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# Introduction to Glycoside Hydrolases: Classification, Identification and Occurrence

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## Abstract

Glycoside hydrolases are group of enzymes belonging to class 3 enzymes as per classification by IUBMB. They specifically break down glycosidic bonds of complex polysaccharides and are generally named upon the substrates on which they act (e.g. lactase acting on lactose; chitinase acting on chitin, sucrase acting on sucrose, etc). Present chapter introduces glycoside hydrolases, its identification and occurrence in nature, highlighting the diverse existence of these enzymes. It covers a detailed report on classification and available three-dimensional structures of the enzymes of the group. Glycoside hydrolases have been classified under 166 families which are grouped in 16 different superfamilies and three added clans. The chapter gives a complete documentation of all available data on glycoside hydrolases, which will be beneficial for researchers working in the domain.

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## Keywords

Glycoside hydrolases · Enzyme classification · Enzyme nomenclature · Glycoside hydrolase superfamilies

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## Introduction

Glycoside hydrolases (Glycosidase or glycosyl hydrolases) are specific catalyst that breaks down glycosidic bonds of complex polysaccharides, such as cellulose, hemicelluloses, starch etc. These enzymes with their specific functions are called cellulase, amylases, xylanases, arabinases and includes several others. In addition to

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degradation of plant polysaccharides they also function in anti bacterial defense mechanism (lysozymes), in normal cellular functioning (such as biosynthesis of N-linked glycoprotein through mannosidases), pathogenesis through viral neuraminidase (Bourne and Henrisatt 2001; Henrisatt and Davies 1997)

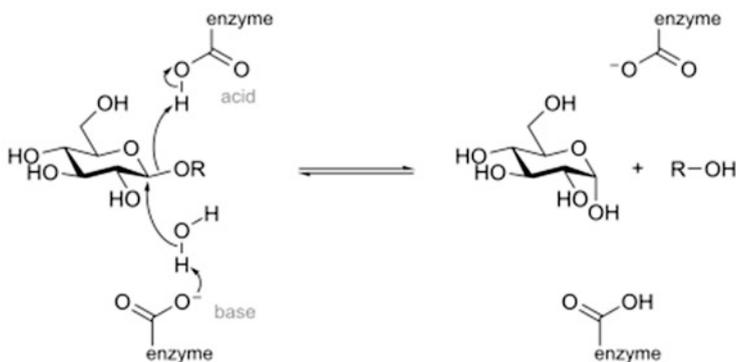
## Occurrence and Importance

Present in almost all domains of life, glycoside hydrolases are found both as intracellular and extracellular enzymes in prokaryotes and are majorly involved in hydrolysis of glycoside molecules, nutrient acquisition, regulation of expression of operon, post translational modification, lysosomal storage in higher organisms, biosynthesis and degradation of glycogen. In prokaryotes and lower eukaryotes they are present intracellular as well as secreted as extracellular enzymes. In higher organisms they are found within endoplasmic reticulum and golgi apparatus (processing of N-linked glycoproteins); in lysosome (degradation of carbohydrate structure) in intestinal tract and in saliva as carbohydrate degraders (amylase), in gut as glycosylphosphatidyl anchored enzymes on endothelial cells as enzyme lactase (degradation of milk sugar lactose), enzyme O-GlcNAcase (removal of N-acetylglucosamine groups from cytoplasmic and nuclear located serine and threonine residues)

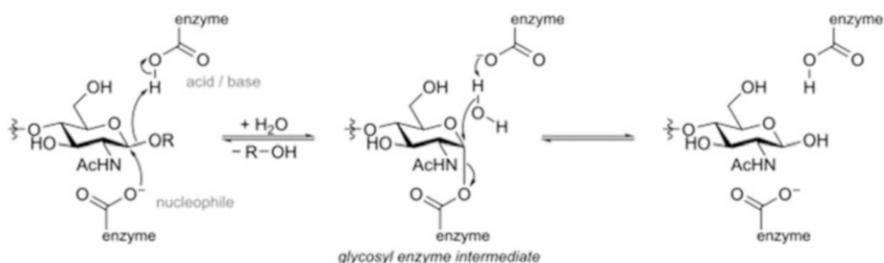
## Mechanisms of Glycoside Hydrolases

Basis their mechanism of action, Glycoside hydrolases are broadly classified as inverting glycoside hydrolases and retaining glycoside hydrolases.

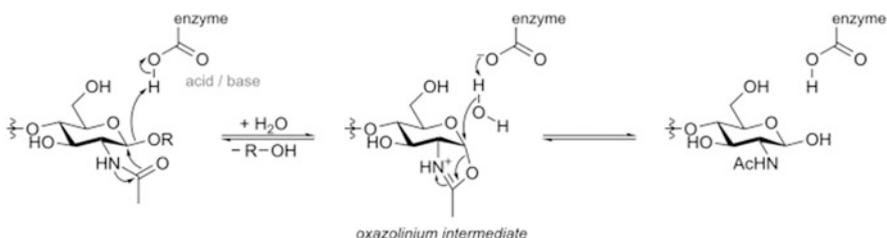
Inverting enzymes utilize two enzymic residues (generally carboxylate), one act as acid and other as base. Figure 1 shows mechanism of action of a  $\beta$ -glucosidase.



**Fig. 1** Mechanism of action of a  $\beta$ -glucosidase



**Fig. 2** Mechanism of action for hen egg white lysozyme



**Fig. 3** Mechanism of action of N-acetyl Hexoaminidases

Retaining glycoside hydrolases conduct hydrolysis through two main mechanisms, with each step resulting in inversion. Two residues involved are generally enzyme accepted carboxylates; of which one acts as a nucleophile and the other as acid/base. First step involves attack of nucleophile to the anomeric center forming a glycosyl enzyme intermediate (acidic assistance provided by acid carboxylate), followed by hydrolysis of glycosyl-enzyme transitional state through nucleophilic water (assisted by deprotonated acidic carboxylate, that acts as a base). This process results in net retention of stereochemistry. Mechanism has been illustrated as example for hen egg white lysozyme (Fig. 2) (Vocadlo et al. 2001).

Enzyme mechanisms that carried out hydrolysis with retention of stereochemistry occurs through a substrate bound nucleophilic residue, rather than being directly attached to the enzyme. Such mechanism can be seen for certain N-acetylhexosaminidases (Fig. 3), where an acetamido group present on the enzyme can participate with neighboring group and forms an intermediate oxazoline or oxazolinium ion following two steps mechanism of distinct inversions making net retention of configuration.

## Applications of Glycoside Hydrolases

Glycoside hydrolases are one of the major catalysts of ensuing generation owing to its varied applications in biorefining processes. These includes hydrolysis of plant materials (cellulases, xylanases) for production of value added components,

applications in food industries (invertase for production of invert sugars; amylase for production of maltodextrins), usage in paper and pulp industry, in detergent manufacturing (cellulases for washing of cotton fabrics for maintenance of fabric colour by removing microfibre) (Linares-Pastén et al. 2014).

These enzymes are used as synthetic catalysts (performing reverse hydrolysis/transglycosylation); reversing equilibrium and enabling the retaining glycoside hydrolase to catalyze transfer of glycosyl moiety from an activated glycoside to an acceptor alcohol. Glycosynthases (formed from retaining glycoside hydrolases by site directed mutagenesis of enzymic nucleophile to some less nucleophilic group like alanine/glycine) are enzymes catalyzing high yield of glycosides from activated glycosyl donors like glycosyl fluorides. Thioglycoligases also are mutant glycoside hydrolases, formed by site directed mutagenesis of the acid base residue of retaining glycoside hydrolases and catalyze condensation of activated glycosides and thiol comprising acceptors.

Glycoside hydrolases show usefulness in matrix polysaccharide within extracellular polymeric substances (EPS) of microbial biofilm (Fleming and Rumbaugh 2017). Degrading microbial biofilm, increases antibiotic efficacy, potentiating host immune function (Fleming et al. 2017).

