_221C		PRVLSRWGN		TKYRRKSA	AL	LL	HMHN	CT	TV	TC	EE
31_1UOK		PRVLSRWGN		DOMYRRSA	AL	LL	HMHN	CT	TV	TC	EE
20_2WCS		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1SMA		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1J0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1E9		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
20_1B2		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AA19800		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
39_AFI70750		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2E8Y		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
14_2W0H		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2YOC		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_2F6Z		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
13_4A1O		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_2YA0		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_CAR47543		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_3FAW		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
12_41_AAS36537		PRVLSRWGN		DRKSVLST	LL	HMHN	CT	TV	TC	EE	TCMT
11_2VNC		PRV									

	500	510	520	530
31_1A1E	ARLSNEQNFVNSD...	...QIE.TILSNY.	...PERN.	...NVONITITDKPYEAFISKVI
31_121C	VNSDQEEVLSDV...	...DKQE.TILSNY.	...NESA.	...AJANHKIQPDAFCIKIL
31_130K	AMTAECEIFELPED.	...SYGEVE.LILNY.	...DVENG.	...PENITITKPYEAMVFKLK
20_2WCS	INAGTSATANDVASLHQPKN.LLLY.	...2WCS.	...TAEAWN	...GERGEOQQUSLITPARSGC
20_15NA	IRNSNAAEIEPPIIDA.	...RGKWL.V.NLLT.	...GERFAE.	...AETICVSDPPYFIVLAVESW
20_15NA	IRNSDAAEIEPPIIDA.	...RGKWL.V.NLLT.	...GERFAE.	...AETICVSDPPYFIVLAVESW
20_1E49	LNDRAGCHILILPVHR...	...QWMI.HLWQ.	...DEVLTRA.	...HGQULIKVPAISGAVLKASSD
39_1B79363	LNDRGQAQVILQVSGGKWL.DCLT.	...	...GEEVHKA.	...QGQUKLITPAISGAVILANGR
39_1AA18800	...	...	...	...
39_1A17050	...	...	...	...
14_288Y	HNASPSQVEMLPND.	...TPYR.LLCBPSG	...FQDPTPE	...IKKTAVANGICATVILVLC
14_3WHD	VNEEKKAMVLAD.	...GKWK.VMFSE.	...LGNV.	...CEOFIEANGICATVILVLC
13_2VAC	VYENKTSOTLNEPAG.	...DWTAVGLDGOIG	...EKSUGH	...VWGNVOVPAISTILKO
13_2VAC	TLASAPESRTLNEPAG.	...ETLQSLAIOQTG	...ENS.LANGVOIA	...ADGSTVTPAWSVAVLELPOEAGAGLPPVSK
13_2RGZ	TLASAPESRTLODFAG.	...TSLQSLAIOQAAG.	...DRLS.LASGVOIA	...ADGSTVTPAWSVAVLELPOEAGAGLPPVSK
13_4AIO	FVACPHVSRMBDIPAA.	...SMGFEHLPVQVNSST	...LVKSAVEA.	...AGTRFTVPEGRVTSVVFVEPR
12_2YAO	VNADEKAREPNLGTAF.	...AHURNAEVLADEN	...VGIA.NPKGLEW	...TEKGLKNALATVILVRSQNGTISHST
12_41_CAR47543	VNADEKAREPNLGTAF.	...AHURNAEVLADEN	...QAGP.	...
12_3FAM	VNADSRARNVLGEXY.	...KHLKGVQIVDAD	...QAGI.	...EKDSSLIDPLATVILKVG
12_41_AAS36537	...	...	...	...
11_2VNC	LNANPNNVKVPFK.	...GKWE.LIVISSY	...LREI.	...GEKELEJEGRALVVRREL
11_2WSK	LNATLEVEIEVLPA.	...GEWH.AIPPPAG	...EDNPVI.	...TAVWQOQPAHGLCVFPR
11_1BF2	YNGWSSVFTFLPAP.	...SGTAVQ.RVTDCTDMNDGASTFA.	...PGS.ETL.IGG	...AGTYGCGQSSILILFSK
11_4B7R	FTLSTHPKLLLOPKW.	...GGQVWQ.PLDSTSKVAP	...YDFLAVDQVLSAEDVAAARQMAWMT	...ADITYPLPWPSWILLOSAPEDPAATSMIK

Table S1. Overview of the kinetic constants of members of GH13 subfamilies 11–14, and 39, and GH57 members with α -glucan debranching activity. GH13 proteins, which experimentally have been shown to have α -glucan debranching activity, but which are not included in a subfamily are included.

GH13_11						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Escherichia coli</i> str. K-12 substr. MG1655, GDE, AAC76456	pH 7.0, 37°C					[1]
	Maltosyl- β -CD	ND	ND		ND	
	Maltotriosyl- β -CD	1.51 $\pm$ 0.14	38.0 $\pm$ 0		26.0 $\pm$ 2.5	
	Maltotetrasyl- β -CD	0.15 $\pm$ 0.03	41.7 $\pm$ 2.9		300 $\pm$ 45	
	Maltopentasy- β -CD	ND	ND		ND	
<i>Sulfolobus shibatae</i> B12, GDE, AAM81590	pH 5.5, 70°C	mM				[2]
	Maltopentaose	246.3				
	Maltosyl- β -CD	12.2				
	Pullulan	19				
<i>Sulfolobus solfataricus</i> P2, GDE, AAK42273	pH 5.5, 70°C	mg/ml	1/s		ml/(mg s)	[3]
	Amylopectin	31.1 $\pm$ 0.23 (88.0 $\pm$ 5.63) ^a	0.923 $\pm$ 0.006 (17.35 $\pm$ 0.96) ^a		(29.6 $\pm$ 0.294)·10 ⁻³ ((197 $\pm$ 16.7)·10 ⁻³) ^a	
	pH 6.5, 70°C	mg/ml	1/s		ml/(mg s)	
	Amylopectin	92.3 $\pm$ 7.41 (345 $\pm$ 28.3) ^a	0.48 $\pm$ 0.05 (7.57 $\pm$ 0.23) ^a		(5.21 $\pm$ 0.654)·10 ⁻³ ((21.9 $\pm$ 1.92)·10 ⁻³) ^a	
	pH 5.5, 70°C	mM	1/s		1/(mM s)	[4]
	Maltotetrasyl- β -CD	0.206 $\pm$ 0.003	2570 $\pm$ 334		1242 $\pm$ 162	
GH13_12						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Bacillus</i> sp. KSM-1378, PULII_II, AAS36537	pH 9.0, 50°C	mg/ml		μ g/ml/min		[5]
	Pullulan	0.72		169		
	Amylose	0.27		51		
<i>Streptococcus pneumoniae</i> , PULI, SpuA, AAK74446	25°C	mM	1/s			[6]
	pNP- α -maltopentaoside	0.36 $\pm$ 0.06	4.5 $\pm$ 0.2			