## Inhibitors of Glycoside Hydrolases

There are various natural and synthetic compounds that have been reported to act as inhibitors of glycoside hydrolases. This includes naturally occurring nitrogen containing sugar shaped heterocycles (deoxynojirimycin, swainsonine, australine, castanospermine) working as natural templates for developing modified inhibitors (e.g. isofagomine, deoxygalactonojirimycin, unsaturated compounds such as PUGNAc). Glycoside hydrolase inhibitors finding clinical usage includes anti-diabetic drugs (acarbose and miglitol) and antiviral drugs (oseltamivir and zanamivir). Few proteins have also been identified as Glycoside hydrolase inhibitors.

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## Classification of Glycoside Hydrolases

According to enzyme nomenclature by International Union of Biochemistry and Molecular Biology (IUBMB), Glycoside hydrolases are classified into EC 3.2.1 as enzymes catalyzing the hydrolysis of O- or S-glycosides (Sinnott 1990). They are also classified based on stereochemical outcome of hydrolysis reaction (retaining or inverting enzymes); on exo (non-reducing end) and endo (middle of molecule) acting, on sequence and structure based classification. Sequence based classification has suggested more than 150 different families of glycoside hydrolases (Henrissat et al. 1995; Henrissat and Davies 1995; Bairoch 1999), these are available on CAZy Carbohydrate-Active Enzymes web site, supported by CAZyedia [Cazy family Glycoside hydrolase; CAZyedia; Henrissat and Coutinho 1999]. Sequence based

classification significantly helps in prediction of mechanism of action of enzyme, active site residues and possible substrates. Enzymes further classified as clans of related structure based on three dimensional structural similarities obtained from available sequences (Naumoff 2006, 2011).

Glycoside hydrolases (O-Glycosyl hydrolases) EC 3.2.1.X catalyzing hydrolysis of glycosidic bond are classified based on sequence similarity to be most reliable and led to the definition of 128 families and 14 clans (based on folds of proteins) of the same (Henrissat et al. 1995; Henrissat and Davies 1995; Henrissat and Bairoch 1996). This sequence based classification is available on the CAZy (Carbohydrate-Active Enzymes) (Cantarel et al. 2009). Glycoside hydrolases are also classified based on their localization in cell (secreted, monotopic, peripheral, lysosomal, located in eukaryotic plasma, inner membrane of Gram positive bacteria).

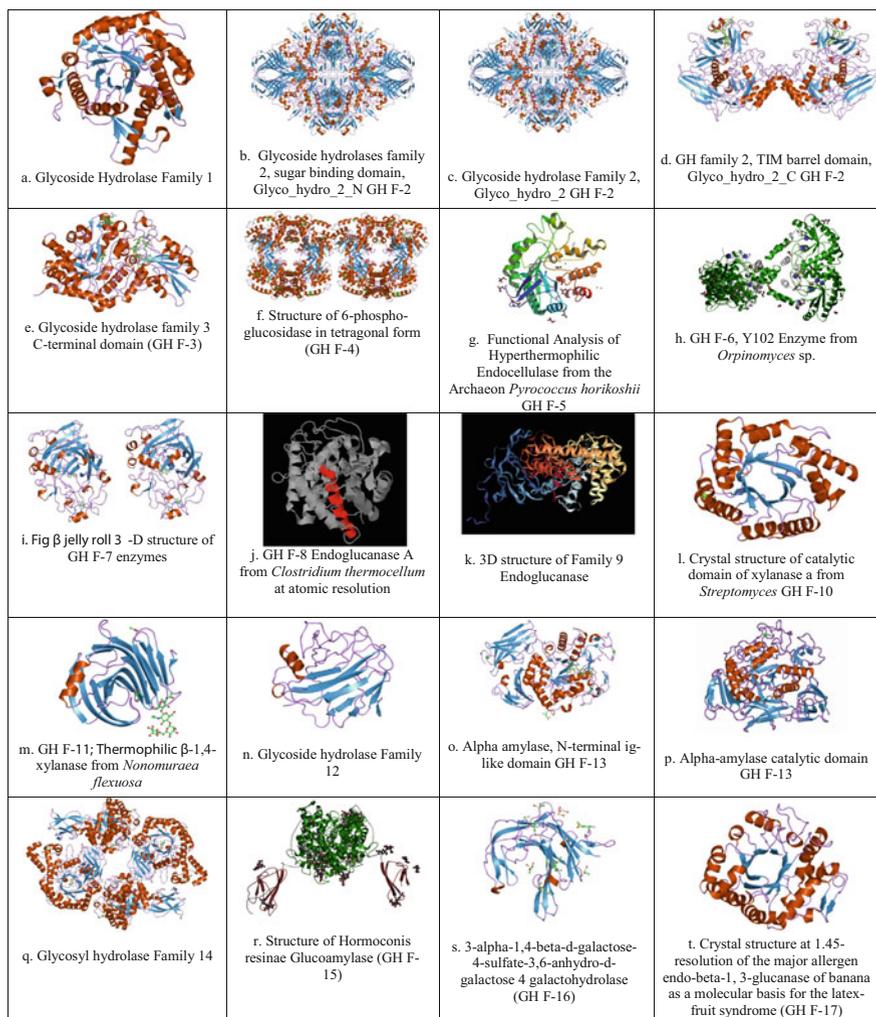
Following section in the chapter will deal with the major features and mode of action of all glycoside hydrolases classified. One prominent known structure from each family is depicted in Fig. 4a-r5.

## Glycoside Hydrolase Family 1

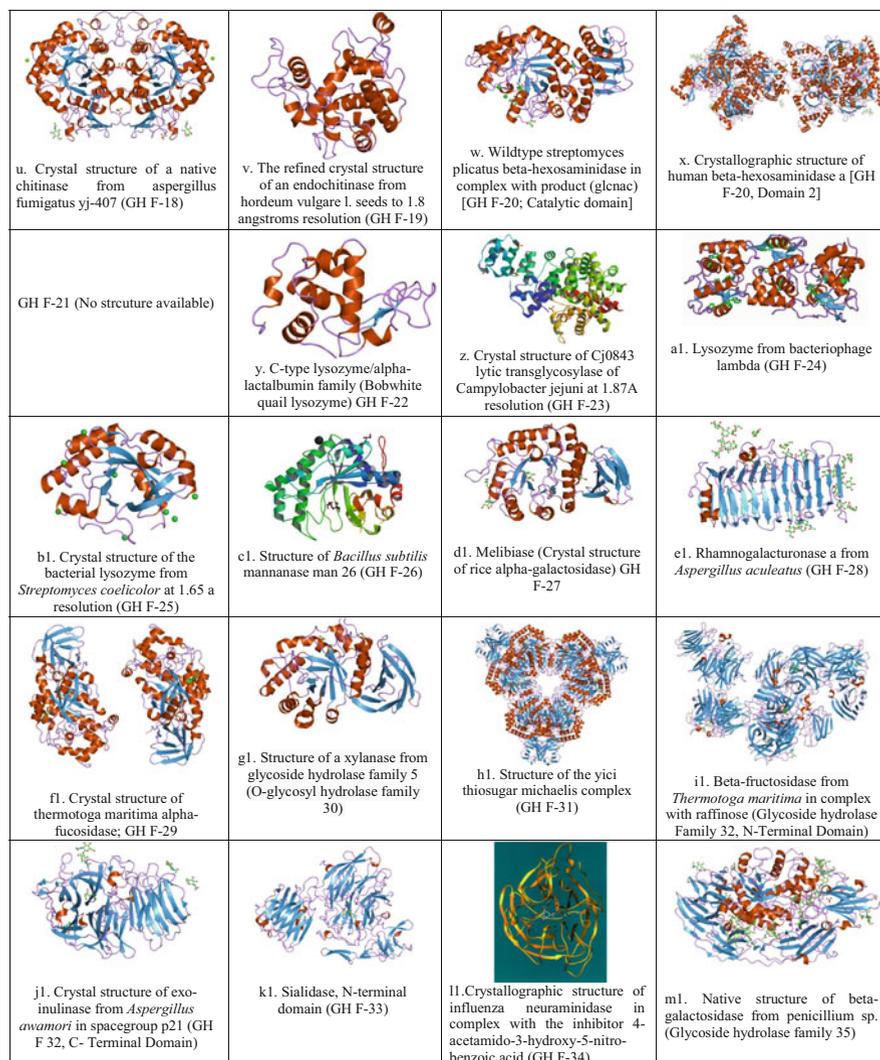
Enzymes of family 1 follow the IUBMB EC 3.2.1 nomenclature and catalyzes hydrolysis of glycosidic bond between two carbohydrate molecules or a carbohydrate and non carbohydrate molecule (Lombard et al. 2014; [Cazypedia.org](http://Cazypedia.org) and Cazypedia Consortium). Major enzymes of Glycoside Hydrolase family 1 are; beta-glucosidase (EC 3.2.1.21); beta-galactosidase (EC 3.2.1.23); 6-phospho-beta-galactosidase (EC 3.2.1.85); 6-phospho-beta glucosidase (EC 3.2.1.86); lactase-phlorizin hydrolase (EC 3.2.1.62), lactase (EC 3.2.1.108); beta mannosidase (EC 3.2.1.25); myrosinase (EC 3.2.1.147). Figure depicts general structure of Glycoside hydrolase family 1. According to the PROSITE documentation (<https://prosite.expasy.org/cgi-bin/prosite/prosite-search-ac?PDOC00495#description>) (Henrissat 1991a, b; Gonzalez-Candelas et al. 1990; El Hassouni et al. 1992) classifies  $\beta$ -glucosidases (EC 3.2.1.21);  $\beta$ -Galactosidases (EC 3.2.1.23); 6-phospho- $\beta$ -Galactosidases (EC 3.2.1.85);  $\beta$ -Glucosidases (EC 3.2.1.86); plant myrosinases (EC 3.2.1.147; e.g. synigrinases or thioglucosidases); Mammalian lactase-phlorizin hydrolase (LPH; EC 3.2.1.108/EC 3.2.1.62) in family 1 of Glycoside hydrolases. Conserved regions are central glutamic acid residues, acting as nucleophile during glycosidic bond cleavage. This is marked as signature pattern for this group of enzymes with another along with a conserved region containing glutamic acid found at their N-terminal extremity (Withers et al. 1990). This group belongs to the TIM Barrel glycoside hydrolase superfamily contains the range of enzymes belonging to group that possess a TIM barrel fold merging clans GH-A, GH-D, GH\_H and GH-K. It contains 57 families and 259,156 domains and was built by A Bateman (Naumov and Karrera 2009). All members of family 1 glycoside hydrolases for which localization is known are typically restricted to plasma membrane and endoplasmic reticulum membrane (Davies and Henrissat 1995; Henrissat et al. 1995) (Fig. 4a).

## Glycoside Hydrolase Family 2

Glycoside hydrolases family 2 enzymes comprises enzymes with  $\beta$ -galactosidases (EC 3.2.1.23);  $\beta$ -mannosidases (EC 3.2.1.25),  $\beta$ -glucuronidase (EC 3.2.1.31). Glutamic acid residue is the general acid/base catalyst at active sites of these enzymes (Gebler et al. 1992). The catalytic domain of  $\beta$ -galactosidases contains TIM barrel core surrounded beta domains, with sugar binding domain forming jelly-roll fold, containing immunoglobulin like beta-sandwich domain (Jacobson et al. 1994). General structure of glycoside hydrolase family 2 enzymes is elaborated as its



**Fig. 4** Glycoside hydrolase family 1-Family 166 (One PDB accession for each family)



**Fig. 4** (continued)

complete picture, sugar binding domain and TIM Barrel domain as depicted in Fig. 4b–d respectively.

Sugar binding domain belongs to the galactose binding domain like superfamily, the clan that contains 70 families and 124,105 domains. This superfamily has prominent sandwich domains with a jelly roll topology and is mainly involved in

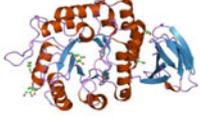
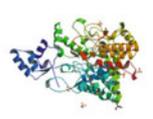
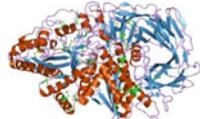
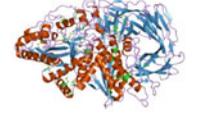
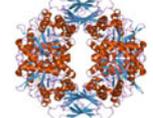
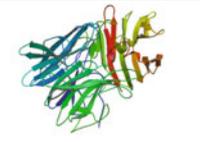
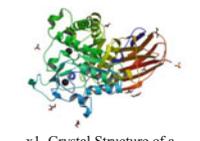
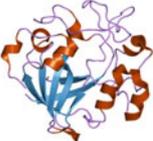
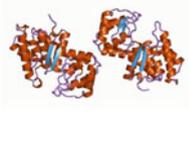
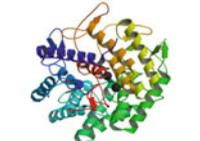
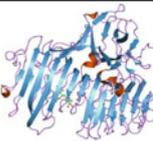
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| <br>n1. Crystal structure of rice alpha-galactosidase (Melibiase, GH F-36)  | <br>o1. Structure of periplasmic trehalase from Diamondback moth gut bacteria complexed with validoxylamine (GH F-37)                        | <br>p1. Golgi alpha-mannosidase II (GH F-38, N terminal domain)                              | <br>q1. Golgi alpha-mannosidase II (Alpha mannosidase middle domain; GH F-38)  |
| <br>r1. Golgi alpha-mannosidase II (GH F-38, C-terminal domain)   | <br>s1. Crystal structure of beta-d-xylosidase from <i>Thermoanaerobacterium saccharolyticum</i> , a family 39 glycoside hydrolase (GH F-39) | GH F-40 (No structure available)  | GH F-41 (No structure available)   |
| <br>t1. Crystal structure of thermophilus a4 beta-galactosidase (GH F-42)   | <br>u1. crystal structure of thermophilus a4 beta-galactosidase (Trimerization domain; GH F-42)  | <br>v1. Crystal structure of thermophilus a4 beta-galactosidase (C-terminal domain; GH F-42) | <br>w1. Crystal structure of xylan beta-1,4-xylosidase from <i>Bacillus Halodurans</i> C-125 (GH F-43)   |
| <br>x1. Crystal Structure of a Glycoside Hydrolase Family 44 Endoglucanase produced by <i>Clostridium acetobutylicum</i> ATCC 824 (GH F-44) | <br>y1. Endoglucanase from <i>Humicola insolens</i> at 1.7a resolution (GH F-45)   | <br>z1. <i>Streptomyces</i> n174 chitosanase ph5.5 298k (Glycoside hydrolase family 46)      | <br>a2. Structure of The GH47 processing alpha-1,2-mannosidase from <i>Caulobacter</i> strain K31 (GH F-47)  |
| <br>b2. X-tal structure of the mutant e44q of the cellulase cel48f in complex with a thiooligosaccharide (GH F-48)                        | <br>c2. dex49a from <i>Penicillium minioluteum</i> complex with isomaltose (GH F-49)   | <br>d2. The crystal structure of an agarase, AgWH50C (GH F-50)                             | <br>e2. Elucidation of the substrate specificity and protein structure of AbfB, a family 51 alpha-L-arabinofuranosidase from <i>Bifidobacterium longum</i> (GH F-51) |

Fig. 4 (continued)

carbohydrate recognition. They share very little sequence similarity and weak sequence motif with conserved bulge (possibly helps in bending of beta sheets) in the C-terminal beta sheet, enabling curvature of sheet that forms a sugar binding site (Murzin and Bateman 1998).