^aNumbers in paranthesis are values from assay with DMSO

GH13_13						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
Barley (<i>Hordeum vulgare</i>), PULI, AAD04189	pH 5.5, 37°C	mg/ml	1/s		ml/(mg s)	[7, 8]
	Pullulan	0.081±0.003 (0.16±0.02) ^b	61±13 (78±10) ^b		753 (488) ^b	
	Amylopectin	6.9±1.0	15.6±1.2		2.3	
	pH 5.0, 40°C	mg/ml	1/s		ml/(mg s)	[9]
	Pullulan	0.44	60.0		140	
	6 ² -α-maltotriosyl-maltotetraose	0.81 (0.78 mM)	215		280 (273 1/(mM s))	
	pH 5.0, 40°C	mM	1/s		1/(mM s)	[10]
	6 ³ -α-maltosyl-maltotetraose	0.49	164		335	
	6 ⁴ -α-maltosyl-maltopentaose	0.47	85		181	
	6 ³ -α-maltotriosyl-maltotetraose	0.78	218		279	
	6 ³ -α-maltosyl-maltotriose	3.3	157		48	
	G ₂ -α-cyclodextrin	0.087	10		113	
	G ₂ -β-cyclodextrin	0.47	5		11	
	G ₂ -γ-cyclodextrin	0.19	39		205	
	pH 5.0, 40°C	mM	1/s		1/(mM s)	[10]
	6 ³ -α-maltosyl-maltotetraose	0.98	2071		2100	
<i>Klebsiella pneumoniae</i> (<i>Aerobacter aerogenes</i>), PULI, AAA25124	6 ⁴ -α-maltosyl-maltopentaose	1.19	893		750	
	6 ³ -α-maltotriosyl-maltotetraose	0.7	1409		2000	
	6 ³ -α-maltosyl-maltotriose	1.7	772		450	
	G ₂ -α-cyclodextrin	0.083	8		96	
	G ₂ -β-cyclodextrin	1.1	10		9	
	G ₂ -γ-cyclodextrin	0.12	241		2000	
	pH 5.0, 40°C	mg/ml	1/s		ml/(mg s)	[11]
	Pullulan	0.017	103.3		6076	
	Potato amylopectin	10.1	14.1		1.4	
	Sweet potato amylopectin	8.3	8.8		1.1	
	Wheat amylopectin	7.6	10.3		1.4	
	Maize amylopectin	9.9	8.6		0.9	
	Waxy maize amylopectin	9.8	9.0		0.9	
	Glutinous rice amylopectin	9.1	9.4		1.0	
	Waxy maize amylopectin, acid treated	8.9	19.9		2.2	
	Glutinous rice amylopectin, acid treated	7.7	13.7		1.8	
	Oyster glycogen	33	4.5		0.1	
	Rabbit liver glycogen	20	1.8		0.1	
	Sweet corn phytoglycogen	50	8.2		0.2	

<i>Klebsiella pneumoniae</i> (<i>Aerobacter aerogenes</i>), PULI (commercial)	pH 5.4, 37°C	mg/ml	1/s	ml/(mg s)	[12]
	Pullulan	0.617	116	188	
Maize (<i>Zea maize</i>) PULI, AAD11599	pH 6.0, 37°C	mM	μmol/min		[13]
	Pullulan	0.37±0.05	95.0±4.1		
	β-limit dextrin from amylopectin	4.10±0.95	344.4±55.2		
	pH 6.0, 37°C	mg/ml	1/s	ml/(mg s)	[14]
	Pullulan	0.625	23.1	37.0	
	Amylopectin	1.538	2.18	1.42	
	Soluble starch	1.538	2.80	1.88	
	Glycogen β-limit dextrin	0.909	3.31	3.64	
	Rice (<i>Oryza sativa</i> L. <i>japonica</i>), PULI, BAA28632	pH 5.0, 30°C	mg/ml	Relative to pullulan	
Pullulan		-	100		
Amylopectin		6	35		
Amylopectin β-limit dextrin		4	150		
Rabbit liver glycogen β-limit dextrin		7	55		
Phytoglycogen β-limit dextrin		10	135		
6 ³ -α-maltotriosyl-maltotetraose		0.67 (0.6 mM)	260		
<i>Shewanella arctica</i> , PULI, CED95594		%	1/s	μmol/min/ mg	[16]
	Pullulan	0.1	86.9	33.8	
<i>Sorghum bicolor</i> , PULI, ABK63595 (fragment)	pH 5.0, 30°C	mg/ml	Relative to pullulan		[17]
	Pullulan	0.2	1.0		
	Amylopectin	-	0.14		
	Amylopectin β-limit dextrin	2.5	2.6		
	Glycogen β-limit dextrin	30	3.0		
	6 ³ -α-maltotriosyl-maltotetraose	1.7	5.3		
Spinach (<i>Spinacia oleracea</i>), PULI, AAN26412	pH 6.0, 37°C	mg/ml	U/mg		[18]
	Pullulan	0.78	69		
	Amylopectin	7	22		
	pH 6.0, 37°C	mg/ml	U/mg		[19]
	Pullulan	0.70	62		
	Amylopectin	7.2	16		

^bResults in brackets are based on the Michaelis-Menten equation for uncompetitive substrate inhibition

GH13_14						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Anaerobranca gottschalkii</i> , PULI, AAS47565	pH 8, 60°C	mg/ml		U/mg		[20]
	Pullulan	0.75		61		
	pH 5.0, 70°C	mg/ml		U/min		[21]
	Pullulan	4.0		0.23		
	Soluble starch	11.1		0.2		
	Dextran	40.0		1.25		
	pH 5.0, 40°C	mM	1/s		1/(mM s)	[10]
<i>Bacillus acidopullulyticus</i> , PULI, CAC60156	6 ³ - α -maltosyl-maltotetraose	0.3	164		550	
	6 ⁴ - α -maltosyl-maltopentaose	0.47	61		130	
	6 ³ - α -maltotriosyl-maltotetraose	0.23	87		380	
	6 ³ - α -maltosyl-maltotriose	1.5	65		43	
	G ₂ - α -cyclodextrin	0.21	8		38	
	G ₂ - β -cyclodextrin	1.4	50		36	
	G ₂ - γ -cyclodextrin	0.08	31		390	
<i>Bacillus deramificans</i> T89.117D, PULI,	pH 4.5, 37°C	mg/ml	1/s		ml/(mg s)	[22]
	Pullulan	0.7±0.02	1900.4±103.5		2712.9±121.6	
	pH 5.4, 60°C	mg/ml	1/s	U/mg	ml/(mg s)	[23]
<i>Bacillus subtilis</i> strain 168, PULI, AAC00283	Pullulan	0.38±0.02	2031±97.5	436±21.6	5403±270.1	
	pH 5.4, 37°C	mg/ml	1/s		ml/(mg s)	[12]
<i>Exiguobacterium acetylium</i> a1/YH5, PULI	Pullulan	1.28	97	75.5		
	pH 6.0, 50°C	mg/ml		U/mg		[24]
	Pullulan	0.12±0.02		33.79±0.26		
<i>Exiguobacterium</i> sp. SH3, PULI, AJG04842	Soluble starch	0.69±0.04		20.17±0.28		
	pH 7.0, 37°C	mg/ml		U/ml		[25]
	Pullulan	0.069		967		
<i>Fervidobacterium pennavorans</i> Ven5, PULI, AAD30387	pH 8.5, 45°C	mg/ml	1/s	U/mg	ml/(mg s)	[26]
	Pullulan	2.8	37	20.3	13.2	
	pH 6.0, 80°C	mg/ml		U/mg		[27]
<i>Lactobacillus plantarum</i> L137, PULII_I, BAF93906	Pullulan	0.4		1.58		
	pH 4.0, 40°C	mg/ml	1/s	U/mg	ml/(mg s)	[28, 29]
	Pullulan	6.9	18.0	37.9	3.3	
	Soluble starch	7.7	25.5	53.4	5.9	
	Amylose	2.6	15.3	32.3	2.6	
<i>Paenibacillus polymyxa</i> Nws-pp2, PULI, AIE88189	pH 6.0, 35°C	mg/ml		U/mg		[30]
	Pullulan	15.25		20.1		