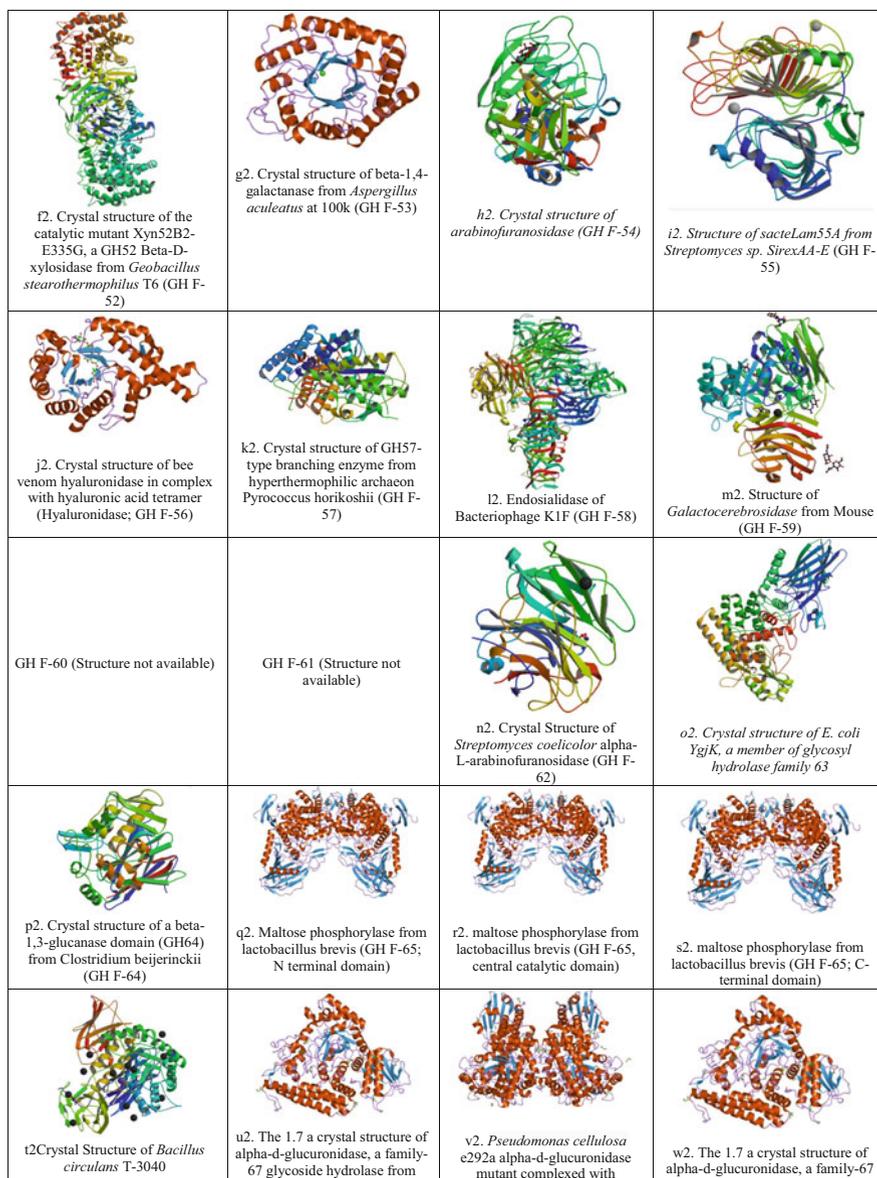
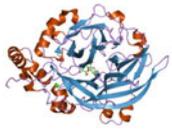
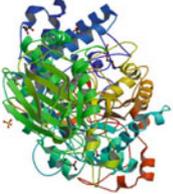
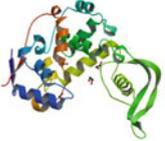
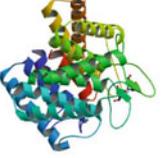
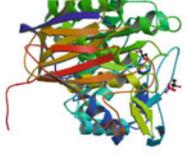
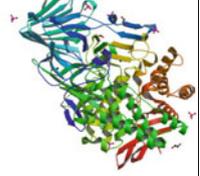
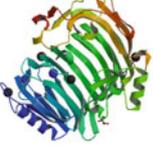


Fig. 4 (continued)

### Glycoside Hydrolase Family 3

Glycoside hydrolase family 3 enzymes are two domain globular proteins N-glycosylated at 3 sites (Varghese et al. 1999). This family comprises of

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| <p>cycloisomaltooligosaccharide glucanotransferase (GH F 66)</p>  <p>x2. crystal structure of levansucrase (c342a) complexed with sucrose (GH F-68)</p> | <p>bacillus stearotherophilus t-1 (GH F-67, N-terminal domain)</p> <p>GH F-69 (No structure available)</p>   | <p>aldotriuronic acid (GH F-67 Middle domain)</p>  <p>y2. 4,6-alpha-glucanotransferase GTFB from <i>Lactobacillus reuteri</i> 121 (GH F-70)</p> | <p>glycoside hydrolase from bacillus stearotherophilus t-1 (GH F-67; C Terminal domain)</p> <p>GH F-71 (No structure available)</p>  |
|  <p>Z2. <i>Saccharomyces cerevisiae</i> gas2p (e176q mutant) in complex with laminaritetraose and laminaripentaose (gh f-72)</p>                        |  <p>a3. X-ray structure of catalytic domain of autolysin from <i>Clostridium perfringens</i> (GH F73)</p>                 |  <p>b3. The structure of <i>Acidothermus cellulolyticus</i> family 74 glycoside hydrolase (GH F-74)</p>   | <p>GH F-75 (No structure available)</p>  |
|  <p>c3. Crystal structure of <i>Bacillus circulans</i> TN-31 Aman6 in complex with mannobiose (GH F 76)</p>   |  <p>d3. structure determination and refinement at 1.8 a resolution of disproportionating enzyme from potato (GH F-77)</p> |  <p>e3. Crystal structure of GH78 family rhamnosidase of <i>Bacillus</i> SP. GL1 AT 1.9 A (GH F-78)</p>   |  <p><math>\beta</math>3. Crystal structure of beta-glucuronidase from <i>Acidobacterium capsulatum</i> (GH F-79)</p> |
|  <p>g3. Crystal Structure of GH80 chitosanase from <i>Mitsuraria chitosanitabida</i> (GH F-80)</p>   |  <p>h3. Crystal structure of SeMet derivative BhGH81 (GH F-81)</p>   |  <p>i3. 1,3-Alpha-1,4-Beta-D-Galactose-4-Sulfate- 3,6-Anhydro-D-Galactose-2-Sulfate 4 Galactohydrolase (GH F-82)</p>                           |  <p>j3. Structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase (GH F-83)</p>                    |

**Fig. 4** (continued)

$\beta$ -glucosidases (EC 3.2.1.21);  $\beta$ -xylosidase (EC 3.2.1.37), N-acetyl  $\beta$ -glucosaminidase (EC 3.2.1.52), Glucan  $\beta$ -1,3-glucosidase (EC 3.2.1.58), celloextrinase (EC 3.2.1.74); exo 1,3-1,4-glucanase (EC 3.2.1.). Crystal structures for N-terminal and C-terminal domain of family 3 enzymes are available (Fig. 4e). Conserved region of these enzymes have been centered on a conserved aspartic acid residue depicted in  $\beta$ -glucosidase A3 in *Aspergillus wentii* (Bause and Legler 1980).

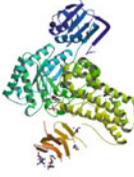
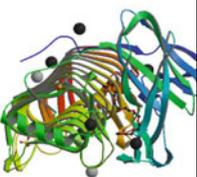
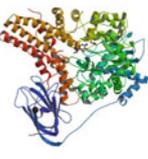
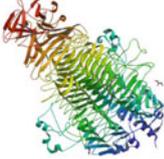
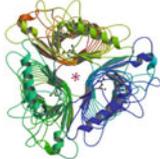
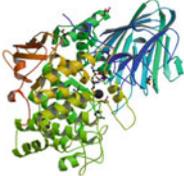
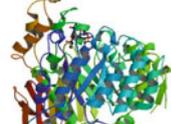
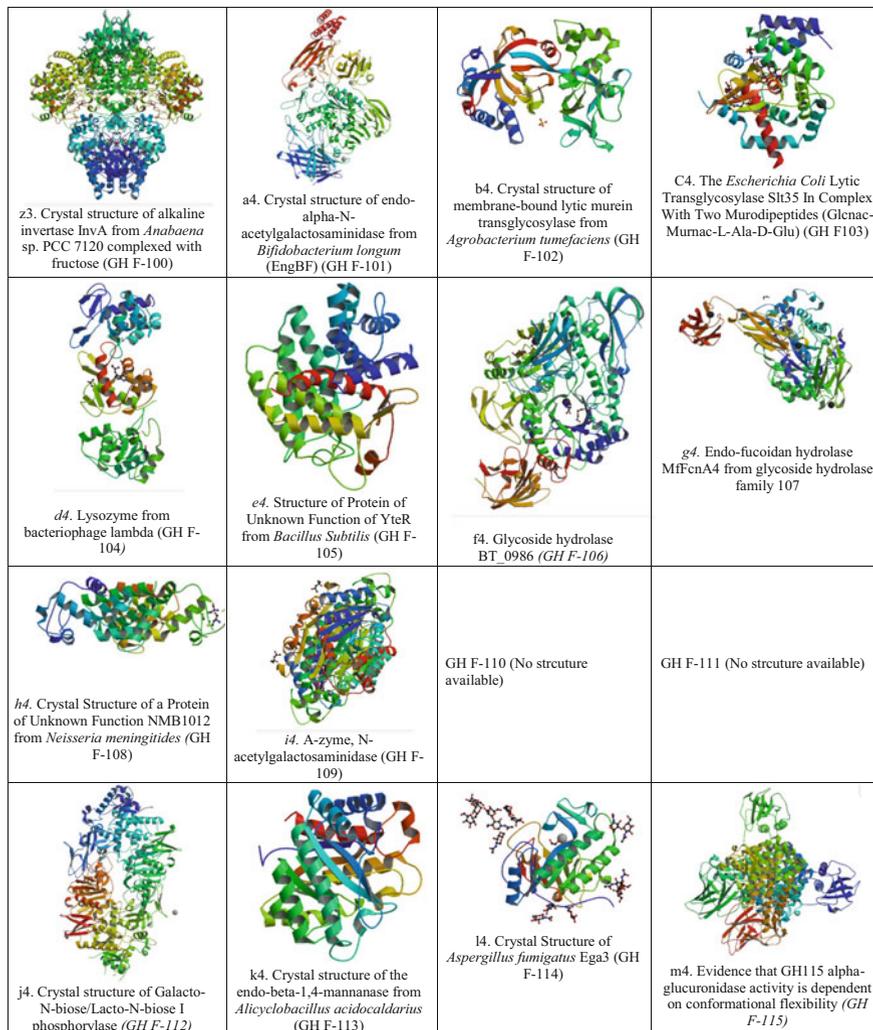
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|  <p>k3. <i>Bacteroides thetaiotaomicron</i> hexosaminidase with O-GlcNAcase activity- NAG-thiazoline complex (GH F-84)</p>  |  <p>l3. X-ray crystal structure of the Endo-beta-N-acetylglucosaminidase from <i>Arthrobacter protophormiae</i> E173Q mutant reveals a TIM barrel catalytic domain and two ancillary domains (GH F-85)</p> |  <p>m3. BpGH86A: A beta-porphyrinase of glycoside hydrolase family 86 from the human gut bacterium <i>Bacteroides plebeius</i> (GH F-86)</p>  |  <p>n3. Crystal structure of AgI-KA catalytic domain (GH F-87)</p>  |
|  <p>o3. Crystal Structure of Unsaturated Glucuronyl Hydrolase, Responsible for the Degradation of Glycosaminoglycan, from <i>Bacillus</i> sp. GL1 at 1.8 Å Resolution (GH F-88)</p> |  <p>p3. Family 89 Glycoside Hydrolase from <i>Clostridium perfringens</i> in complex with 2-acetamido-1,2-dideoxynojmycin (GH F-89)</p>  |  <p>q3. Siphovirus 9NA tailspike receptor binding domain (GH F-90)</p>  |  <p>r3. Crystal structure of insulin fructotransferase in the absence of substrate (GH F-91)</p>                                 |
|  <p>s3. Structure of the Family GH92 Inverting Mannosidase BT3990 from <i>Bacteroides thetaiotaomicron</i> VPI-5482 (GH F-92)</p>   |  <p>t3. structure of the GH93 alpha-L-arabino furanosidase of <i>Fusarium graminearum</i> (GH F-93)</p>  |  <p>u3. Crystal Structure of <i>Cellvibrio gilvus</i> Cellobiose Phosphorylase Crystallized from Ammonium Sulfate (GH F-94)</p>   |  <p>v3. Crystal structure of a putative glycoside hydrolase family protein from <i>Bacillus halodurans</i> (GH F-95)</p>         |
| <p>GH F-96 (No structure available)</p>  |  <p>w3. Crystal structure of BT1871 retaining glycosidase (GH F-97)</p>  |  <p>x3. Crystal structure of the catalytic module of a family 98 glycoside hydrolase from <i>Streptococcus pneumoniae</i> SP3-B571 in complex with the A-trisaccharide blood group antigen. (GH F-98)</p> |  <p>y3. Selenomethionine derivative of the GH99 endo-alpha-mannosidase from <i>Bacteroides thetaiotaomicron</i> (GH F-99)</p> |

Fig. 4 (continued)

## Glycoside Hydrolase Family 4

Major enzyme activities of this group comprise 6-phospho- $\beta$ -glucosidase (EC 3.2.1.86); 6-phospho- $\alpha$ -glucosidase (EC 3.2.1.122),  $\alpha$ -galactosidase (EC 3.2.1.22); of these 6-phospho- $\alpha$ -glucosidase requires both NAD(H) and divalent



**Fig. 4** (continued)

metal ( $Mn^{2+}$ ,  $Fe^{2+}$ ,  $Co^{2+}$ , or  $Ni^{2+}$ ) for activity (Thompson et al. 1998). Figure shows structure of 6-phospho- $\beta$ -glucosidase from *Thermotoga maritime* in tetragonal form with manganese, NAD<sup>+</sup> and glucose-6-phosphate (GH F-4), belonging to FAD/NAD(P)-binding Rossmann fold Superfamily (CL0063) (Fig. 4f).

These are redox enzymes containing a catalytic domain (provides substrate specificity) and Rossmann-fold domain (contains alpha-beta folds with central beta-sheet surrounded by 5 alpha-helices in order 654123; binds to NAD<sup>+</sup>), where NAD<sup>+</sup> reversibly binds to hydride ion (lost/gained during redox process). Inter sheet

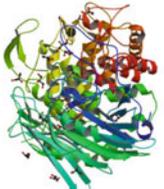
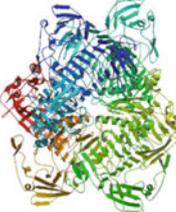
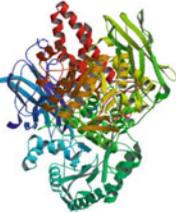
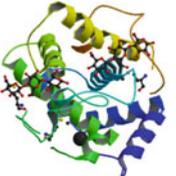
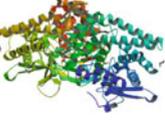
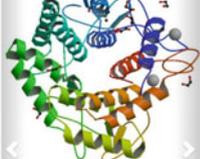
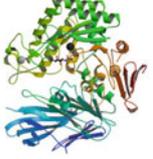
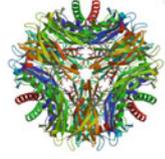
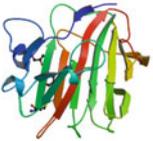
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| <br>n4. Crystal structure of <i>Thermoanaerobacterium xylolyticum</i> GH116 beta-glucosidase (GH F-116) | <br>o4. Native crystal structure of <i>BpGH117</i> (GH F-117)   | GH F-118 (No structure available)  | GH F-119 (No structure available)  |
| <br>p4. The complex structure of XylC with Tris (GH F-120)  | GH F-121 (No structure available)  | GH F-122 (No structure available)  | <br>q4. Crystal structure of BvGH123 (GH F-123)  |
| <br>r4. CtCel124: a cellulase from <i>Clostridium thermocellum</i> (GH F-124)                           | <br>s4. Crystal structure of an exo-alpha-1,6-mannosidase (bacova_03347) from <i>bacteroides ovatus</i> at 1.60 a resolution (GH F-125) | <br>t4. CPF_2247, a novel alpha-amylase from <i>Clostridium perfringens</i> (GH F-126)                              | <br>u4. Glycoside hydrolase BT_1003 (GH F-127)  |
| GH F-128 (No structure available)  | <br>v4. Alpha-N-acetylgalactosaminidase NagBb from <i>Bifidobacterium bifidum</i> - GalNAc complex (GH F-129)                          | <br>w4. Crystal structure of 4-O-beta-D-mannosyl-D-glucose phosphorylase MGP complexed with Man-Glc+PO4 (GH F-130) | <br>x4. Crystal structure of the catalytic domain of the glycoside hydrolase family 131 protein from <i>Coprinopsis cinerea</i> (GH F-131) |

Fig. 4 (continued)

crossover of the strands in the sheet form the NAD<sup>+</sup> binding site (Bashton and Chothia 2002) and in some distantly related Rossmann NAD<sup>+</sup> is replaced by FAD. Clan has been built by RD Finn.