GH13_39						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Geobacillus thermoleovorans</i> NP33, PULII_I, AFI70750		mg/ml	1/s	U/mg	ml/(mg s)	[31]
	Pullulan	3.3	1941	640	2426	
	Starch	0.8	2021	666	588	
<i>Thermoanaerobacter pseudethanolicus</i> ATCC33223 (<i>Clostridium thermohydrosulfuricum</i>), PULII_I, ABY95795		mg/ml	1/s			[32]
	Pullulan	0.35	267			
	Amylose	1.00				
	pH 6.0, 60°C	mg/ml	1/s		ml/(mg s)	[33]
	Pullulan	0.675	271		410	
	pH 6.0, 70°C	mg/ml	1/s		ml/(mg s)	
<i>Thermoanaerobacterium saccharolyticum</i> NTOU1, PULII_I	Pullulan	13.0±0.4	1751.3±38.0		135.1	[34]
	Starch	7.4±0.4	877.9±2.0		119.0	
	65°C	mg/ml				
<i>Thermoanaerobacterium thermosulfurigenes</i> EM1 (<i>Clostridium thermosulfurogenes</i>), PULII_I, AAB00841	Pullulan	1.5				[35]
	Soluble starch	0.70				
GH13 debranching enzymes not classified into subfamily						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Desulfurococcus mucosus</i> , PULII_I, AAG31033	pH 5.5, 80°C	M	1/s		1/(M s)	[36]
	Pullulan	0.25	170		680	
	Starch	5.88	102		12.3	
<i>Thermococcus kodakarensis</i> KOD1, PULII_I/pullulan hydrolase type III, BAD85166	pH 5.5	mg/ml	1/s	U/mg	ml/(mg s)	[37]
	Pullulan	0.37±0.02	157.8	118.39±1.76	424.3	
	pH 6.0	mg/ml	1/s	U/mg	ml/(mg s)	[38]
	Soluble starch	0.36±0.05	70.9	53.19±11.66	196.9	
	pH 4.2, 90°C	mg/ml		U/mg		
Uncultured bacterium, PULII_II, AGL50935	Pullulan	0.2		109		[39]
	pH9.0, 50°C	mg/ml		U/mg		
	Pullulan	1.5±0.1		306.8±5.9		
	Amylose (potato)	3.6±0.2		254.3±2.7		
	Soluble starch	4.4±0.2		230.6±4.1		
	Amylopectin (potato)	5.1±0.4		142.5±2.9		
	Glycogen (oyster)	9.7±0.8		58.9±2.3		

GH57

Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Pyrococcus furiosus</i> DSM 3638, PULII_I, ABA33719	90°C	mg/ml		U/mg		[40]
	Pullulan	0.13±0.03		156.7±9.9		
	Soluble starch	1.17±0.15		63.9±3.0		
	pH 5.0, 90°C	mg/ml	1/s		ml/(mg s)	
<i>Staphylothermus marinus</i> F1, PULII_I, ABN70497	Pullulan	0.55±0.02	187.55±10.76		342.34±12.10	[41]
	Amylopectin	16.24±2.56	181.45±10.19		11.38±1.31	
	Amylose	4.71±0.26	39.32±1.43		8.36±0.20	
	Starch	0.54±0.07	59.69±2.90		112.46±10.25	
	Glycogen	0.07±0.01	1.79±0.14		26.30±1.73	
		mM	1/s		1/(mM s)	
	α-CD	5.32±0.24	235.22±19.30		44.23±3.15	
	β-CD	5.72±1.53	196.60±31.16		35.46±4.54	
	γ-CD	5.76±0.19	445.04±5.68		77.33±2.61	
	Glucosyl-β-CD	5.81±0.04	78.07±3.93		13.45±0.71	
	Maltosyl-β-CD	3.88±0.13	241.84±5.80		62.33±1.21	
	Maltotriosyl-β-CD	0.22±0.05	77.82±13.10		361.38±26.81	
	Maltotriose	0.56±0.004	12.44±0.33		223.13±11.85	
	Maltopentaose	0.11±0.024	32.07±3.45		301.38±29.54	
<i>Thermococcus kodakarensis</i> KOD1, PULII_I, BAD85963	pH 5.5, 105°C	mg/ml	1/s	U/mg	ml/(mg s)	[42]
	Pullulan	2.2±0.2	192.3	92.3±1.5	87.4	
	pH 5.0, 110°C	mg/ml	1/s	U/mg	ml/(mg s)	
	Starch	1.9±0.4	27.6	13.2±1.3	14.5	