Structure of GH F-4 C terminal domain belongs to LDH C-terminal domain-like superfamily (CL 0341). This superfamily includes the C-terminal domain of lactate/malate dehydrogenase as well as the C-terminal domain of the GH F-4.

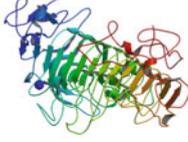
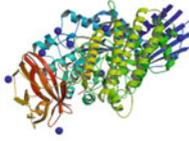
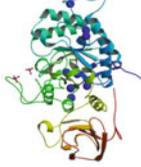
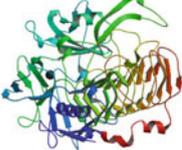
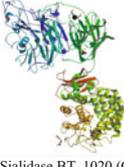
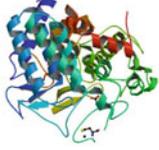
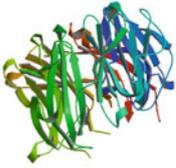
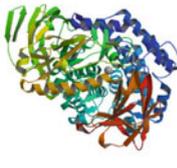
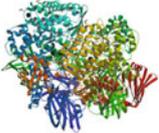
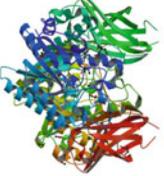
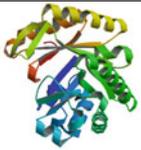
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| GH F-132 (No structure available)   |  <p>y4. Crystal Structure of the <i>Candida Glabrata</i> Glycogen Debranching Enzyme (GH F-133)</p> |  <p>z4. Structure of a beta-1,4-mannanase, SsGH134 (GH F-134)</p>                 |  <p>a5. Crystal Structure of <i>Aspergillus clavatus</i> Sph3 (GH F-135)</p> |
|  <p>b5. Crystal structure of lacto-N-biosidase LnbX from <i>Bifidobacterium longum</i> subsp. longum, ligand-free form (GH F-136)</p>              |  <p>e5. Glycoside hydrolase BT_0996 (GH F-137)</p>  |  <p>d5. BP0997, GH138 enzyme targeting pectin rhamnogalacturonan II (GH F138)</p> | GH F-139 (No structure available)   |
|  <p>e5. Glycoside hydrolase BT_1012 (GH F-140)</p>   |  <p>f5. Glycoside hydrolase BT_1002 (GH F-141)</p>  |  <p>g5. Sialidase BT_1020 (GH F-142)</p>  |  <p>h5. Sialidase BT_1020 (GH F-143)</p>                                     |
|  <p>i5. Crystal structure of a putative glucoamylase (BACCAC_03554) from <i>Bacteroides caccae</i> ATCC 43185 at 2.05 Å resolution (GH F-144)</p> |  <p>j5. Glycoside Hydrolase BACCELL_00856 (GH F-145)</p>   |  <p>k5. Beta-L-arabinofuranosidase (GH F-146)</p>                                | GH F-147 (No structure available)   |
| GH F-148 (No structure available)   |  <p>l5. Bacterial beta-1,3-oligosaccharide phosphorylase from GH F-149</p>                        | GH F-150 (No structure available)  | GH F-151 (No structure available)   |

Fig. 4 (continued)

## Glycoside Hydrolase Family 5

They are membrane localized enzymes, basically involved in polysaccharides like cellulose and xylan and have been reported to be produced from fungi as well

|   |   |  |   |
|---|---|--|---|
|  <p>m5. Recombinant thaumatin I at 0.9 Angstrom (GH F-152)</p>   |  <p>n5. Structure of the PgaB (BpsB) glycoside hydrolase domain from <i>Bordetella bronchiseptica</i> (GH F-153)</p> | GH F-154 (No structure available)  | GH F-155 (No structure available)   |
|  <p>o5. Crystal structure of an inverting family GH156 exosialidase from uncultured bacterium pG7 (GH F-156)</p>                 | GH F-157 (No structure available)   | GH F-158 (No structure available)  | GH F-159 (No structure available)   |
| GH F-160 (No structure available)   | GH F-161 (No structure available)   |  <p>p5. The apo-structure of endo-beta-1,2-glucanase from <i>Talaromyces funiculosus</i> (GH F-162)</p> | GH F-163 (No structure available)   |
| GH F-164 (No structure available)   | GH F-165 (No structure available)   | GH F-166 (No structure available)  |  <p>Fig. 4q5. Structure of the glycoside hydrolase domain of PelA from <i>Pseudomonas aeruginosa</i> (GH F-166)</p> |
|  <p>r5. Crystal structure of glucosidase from <i>Croceicoccus marinus</i> at 1.8 Angstrom resolution (GH F-Non classified)</p> |   |  |   |

**Fig. 4** (continued)

bacteria. This family comprises of enzymes namely endoglucanase (EC 3.2.1.4); beta-mannanase (EC 3.2.1.78); exo-1,3-glucanase (EC 3.2.1.58); endo-1,6-glucanase (EC 3.2.1.75); xylanase (EC 3.2.1.8); endoglycoceramidase (EC 3.2.1.123). Active site includes conserved glutamic acid residue (Henrissat

1991a; Py et al. 1991). Relationship between thermal stability and structural rigidity of members of GH F-5 enzymes have been reported after being studied through molecular dynamics (Bedieyan et al. 2012). It belongs to TIM Barrel Glycoside hydrolase Superfamily (CL0058) (Fig. 4g).

## Glycoside Hydrolase Family 6

They were formerly known as Cellulase family B including endoglucanases (EC 3.2.1.4) and cellobiohydrolases (EC 3.2.1.91). They are alpha-beta proteins (Fig. 4h) having similar folds as that seen in triose phosphate isomerase. This was studied in 3D structure of cellobiohydrolase II (CBHII) from the fungus *Trichoderma reesei*, having active site located at C-terminal end of a parallel beta barrel present in an enclosed tunnel. Probable catalytic residues are two aspartic acid residues (positioned in center of tunnel) (Rouvinen et al. 1990).

## Glycoside Hydrolase Family 7

Known activities of enzymes (formerly known as cellulase family C) classified under this group are endoglucanase (EC 3.2.1.4) and cellobiohydrolase (EC 3.2.1.91, end-acting cellobiohydrolase (EC 3.2.1.176); chitosanase (EC 3.2.1.132); endo- $\beta$ -1,3-1,4-glucanase (EC 3.2.1.73) from eukaryotes catalyzing glycosidic bond using double-displacement mechanism. This mechanism helps in net retention of the conformation at the anomeric carbon. Endoglucanases cleaves the beta-1,4 linkages of cellulose and CBH cleaves off cellobiose disaccharide units from the reducing end of the chain. Their catalytic region is generally bound to cellulose binding domain (CBD) via a proline and/or hydroxy-amino acids rich linker region. In type I exoglucanases, the CBD domain is found at the C-terminal extremes (this domain forms a hairpin loop structure stabilized by 2 disulphide bridges). Active site contains glutamic acid residue as catalytic nucleophile/base as well as proton donor and catalysis progress through retaining mechanism (Divne et al. 1994; Mackenzie et al. 1998; Ducros et al. 2003; Klarskov et al. 1997; Sulzenbacher et al. 1997; Viladot et al. 1998).

GH F-7 enzymes belong to Concanavalin-like lectin/glucanase superfamily (contains 49 families and 153254 domains) (Bateman..). Conserved protein domain of endoglucanase and cellobiohydrolase are grouped under O-glycosyl hydrolases domain family (cd07999) contains a total of 95 families which includes enzymes of glycoside hydrolase family 7 as well.

Beta-jellyroll folded framework, containing two antiparallel beta-sheet packed face-to-face forming a curved beta-sandwich is the predominant three dimensional feature (Fig. 4i). These sandwich structures are extended along both the ends through several loops, resulting in an elongated assembly of  $\sim 50$  Å, with a substrate binding structure running perpendicular to the  $\beta$ -strands of the inner, concave  $\beta$ -sheet. Some loops have short alpha-helical segments present at the periphery. Endoglucanases

specifically contains open substrate binding domain, while cellobiohydrolases have additional elongated loops that bend around the active site giving it a closed tunnel form. Some key studies include, boat confirmation required prior to hydrolysis (Sulzenbacher et al. 1997), presence of discrete glycoside binding subsites (Divne et al. 1998); product ejection mechanism during hydrolysis of cellulose also suggesting prior release of cellobiose products (Ubhayasekera et al. 2005); flexibility of sugar binding within tunnel of cellobiohydrolase (Parkkinen et al. 2008). Numerous commercial enzyme of this group is also available produced through Megazymes (Company).

## Glycoside Hydrolase Family 8

These enzymes are of prokaryotic/eukaryotic origin belonging to clan GH-M of six hairpin glycosidase superfamily (CL0059). Enzymes classified under this superfamily share common structure composed of six helical hairpins and are chitosanase (EC 3.2.1.132), cellulase (EC 3.2.1.4), licheninase (EC 3.2.1.73), endo-1,4- $\beta$ -xylanase (EC 3.2.1.8) and reducing-end-xylose releasing exo-oligoxyylanase (EC 3.2.1.156). GH F-8 enzymes cleaves  $\beta$ -1,4 linkages in  $\beta$ -1,4 glucans, xylans (or xylooligosaccharides), chitosans, and lichenans (1,3-1,4- $\beta$ -D-glucan). They are majorly endo acting with few having exo-activity. They were classified by hydrophobic cluster analysis, and was previously known as “Cellulase Family D” (Henrissat et al. 1989; Gilkes et al. 1991). These enzymes have inverting mechanism of action (Fierobe et al. 1993; Petersen et al. 2009). General acid (proton donor to the leaving group; located at N-terminal end of  $\alpha$ 4 helix), base of GH8a (proton acceptor from the nucleophilic water) and base of GH8b subfamily was first identified in CelA from *C. thermocellum* as Glu95 (Alzari et al. 1996; Adachi et al. 2004), CelA from *C. thermocellum* as Asp278 (Alzari et al. 1996) and chitosanase from *Bacillus* sp. K17 as Glu309 (based on its crystal structure and by making E309Q mutant) (Adachi et al. 2004) respectively. They are divided in three subfamilies based on positions of general base. Subfamily ‘a’ has Aspartate at the N-terminal end of  $\alpha$ 8 helix (cellulases, xylanases); ‘b’ has Asparagine, and the general base is a Glu residue located in a long loop inserted between  $\alpha$ 7 and  $\alpha$ 8 helices (chitinases, licheninases, cellulases). They have ( $\alpha/\alpha$ )<sub>6</sub> fold like Glycoside Hydrolase Family 48. Atomic (0.94 Å) resolution structure of CelA in complex with substrate (PDB ID 1kwf) and has been determined (Guérin et al. 2002; Honda and Kitaoka 2006; Béguin et al. 1985) (Fig. 4j).

## Glycoside Hydrolase Family 9

These enzymes were earlier known as cellulase family E. Enzymes include endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) (Béguin, 1990; Gilkes et al. 1991). Enzymes from diverse group of organisms (fungi, bacteria, amoebozoia, invertebrate metazoan, ferns,

mosses, gymnosperms and angiosperms) are reported to belong to this family. They belong to six-hairpin glycosidase super family and major localized to plasma membrane (Fig. 4k).

### **Glycoside Hydrolase Family 10**

Enzymes of this family were formerly known as cellulase family F and comprise of endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) (exoglucanases), or xylanases (EC 3.2.1.8) activities. All family 10 xylanases hydrolyze the glycosidic bond in a double-displacement ‘retaining’ mechanism using two catalytic acidic residues, where one residue acts a nucleophile (base) and the other acts as a general acid/base (Fig. 4l). They undergo retaining type catalytic mechanism, with conserved glutamic acid residues acting as catalytic nucleophile/base and catalytic proton donor. Three dimensional structure has prominent ( $\beta/\alpha$ )<sub>8</sub> barrels.

### **Glycoside Hydrolase Family 11**

This family was earlier known as cellulase family G (Henrissat and Bairoch 1996) and is nonspecific, consisting of xylanases. They belong to Concanavalin-like lectin/glucanase superfamily (CL0004). Major enzymes are xylanases from *Aspergillus awamori*, *Bacillus circulans*, *Clostridium stercorarium*, *Fibrobacter succinogenes* etc. Domains folds into a jelly roll shaped, and anti-parallel  $\beta$  strands bending almost 90 °C to produce a substrate binding grooves are characteristics of this family. Three dimensional structure has two catalytic Glu residues facing each other from opposite sides of the groove and catalysis is carried through double-displacement mechanism, with one Glu residue acting as a general acid/base catalyst and the other as a nucleophile. Two catalytically active conserved regions are centrally located on glutamic acid residues (studied in *Bacillus pumilis*, Lombard et al. 2014) (Fig. 4m).

### **Glycoside Hydrolase Family12**

Family 12 comprises of enzymes Concanavalin-like lectin/glucanase superfamily (CL0004). These include endoglucanase (EC 3.2.1.4), xyloglucan hydrolase (EC 3.2.1.151),  $\beta$ -1,3-1,4-glucanase (EC 3.2.1.73) and xyloglucan endotransglycosylase (EC 3.2.1.207). These enzymes were formerly known as cellulase family H. Three dimensional structures has  $\beta$ -jelly roll and glutamic acid residues acts as catalytic site nucleophile/base and proton donor (Fig. 4n).

### **Glycoside Hydrolase Family 13**

These are maltogenic alpha-amylase, catalyzing hydrolysis of (1-4)-alpha-D-glucosidic linkages in polysaccharides that helps in removal of successive alpha-maltose residues from the non-reducing ends of chains, during conversion of starch to maltose. Other enzymes in this family include neopullulanase, which hydrolyses pullulan to panose, and cyclomaltodextrinase, which hydrolyses cyclodextrins. They belong to TIM Barrel glycosyl hydrolase family (CL0058) (Fig. 4o,p).

### **Glycoside Hydrolase Family 14**

Enzymes of this family have  $\beta$ -amylase (EC 3.2.1.2) activities. They belong to TIM Barrel glycoside hydrolase superfamily (CL0058) containing catalytic glutamic acid residues and have three highly conserved regions (Mikami et al. 1988; Friedberg and Rhodes 1988). First on N-terminal (contains catalytic aspartic acid, Nitta et al. 1989) and second conserved domain is centrally located (catalytic glutamic acid, Totsuka et al. 1994) (Fig. 4q). Various three dimensional structures of enzymes belonging to this family have been studied; like soyabean beta-amylase with an inhibitor, alpha-dextrin etc (Table 1).

### **Glycoside Hydrolase Family 15**

Major enzymes of family 15 are glucoamylase (EC 3.2.1.3; catalyze release of D-glucose from the non-reducing end of starch and other oligo and polysaccharaides); alpha-glucosidase (EC 3.2.1.20) and glucodextranase (EC 3.2.1.70). Glucoamylases have three closely clustered acidic conserved residues at catalytic site (Sierks et al. 1990 and Ohnishi et al. 1992) and 3-D structure have been reported to belong to mainly alpha-class and contains 19 helices and 9 strands (Aleshin et al. 1994). They belong to Six- hairpin glycosidase superfamily (Fig. 4r).

### **Glycoside Hydrolase Family 16**

Enzymes of family 16 belong to Concanavalin-like lectin/glucanase superfamily (CL0004) and are group with functionally heterogenous members, like lichenase (EC 3.2.1.73); xyloglucan xyloglucosyltransferase (EC 2.4.1.207); agarase (EC 3.2.1.81); kappa-carrageenase (EC 3.2.1.83); endo-beta-1,3-glucanase (EC 3.2.1.39); endo-beta-1,3-1,4-glucanase (EC 3.2.1.6); endo-beta-galactosidase (EC 3.2.1.103). All these enzymes have a common ancestor and have diverged significantly in their primary sequences (Fig. 4s).