No sequence, but pullulanase activity						
Source, enzyme type, GenBank accession no.	Substrate and assay conditions	K_M	k_{cat}	V_{max}	k_{cat}/K_M	Reference
<i>Bacillus</i> sp. AN-7, PULI	pH 6.0, 80°C	mg/ml		U/mg		[43]
	Pullulan	1.3		154		
<i>Bacillus</i> sp. S-1, alkaline PULI	pH 9.0, 50°C	mg/ml		mg/s/ml		[44]
	Pullulan	7.92		6.6		
	Amylopectin	1.63		0.63		
	α,β -limited dextran	3.1		0.66		
<i>Bacillus flavocaldarius</i> KP1228, PULI, BAB18516 (Classified into GH13_20, but with EC no 3.2.1.41)	pH 9.0, 75°C	(%)-weight/volume	1/s	volume/ (%-weight s)		[45]
	Pullulan	1.7	48.5	28.5		
	Amylopectin	1.8	12.6	7.0		
	β -limit dextrin	1.1	80.0	72.7		
	Soluble starch	13.0	51.2	3.9		
Broad bean, <i>Vicia faba</i> L., PULI	30°C	mg/ml		Relative units		[46]
	Amylopectin	1.2		0.1		
	Amylopectin β -limit dextrin	1		0.65		
	Rabbit-liver glycogen β -limit dextrin	17		0.5		
	6 ³ - α -maltotriosyl-maltotetraose	0.18		-		
<i>Lactococcus lactis</i> IBB 500, PULI	pH 4.5, 45°C	mg/ml		μ M/(ml mg)		[47]
	Pullulan	0.34 $\pm$ 0.02		2.04 $\pm$ 0.14		
Kidney bean, <i>Phaseolus vulgaris</i> L., ISA1/2	pH 6.0, 30°C, 10 mg/ml		μ mol/min/mg protein			[48]
	Pullulan		0.95 $\pm$ 0.15			
	Amylopectin		14.98 $\pm$ 0.51			
	Glycogen		8.95 $\pm$ 1.43			
	β -limit dextrin		6.58 $\pm$ 1.34			
Oat, <i>Avena sativa</i> , PULI	pH 5.0, 30°C	mg/ml		Relative to pullulan (%)		[15]
	Pullulan	0.17		100		
	Amylopectin	1.4		26		
	Amylopectin β -limit dextrin	1.5		160		
	Rabbit-liver glycogen β -limit dextrin	-		-		
	Phytoglycogen β -limit dextrin	-		-		
Oat, <i>Avena sativa</i> , PULI	6 ³ - α -maltotriosyl-maltotetraose	0.23 (0.2 mM)		380		[49]
	pH 5.6, 30°C	mg/ml		Relative (%)		
	Pullulan	0.23		100		
	Glutinous rice starch β -limit dextrin	3.00		71		
Sugar beet, <i>Beta vulgaris</i> var. <i>altissima</i> , PULI from mature roots	Glutinous rice starch	1.00		6.4		[50]
	pH 5.6, 37°C					
	Pullulan	0.31				

	Amylopectin	4.55			
	pH 7.0, 55°C	% (w/v)			[51]
<i>Thermoactinomyces thalpophilus</i> No. 15 (<i>Laceyella sacchari</i>), pullulanase	Pullulan	0.32			
	Amylopectin	0.36			
	Amylopectin β -limit dextrin	0.45			
	Glycogen β -limit dextrin	1.11			
	pH 5.6, 70°C	mg/ml	1/s	ml/(mg s)	[52]
<i>Thermoanaerobium</i> Tok6-B1, PULII_II	Pullulan	0.07	$6.7 \cdot 10^4$	$9.6 \cdot 10^5$	
	α -1,6-maltotriosyl-maltotriose	0.24	$1.8 \cdot 10^4$	$7.5 \cdot 10^4$	
	Corn amylopectin	0.03	$2.3 \cdot 10^4$	$7.7 \cdot 10^5$	
	Potato amylopectin	0.01	$8.3 \cdot 10^4$	$8.3 \cdot 10^6$	
	Rabbit liver glycogen	0.04	$3.7 \cdot 10^4$	$9.3 \cdot 10^5$	
	Oyster glycogen	0.01	$1.0 \cdot 10^5$	$1.0 \cdot 10^7$	
	Lintner's starch	0.05	$2.2 \cdot 10^4$	$4.4 \cdot 10^5$	
	Short-chain amylose	0.02	$1.5 \cdot 10^4$	$7.5 \cdot 10^5$	
	Potato amylose	0.02	$2.6 \cdot 10^4$	$1.3 \cdot 10^6$	
	pH 5.9, 70°C	(%)		nmol/min/ml	[53]
<i>Thermus aquaticus</i> YT-1 (strain ATCC25104)	Pullulan	0.0063		114	
	Amylopectin	0.0025		12	
	Lintner's starch	0.0034		12	
	pH 7.0, 73°C	mg/ml		U/mg	[54]
<i>Thermus caldophilus</i> GK-24, PULI	Pullulan	0.42		1.8	

References

- 1 Song H, Jung T, Park J, Park B, Myung PK, Boos W, Woo E and Park K (2010) Structural rationale for the short branched substrate specificity of the glycogen debranching enzyme GlgX. *Proteins Struct Funct Bioinf* 78(8):1847–1855
- 2 Van TTK, Ryu S, Lee K, Kim E and Lee S (2007) Cloning and characterization of glycogen-debranching enzyme from hyperthermophilic archaeon *Sulfolobus shibatae*. *J Microbiol Biotechnol* 17(5):792–799
- 3 Park J, Park H, Kang H, Hong J, Cha H, Woo E, Kim J, Kim M, Boos W, Lee S and Park K (2008) Oligomeric and functional properties of a debranching enzyme (TreX) from the archaeon *Sulfolobus solfataricus* P2. *Biocatal Biotransfor* 26(1–2):76–85
- 4 Nguyen DHD, Park J, Shim J, Tran PL, Oktavina EF, Nguyen TLH, Lee S, Park C, Li D, Park S, Stapleton D, Lee J and Park K (2014) Reaction kinetics of substrate transglycosylation catalyzed by trex of *Sulfolobus solfataricus* and effects on glycogen breakdown. *J Bacteriol* 196(11):1941–1949
- 5 Ara K, Saeki K, Igarashi K, Takaiwa M, Uemura T, Hagihara H, Kawai S and Ito S (1995) Purification and characterization of an alkaline amylopullulanase with both α -1,4 and α -1,6 hydrolytic activity from alkalophilic *Bacillus* sp. KSM-1378. *Biochim Biophys Acta, Gen Subj* 1243(3):315–324
- 6 Lammerts van Bueren A, Ficko-Blean E, Pluvinaige B, Hehemann J, Higgins MA, Deng L, Ogunniyi AD, Stroehrer UH, El Warry N, Burke RD, Czjzek M, Paton JC, Vocadlo DJ and Boraston AB (2011) The conformation and function of a multimodular glycogen-degrading pneumococcal virulence factor. *Structure* 19(5):640–651
- 7 Vester-Christensen MB, Abou Hachem M, Naested H and Svensson B (2010) Secretory expression of functional barley limit dextrinase by *Pichia pastoris* using high cell-density fermentation. *Protein Expr Purif* 69(1):112–119
- 8 Møller MS, Windahl MS, Sim L, Bojstrup M, Abou Hachem M, Hindsgaul O, Palcic M, Svensson B and Henriksen A (2015) Oligosaccharide and substrate binding in the starch debranching enzyme barley limit dextrinase. *J Mol Biol* 427(6):1263–1277
- 9 Greffe L, Jensen M, Bosso C, Svensson B and Driguez H (2003) Chemoenzymatic synthesis of branched oligo- and polysaccharides as potential substrates for starch active enzymes. *4(12):1307–1311*
- 10 Jensen MT (2002) Structure and function of barley limit dextrinase and mutational analysis of barley α -amylase isozyme differences and interaction with branched oligosaccharides. Dissertation, University of Southern Denmark, DK-5230 Odense M
- 11 Yokobayashi K, Akai H, Sugimoto T, Hirao M, Sugimoto K and Harada T (1973) Comparison of kinetic parameters of *Pseudomonas* isoamylase and *Aerobacter* pullulanase. *Biochim Biophys Acta* 293(1):197–202
- 12 Malle D, Itoh T, Hashimoto W, Murata K, Utsumi S and Mikami B (2006) Overexpression, purification and preliminary X-ray analysis of pullulanase from *Bacillus subtilis* strain 168. *Acta Crystallogr, Sect F: Struct Biol Cryst Commun* 62:381–384
- 13 Wu CY, Colleoni C, Myers AM and James MG (2002) Enzymatic properties and regulation of ZPU1, the maize pullulanase-type starch debranching enzyme. *Arch Biochem Biophys* 406(1):21–32
- 14 Yamasaki Y, Nakashima S and Konno H (2008) Pullulanase from rice endosperm. *Acta Biochim Pol* 55(3):507–510
- 15 Dunn G and Manners DJ (1975) Limit dextrinases from ungerminated oats (*Avena sativa* L.) and ungerminated rice (*Oryza sativa* L.). *Carbohydr Res* 39(2):283–293
- 16 Elleuche S, Qoura FM, Lorenz U, Rehn T, Brueck T and Antranikian G (2015) Cloning, expression and characterization of the recombinant cold-active type-I pullulanase from *Shewanella arctica*. *J Mol Catal B-Enzym* 116:70–77
- 17 Hardie DG, Manners DJ and Yellowlees D (1976) Limit dextrinase from malted sorghum (*Sorghum vulgare*). *Carbohydr Res* 50(1):75–85

- 18 Ludwig I, Ziegler P and Beck E (1984) Purification and properties of spinach leaf debranching enzyme. *Plant Physiol* 74(4):856–861
- 19 Renz A, Schikora S, Schmid R, Kossmann J and Beck E (1998) cDNA sequence and heterologous expression of monomeric spinach pullulanase: multiple isomeric forms arise from the same polypeptide. *Biochem J* 331:937–945
- 20 Bertoldo C, Armbrrecht M, Becker F, Schafer T, Antranikian G and Liebl W (2004) Cloning, sequencing, and characterization of thermoalkalstable type I pullulanase from *Anaerobranca gottschalkii*. *Appl Environ Microbiol* 70(6):3407–3416
- 21 Singh RS, Saini GK and Kennedy JF (2010) Maltotriose syrup preparation from pullulan using pullulanase. *Carbohydr Polym* 80(2):401–407
- 22 Duan X, Chen J and Wu J (2013) Improving the thermostability and catalytic efficiency of *Bacillus deramificans* pullulanase by site-directed mutagenesis. *Appl Environ Microbiol* 79(13):4072–4077
- 23 Duan X and Wu J (2015) Enhancing the secretion efficiency and thermostability of a *Bacillus deramificans* pullulanase mutant (D437H/D503Y) by N-terminal domain truncation. *Appl Environ Microbiol* 81(6):1926–1931
- 24 Qiao Y, Peng Q, Yan J, Wang H, Ding H and Shi B (2015) Gene cloning and enzymatic characterization of alkali-tolerant type I pullulanase from *Exiguobacterium acetylicum*. *Lett Appl Microbiol* 60(1):52–59
- 25 Rajaei S, Heidari R, Zahiri HS, Sharifzadeh S, Torktaz I and Noghabi KA (2014) A novel cold-adapted pullulanase from *Exiguobacterium* sp SH3: Production optimization, purification, and characterization. *Starch-Stärke* 66(3–4):225–234
- 26 Rajaei S, Noghabi K, Sadeghizadeh M and Zahiri H (2015) Characterization of a pH and detergent-tolerant, cold-adapted type I pullulanase from *Exiguobacterium* sp. SH3. *Extremophiles* 19(6):1145–1155
- 27 Bertoldo C, Duffner F, Jorgensen P and Antranikian G (1999) Pullulanase type I from *Fervidobacterium pennavorans* Ven5: Cloning, sequencing, and expression of the gene and biochemical characterization of the recombinant enzyme. *Appl Environ Microbiol* 65(5):2084–2091
- 28 Kim J, Sunako M, Ono H, Murooka Y, Fukusaki E and Yamashita M (2008) Characterization of gene encoding amylopullulanase from plant-originated lactic acid bacterium, *Lactobacillus plantarum* L137. *J Biosci Bioeng* 106(5):449–459
- 29 Kim J, Sunako M, Ono H, Murooka Y, Fukusaki E and Yamashita M (2009) Characterization of the C-terminal truncated form of amylopullulanase from *Lactobacillus plantarum* L137. *J Biosci Bioeng* 107(2):124–129
- 30 Wei W, Ma J, Chen S, Cai X and Wei D (2015) A novel cold-adapted type I pullulanase of *Paenibacillus polymyxa* Nws-pp2: in vivo functional expression and biochemical characterization of glucans hydrolyzates analysis. *BMC Biotechnol* 15:1–13
- 31 Nisha M and Satyanarayana T (2015) The role of N1 domain on the activity, stability, substrate specificity and raw starch binding of amylopullulanase of the extreme thermophile *Geobacillus thermoleovorans*. *Appl Microbiol Biotechnol* 99(13):5461–5474
- 32 Mathupala S, Saha B and Zeikus J (1990) Substrate competition and specificity at the active site of amylopullulanase from *Clostridium thermohydrosulfuricum*. *Biochem Biophys Res Commun* 166(1):126–132
- 33 Saha B, Mathupala S and Zeikus J (1988) Purification and characterization of a highly thermostable novel pullulanase from *Clostridium thermohydrosulfuricum*. *Biochem J* 252(2):343–348
- 34 Lin F, Ma H, Lin H, Liu S and Tzou W (2011) Biochemical characterization of two truncated forms of amylopullulanase from *Thermoanaerobacterium saccharolyticum* NTOU1 to identify its enzymatically active region. *Appl Biochem Biotechnol* 165(3–4):1047–1056
- 35 Spreinat A and Antranikian G (1992) Analysis of the amylolytic enzyme-system of *Clostridium thermosulfurogenes* EM1: Purification and synergistic action of pullulanases and maltohexaose forming α -amylase. *Starch-Stärke* 44(8):305–312

- 36 Duffner F, Bertoldo C, Andersen J, Wagner K and Antranikian G (2000) A new thermoactive pullulanase from *Desulfurococcus mucosus*: Cloning, sequencing, purification, and characterization of the recombinant enzyme after expression in *Bacillus subtilis*. *J Bacteriol* 182(22):6331–6338
- 37 Han T, Zeng F, Li Z, Liu L, Wei M, Guan Q, Liang X, Peng Z, Liu M, Qin J, Zhang S and Jia B (2013) Biochemical characterization of a recombinant pullulanase from *Thermococcus kodakarensis* KOD1. *Lett Appl Microbiol* 57(4):336–343
- 38 Ahmad N, Rashid N, Haider MS, Akram M and Akhtar M (2014) Novel maltotriose-hydrolyzing thermoacidophilic type III pullulan hydrolase from *Thermococcus kodakarensis*. *Appl Environ Microbiol* 80(3):1108–1115
- 39 Lee YS, Seo SH, Yoon SH, Kim SY, Hahn BS, Sim JS, Koo BS and Lee CM (2016) Identification of a novel alkaline amylopullulanase from a gut metagenome of *Hermetia illucens*. *Int J Biol Macromolec* 82:514–522
- 40 Dong G, Vieille C and Zeikus J (1997) Cloning, sequencing, and expression of the gene encoding amylopullulanase from *Pyrococcus furiosus* and biochemical characterization of the recombinant enzyme. *Appl Environ Microbiol* 63(9):3577–3584
- 41 Li X, Li D and Park K (2013) An extremely thermostable amylopullulanase from *Staphylothermus marinus* displays both pullulan- and cyclodextrin-degrading activities. *Appl Microbiol Biotechnol* 97(12):5359–5369
- 42 Guan Q, Guo X, Han T, Wei M, Jin M, Zeng F, Liu L, Li Z, Wang Y, Cheong G, Zhang S and Jia B (2013) Cloning, purification and biochemical characterisation of an organic solvent-, detergent-, and thermo-stable amylopullulanase from *Thermococcus kodakarensis* KOD1. *Process Biochem* 48(5–6):878–884
- 43 Kunamneni A and Singh S (2006) Improved high thermal stability of pullulanase from a newly isolated thermophilic *Bacillus* sp AN-7. *Enzyme Microb Technol* 39(7):1399–1404
- 44 Kim CH, Choi HI and Lee DS (1993) Purification and biochemical properties of an alkaline pullulanase from alkalophilic *Bacillus* sp. S-1. *Biosci Biotechnol Biochem* 57(10):1632–1637
- 45 Suzuki Y, Hatagaki K and Oda H (1991) A hyperthermostable pullulanase produced by an extreme thermophile, *Bacillus flavocaldarius* Kp-1228, and evidence for the proline theory of increasing protein thermostability. *Appl Microbiol Biotechnol* 34(6):707–714
- 46 Gordon RW, Manners DJ and Stark JR (1975) Limit dextrinase of broad bean (*Vicia faba* L.). *Carbohydr Res* 42(1):125–134
- 47 Wasko A, Polak-Berecka M and Targonski Z (2011) Purification and characterization of pullulanase from *Lactococcus lactis*. *Prep Biochem Biotechnol* 41(3):252–261
- 48 Takashima Y, Senoura T, Yoshizaki T, Haniada S, Ito H and Matsui H (2007) Differential chain-length specificities of two isoamylase-type starch-debranching enzymes from developing seeds of kidney bean. *Biosci Biotechnol Biochem* 71(9):2308–2312
- 49 Yamada J (1981) Purification of oat debranching enzyme and occurrence of inactive debranching enzyme in cereals. *Agric Biol Chem* 45(4):1013–1015
- 50 Masuda H, Takahashi T and Sugawara S (1987) Purification and properties of starch hydrolyzing enzymes in mature roots of sugar-beets. *Plant Physiol* 84(2):361–365
- 51 Odibo F and Obi S (1988) Purification and characterization of a thermostable pullulanase from *Thermoactinomyces thalophilus*. *J Ind Microbiol* 3(6):343–350
- 52 Plant AR, Clemens RM, Morgan HW and Daniel RM (1987) Active-site and substrate-specificity of *Thermoanaerobium* Tok6-B1 pullulanase. *Biochem J* 246(2):537–541
- 53 Plant AR, Morgan HW and Daniel RM (1986) A highly stable pullulanase from *Thermus aquaticus* YT-1. *Enzyme Microb Technol* 8(11):668–672
- 54 Kim C, Nashiru O and Ko J (1996) Purification and biochemical characterization of pullulanase type I from *Thermus caldophilus* GK-24. *FEMS Microbiol Lett* 138(2–3):147–152

Table S2. Relative specific activities of characterised members of GH13 subfamilies 11–14 and 39 including non-sequenced enzymes with pullulanase activity as well as GH13 pullulanase members not assigned to a subfamily, and GH57 enzymes.

	GH13 11			GH13 12+44
Organism, enzyme name, GenBank accession no., reference	<i>Flavobacterium</i> sp., GDE, AAB63356 [1]	<i>Pseudomonas</i> , ISA [2]	<i>Sulfolobus shibatae</i> B12, GDE, AAM81590 [3]	<i>Bacillus</i> so. KSM-1378, PULII_II, AAS36537 [4]
Assay conditions: pH, temperature, substrate concentration			pH 5.5, 70°C, 1% (w/v)	pH 9.0, 50°C
Pullulan	2	0.5	36.8	100
Amylopectin	100 (corn)	100 (potato) 106 (sweet potato) 111 (wheat) 124 (maize) 96 (waxy maize) 74 (glutinous rice)	100	55 (potato)
Amylopectin limit dextrin				6 (dextrin)
Glycogen	101 (rabbit liver), 106 (oyster)	73 (oyster) 50 (rabbit liver)	73.5	25 (oyster), 24 (bovine muscle)
Phytoglycogen		105 (sweet corn)		
Starch	70 (potato), 90 (rice)		82.4 (rice)	130 (potato)
Amylose			16.9	28 (potato, DP=17)

	GH13_13					
Organism, enzyme name, GenBank accession no., reference	Barley (<i>Hordeum vulgare</i>), PULI, AAD04189 [5]	<i>Klebsiella pneumoniae</i> (<i>Aerobacter aerogenes</i>), PULI, AAA25124 [2]	<i>Klebsiella pneumoniae</i> (<i>Aerobacter aerogenes</i>), PULI, AAA25124 [6]	Rice (<i>Oryza sativa</i>), PULI, [7]	Rice (<i>Oryza sativa</i>), PULI, [8]	<i>Sorghum bicolour</i> , PULI, ABK63595 [9]
Assay conditions: pH, temperature, substrate concentration	pH 5.2, 37°C, 1 mM	pH 5.5, 40°C, 2% (w/v)	pH 5.0, 35°C, 0.5% (w/v)	pH 5.0, 30°C	pH 6.0, 37°C	pH 5.0, 30°C
Pullulan	100	100	100	100	100	100
Amylopectin	<<1	9.1 (potato), 6.0 (sweet potato), 7.2 (wheat), 5.5 (maize), 6.0 (waxy maize), 6.4 (glutinous rice), 13.6 (waxy maize, acid-treated), 9.4 (glutinous rice, acid-treated)			34.1	
Amylopectin limit dextrin	68				19.5 (β-)	
Glycogen	0	1.7 (oyster), 1.1 (rabbit liver)			6.3	
Phytoglycogen		2.3 (sweet corn)				
Soluble starch					15.8	
Panose				0		0
6 ³ -α-D-glucosyl-maltotriose			0	0		
6 ³ -α-maltosyl-maltotriose	13		55	50		220
6 ³ -α-maltosyl-maltotetraose	123		171	210		300
6 ³ -α-maltotriosyl-maltotriose	119		91	170		250
6 ³ -α-maltotriosyl-maltotetraose	243		112	260		400
maltosyl-α-cyclodextrin						0
maltosyl-β-cyclodextrin						50

	GH13_13, continued			
Organism, enzyme name, GenBank accession no., reference	<i>Spinacia oleracea</i> , spinach, PULI, AAN26412 [10]	<i>Spinacia oleracea</i> , spinach, recombinant PULI, AAN26412 [11]	<i>Zea maize</i> , maize, PULI, AAD11599 [12]	<i>Shewanella arctica</i> , PULI, CED95594 [13]
Assay conditions: pH, temperature, substrate concentration	pH 9.0, 50°C	pH 5.2, 37°C, 1 mM	pH 5.5, 40°C, 2% (w/v)	pH 6.0
Pullulan	100	100	100	100
Amylopectin	17.4	16 (maize)	20	38.7
Amylopectin limit dextrin			135	
Glycogen	0 (oyster), 0 (bovine and rabbit liver)		0 (oyster)	
Phytoglycogen			9	
Soluble starch	37	35		47.8
Amylose	0	0		1.6
β -glucan				2.3
Birchwood xylan				1.2

	GH13_14			
Organism, enzyme name, GenBank accession no., reference	<i>Anaerobranca gottshalskii</i> , PULI, AAS47565 [14]	<i>Bacillus acidopullulyticus</i> , PULII, CAC60156 [15]	<i>Exiguobacterium acetylicum</i> a1/YH5, PULI, AFL46505 [16]	<i>Paenibacillus polymyxa</i> Nws-pp2, PULI, AIE88189 [17]
Assay conditions: pH, temperature, substrate concentration	pH 8.0, 60°C, 0.5% (0.2% for amylose) (w/v)	pH 5.0, 35°C, 0.5% (w/v)	pH 6.0, 50°C, 0.5% (w/v)	pH 6.0, 35°C, 1% (w/v)
Pullulan	100	100	100	100
Amylopectin	14	16 (rice)	6.0	72.2
Amylopectin limit dextrin	24 (β -)		19.7 (β -)	
Glycogen	3	2 (oyster)	6.1	4.1
Glycogen β -limit dextrin		26 (oyster), 32 (rabbit liver)		
Soluble starch	15	10	12.8	67.8
Amylose	N.D.			
	GH13_39			

Organism, enzyme name, GenBank accession no., reference	<i>Geobacillus thermoleovorans</i> NP33, PULII_I, AF170750 [18]	<i>Thermoanaerobacterium saccharolyticum</i> B6A-RI, PULII_I, AAA23205 [19]	<i>Clostridium thermohydrosulfuricum</i> (<i>Thermoanaerobacter thermohydrosulfuricus</i>), PUL, AAA23205 [20]
Assay conditions: pH, temperature, substrate concentration		pH 6.0, 65°C, 0.2%	pH 6.0, 37°C
Pullulan	77	100	100
Amylopectin	90	53	62
Glycogen	13	61	75 (oyster)
Glycogen β -limit dextrin			77 (oyster)
Soluble starch	100	55	52
Amylose	64	56	

	GH57
Organism, enzyme name, GenBank accession no., reference	<i>Caldivirga maquilingensis</i> IC-167, PULII_I ABW02197 [21]
Assay conditions: pH, temperature, substrate concentration	pH 5.0, 100°C, 1% (w/v)
Pullulan	100
Amylopectin	89
Starch	86
Amylose	57
β -cyclodextrin	61

	No sequence/not in a subfamily					
Organism, enzyme name, GenBank accession no., reference	<i>Avena sativa</i> , oat, PULI [7]	<i>Bacillus</i> sp. S-1, PULI [22]	<i>Desulfurococcus muscosus</i> , PULII, recombinant, [23]	<i>Micrococcus</i> sp. Y-1, PULI [24]	<i>Phaseolus vulgaris</i> L., kidney bean, ISA1/2 and ISA3 [25]	<i>Pisum sativum</i> L., pea, PULI [26]
Assay conditions: pH, temperature, substrate concentration	pH 5.0, 30°C	pH 9.0, 50°C, 2% (w/v)	pH 5.0, 30°C	pH 10.0, 50°C, 1% (w/v)	pH 6.0, 30°C, 10 mg/ml	pH 6.0, 30°C
Pullulan	100	100	100	100	100/100	100
Amylopectin		23.5	35	38	1577/456	35
Amylopectin limit dextrin		46.3 (α - and β -), 18.3 (β -)		34	693/3773 (β -)	130
Dextrin						
Glycogen		13.0 (oyster), 17.7 (liver)	0	44 (oyster)	942/2229	0
Glycogen β -limit dextrin						15
Soluble starch		8.6		64		
Starch		3.4 (potato)	62			
Amylose		N.D.	77	62		
Dextran		N.D.	0			
6 ³ - α -maltosyl-maltotriose	120					90
6 ³ - α -maltosyl-maltotetraose	290					230
6 ³ - α -maltotriosyl-maltotriose	220					170
6 ³ - α -maltotriosyl-maltotetraose	380					280
α -cyclodextrin						
β -cyclodextrin						
γ -cyclodextrin						

Organism, enzyme name, GenBank accession no., reference	<i>Pisum sativum</i> L., developing pea embryos, PULI [27]	<i>Thermococcus kodakarensis</i> KOD1, PULII_I/pullulan hydrolase type III, BAD85166 [28]	<i>Thermus caldophilus</i> Gk- 24, PULI [29]	Uncultured bacterium, PULII_II, AGL50935 [30]
Assay conditions: pH, temperature, substrate concentration	pH 6.0, 37°C, 20 mg/ml	pH 4.2, 90°C, 0.25% (w/v)	pH 8.0, 73°C, 2% (w/v)	pH 9.0, 40°C, 0.5% (v/w)
Pullulan	100	100	100	100
Amylopectin	26	37	21.0	45 (potato)
Amylopectin limit dextrin			36.6 (α- and β-), 16.7 (β-)	
Dextrin		43		
Glycogen	0.9	26	14.2 (oyster), 13.5 (liver)	9 (oyster)
Glycogen β-limit dextrin				
Soluble starch			10.6	69
Starch		60 (potato) 41 (corn)	2.4	
Amylose		46	N.D.	82 (potato)
Dextran			N.D.	N.D.
6 ³ -α-maltosyl-maltotriose				
6 ³ -α-maltosyl-maltotetraose				
6 ³ -α-maltotriosyl-maltotriose				
6 ³ -α-maltotriosyl-maltotetraose				
α-cyclodextrin				N.D.
β-cyclodextrin		5		N.D.
γ-cyclodextrin		76		N.D.

References

- 1 Krohn B, Barry G and Kishore G (1997) An isoamylase with neutral pH optimum from a *Flavobacterium* species: Cloning, characterization and expression of the *iam* gene. *Mol Gen Genet* 254(5):469–478
- 2 Yokobayashi K, Akai H, Sugimoto T, Hirao M, Sugimoto K and Harada T (1973) Comparison of kinetic parameters of *Pseudomonas* isoamylase and *Aerobacter* pullulanase. *Biochim Biophys Acta* 293(1):197–202
- 3 Van TTK, Ryu S, Lee K, Kim E and Lee S (2007) Cloning and characterization of glycogen-debranching enzyme from hyperthermophilic archaeon *Sulfolobus shibatae*. *J Microbiol Biotechnol* 17(5):792–799
- 4 Ara K, Saeki K, Igarashi K, Takaiwa M, Uemura T, Hagihara H, Kawai S and Ito S (1995) Purification and characterization of an alkaline amylopullulanase with both α -1,4 and α -1,6 hydrolytic activity from alkalophilic *Bacillus* sp. KSM-1378. *Biochim Biophys Acta, Gen Subj* 1243(3):315–324
- 5 Manners DJ and Yellowlees D (1971) Studies on carbohydrate metabolising enzymes. Part XXVI. Limit dextrinase from germinated barley. *Starke* 23(7):228–234
- 6 Abdullah M, Catley BJ, Lee EYC, Robyt J, Wallenfe.K and Whelan WJ (1966) Mechanism of carbohydrase action.11. Pullulanase an enzyme specific for hydrolysis of α -1,6-bonds in amylaceous oligo- and polysaccharides. *Cereal Chem* 43(1):111–118
- 7 Dunn G and Manners DJ (1975) Limit dextrinases from ungerminated oats (*Avena sativa* L.) and ungerminated rice (*Oryza sativa* L.). *Carbohydr Res* 39(2):283–293
- 8 Yamasaki Y, Nakashima S and Konno H (2008) Pullulanase from rice endosperm. *Acta Biochim Pol* 55(3):507–510
- 9 Hardie DG, Manners DJ and Yellowlees D (1976) Limit dextrinase from malted sorghum (*Sorghum vulgare*). *Carbohydr Res* 50(1):75–85
- 10 Ludwig I, Ziegler P and Beck E (1984) Purification and properties of spinach leaf debranching enzyme. *Plant Physiol* 74(4):856–861
- 11 Renz A, Schikora S, Schmid R, Kossmann J and Beck E (1998) cDNA sequence and heterologous expression of monomeric spinach pullulanase: multiple isomeric forms arise from the same polypeptide. *Biochem J* 331:937–945
- 12 Wu CY, Colleoni C, Myers AM and James MG (2002) Enzymatic properties and regulation of ZPU1, the maize pullulanase-type starch debranching enzyme. *Arch Biochem Biophys* 406(1):21–32
- 13 Elleuche S, Qoura FM, Lorenz U, Rehn T, Brueck T and Antranikian G (2015) Cloning, expression and characterization of the recombinant cold-active type-I pullulanase from *Shewanella arctica*. *J Mol Catal B-Enzym* 116:70–77
- 14 Bertoldo C, Armbrecht M, Becker F, Schafer T, Antranikian G and Liebl W (2004) Cloning, sequencing, and characterization of thermoalkalstable type I pullulanase from *Anaerobranca gottschalkii*. *Appl Environ Microbiol* 70(6):3407–3416
- 15 Kusano S, Nagahata N, Takahashi S, Fujimoto D and Sakano Y (1988) Purification and properties of *Bacillus acidopullulyticus* pullulanase. *Agric Biol Chem* 52(9):2293–2298
- 16 Qiao Y, Peng Q, Yan J, Wang H, Ding H and Shi B (2015) Gene cloning and enzymatic characterization of alkali-tolerant type I pullulanase from *Exiguobacterium acetylicum*. *Lett Appl Microbiol* 60(1):52–59
- 17 Wei W, Ma J, Chen S, Cai X and Wei D (2015) A novel cold-adapted type I pullulanase of *Paenibacillus polymyxa* Nws-pp2: in vivo functional expression and biochemical characterization of glucans hydrolyzates analysis. *BMC Biotechnol* 15:1–13

- 18 Nisha M and Satyanarayana T (2013) Characterization of recombinant amylopullulanase (gt-apu) and truncated amylopullulanase (gt-apuT) of the extreme thermophile *Geobacillus thermoleovorans* NP33 and their action in starch saccharification. *Appl Microbiol Biotechnol* 97(14):6279–6292
- 19 Ramesh M, Podkovyrov S, Lowe S and Zeikus J (1994) Cloning and sequencing of the *Thermoanaerobacterium saccharolyticum* B6A-RI apu gene and purification and characterization of the amylopullulanase from *Escherichia coli*. *Appl Environ Microbiol* 60(1):94–101
- 20 Saha B, Mathupala S and Zeikus J (1988) Purification and characterization of a highly thermostable novel pullulanase from *Clostridium thermohydrosulfuricum*. *Biochem J* 252(2):343–348
- 21 Li X and Li D (2015) Preparation of linear maltodextrins using a hyperthermophilic amylopullulanase with cyclodextrin- and starch-hydrolysing activities. *Carbohydr Polym* 119:134–141
- 22 Kim CH, Choi HI and Lee DS (1993) Purification and biochemical properties of an alkaline pullulanase from alkalophilic *Bacillus* sp. S-1. *Biosci Biotechnol Biochem* 57(10):1632–1637
- 23 Duffner F, Bertoldo C, Andersen J, Wagner K and Antranikian G (2000) A new thermoactive pullulanase from *Desulfurococcus mucosus*: Cloning, sequencing, purification, and characterization of the recombinant enzyme after expression in *Bacillus subtilis*. *J Bacteriol* 182(22):6331–6338
- 24 Kim C, Choi H and Lee D (1993) Pullulanases of alkaline and broad pH range from a newly isolated alkalophilic *Bacillus* sp. S-1 and a *Micrococcus* sp. Y-1. *J Ind Microbiol* 12(1):48–57
- 25 Takashima Y, Senoura T, Yoshizaki T, Haniada S, Ito H and Matsui H (2007) Differential chain-length specificities of two isoamylase-type starch-debranching enzymes from developing seeds of kidney bean. *Biosci Biotechnol Biochem* 71(9):2308–2312
- 26 Yellowlees D (1980) Purification and characterization of limit dextrinase from *Pisum sativum* L. *Carbohydr Res* 83(1):109–118
- 27 Zhu Z, Hylton C, Rössner U and Smith A (1998) Characterization of starch-debranching enzymes in pea embryos. *Plant Physiol* 118(2):581–590
- 28 Ahmad N, Rashid N, Haider MS, Akram M and Akhtar M (2014) Novel maltotriose-hydrolyzing thermoacidophilic type III pullulan hydrolase from *Thermococcus kodakarensis*. *Appl Environ Microbiol* 80(3):1108–1115
- 29 Kim C, Nashiru O and Ko J (1996) Purification and biochemical characterization of pullulanase type I from *Thermus caldophilus* GK-24. *FEMS Microbiol Lett* 138(2-3):147–152
- 30 Lee YS, Seo SH, Yoon SH, Kim SY, Hahn BS, Sim JS, Koo BS and Lee CM (2016) Identification of a novel alkaline amylopullulanase from a gut metagenome of *Hermetia illucens*. *Int J Biol Macromolec* 82:514–522