Catalytic domain have this family has sandwich-like  $\beta$ -jelly roll fold formed by two anti-parallel beta sheet that are closely packed. All enzymes of GH F-16 feature a common catalytic motif E-[ILV]-D-[IVAF]-[VILMF](0,1)-E. The two glutamic

**Table 1** Glycoside hydrolase superfamilies

| Sl no: | Super family                                    | Clan number | Clans included                  | Family  | Number of families | Number of domains |
|--------|---|-------------|---------------------------------|---|--------------------|-------------------|
| 1      | TIM Barrel glycoside hydrolase superfamily      | CL0058      | GH-A, GH-D, GH-H and GH-K, GH-R | GH F-1, GH F-2<br>TIM Barrel Domain, GH F-3 N terminal domain; GH F-5; GH F-10; GH F-13; GH F-14; GH F-17; GH F-18, GH F-20; GH F-25; GH F-26; GH F-27; GH F-29; GH F-30; GH F-31; GH F-35; GH F-36; GH F-39; GH F-42; GH F-44; GH F-50; GH F-51; GH F-53; GH F-56; G F-59; GH F-66; GH F-70; GH F-72; GH F-77; GH F-79; GH F-85; GH F-86; GH F-89; GH F-97; GH F-101; GH F-107; GH F-113; GH F-128; GH F-147; GH F-148; GH F-157; GH F-158 | 57                 | 259156            |
| 2      | Galactose binding domain like superfamily       | CL0202      |                                 | GH F-2 Sugar Binding Domain   | 70                 | 124105            |
| 3      | FAD/NAD(P)-binding Rossmann fold superfamily    | CL0063      |                                 | GH F-4  | 204                | 2203085           |
| 4      | LDH C-terminal domain-like superfamily          | CL0341      |                                 | GH F-4 C Terminal Domain  | 2                  | 17217             |
| 5      | –   | –           |                                 | GH F-6  | –                  | 1 (PF01341)       |
| 6      | Concanavalin-like lectin/ glucanase superfamily | CL0004      |                                 | GH F-7; GH F-11; GH F-12; GH F-16   | 49                 | 153254            |

(continued)

**Table 1** (continued)

| Sl no: | Super family                                   | Clan number | Clans included      | Family   | Number of families | Number of domains |
|--------|--|-------------|---------------------|--|--------------------|-------------------|
| 7      | Six-hairpin glycosidase superfamily            | CL0059      | GH-L, GH-M and GH-G | GH F-8; GH F-9, GH F-15; GH F-37; GH F-47; GH F-48; GH F-63; GH F-65 (central catalytic domain); GH F-76; GH F-78; GH F-88; GH F-100; GH F-125 | 29                 | 105072            |
| 8      | Lysozyme-like superfamily                      | CL0037      | GH-I                | GH F-19; GH F-22, GH F-24; GH F-46; GH F-73; GH F-108  | 19                 | 71609             |
| 9      | Pectate lyase-like beta helix superfamily      | CL0268      | GH-N; GH-I          | GH F-28; GH F-49; GH F-80; GH F-92   | 29                 | 92337             |
| 10     | Beta-fructosidase/ Furanosidase superfamily    | CL-0143     | GH-J, GH-F          | GH F-32; GH F-62; GH F-68  | 9                  | 30592             |
| 11     | Sialidase superfamily                          | CL0434      | GH-E                | GH F-33, GH F-34; GH F-83; GH F-93   | 16                 | 21224             |
| 12     | Glycoside hydrolase/ deacetylase superfamily   | CL0158      |                     | GH F-38 (N-terminal domain); GH F-57   | 11                 | 48380             |
| 13     | Galactose mutarotase like superfamily          | CL0103      |                     | GH F-38 (C-terminal domain); GH F-65 (N-Terminal domain)   | 31                 | 78752             |
| 14     | Class-I Glutamine amidotransferase superfamily | CL0014      |                     | GH F-42 (Trimerisation domain)   | 18                 | 138858            |
| 15     | Glycosyl hydrolase domain superfamily          | CL0369      |                     | GH F-42 (C-terminal Domain)  | 39                 | 76165             |
| 16     | Double Psi beta barrel glucanase superfamily   | CL0199      |                     | GH F-45  | 5                  | 16903             |
| 17     |  |             | GH-O                | GH F_52; GH F-116  |                    |                   |

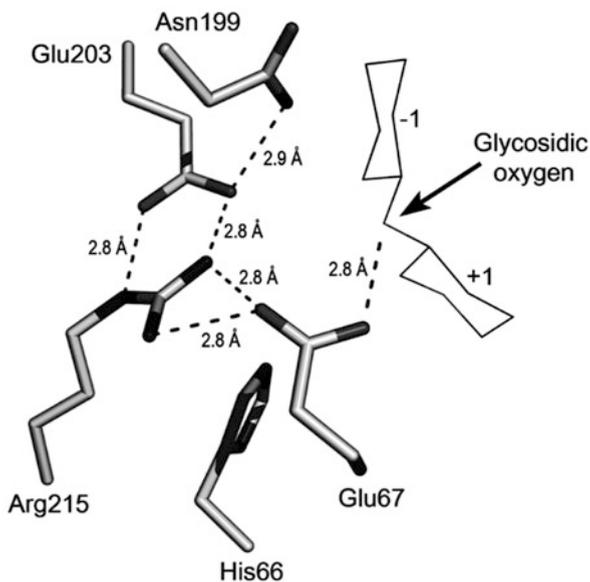
(continued)

**Table 1** (continued)

| Sl no: | Super family | Clan number | Clans included | Family                      | Number of families | Number of domains |
|--------|--------------|-------------|----------------|-----------------------------|--------------------|-------------------|
| 18     |              |             | GH-Q           | GH F-94; GH F-149; GH F-161 |                    |                   |
| 19     |              |             | GH-P           | GH F-127; GH F-146          |                    |                   |

Clan and Superfamily not known for GH F-40, GH F-41, GH F-54, GH F-55, GH F-58, GH F-60; GH F-61; GH F-64; GH F-67; GH F-69; GH F-71; GH F-74, GH F-75; GH F-81; GH F-82; GH F-84; GH F-87; GH F-90; GH F-91; GH F-95; GH F-96; GH F-98; GH F-99; GH F-102; GH F-103; GH F-104; GH F-105; GH F-106; GH F-109; GH F-110; GH F-111; GH F-112; GH F-114; GH F-115; GH F-117; GH F-118; GH F-119; GH F-120; GH F-121; GH F-122; GH F-123; GH F-124; GH F-126; GH F-129; GH F-130; GH F-131; GH F-132; GH F-133; GH F-134; GH F-135; GH F-136; GH F-137; GH F-138; GH F-139; GH F-140; GH F-141; GH F-142; GH F-143, GH F-144; GH F-145; GH F-150; GH F-151; GH F-152; GH F-153; GH F-154; GH F-155; GH F-156; GH F-159; GH F-160; GH F-162; GH F-163; GH F-164; GH F-165; GH F-166; GH F-Non classified

**Fig. 5** Active site of glycoside hydrolase family 19 papaya chitinase (PDB: 3cql). Conserved residues are shown surrounding the catalytic acid, Glu67. A GlcNAc2 unit binding in the -1 and +1 subsites is shown in narrow stick representation and an arrow indicates the position of the glycosidic oxygen (Eijsink et al. 2010)



acid residues in the conserved motif act as nucleophile and base in the catalytic reaction and aspartic acid residue maintains relative position of catalytic amino acids (Allouch et al. 2003; Michel et al. 2001) (Fig. 5).

## Glycoside Hydrolase Family 17

This family comprise of enzymes endo-1,3-beta-glucosidase (EC 3.2.1.39); lichenase (EC 3.2.1.73); exo-1,3-glucanase (EC 3.2.1.58) and have been reported only in eukaryotes namely plants and fungi. Central section has the conserved region, containing tryptophan residue, which is involved in interaction with the glucan substrate (Ori et al. 1990). It also contains a glutamate residue acting as nucleophile in the catalytic reaction (Varghese et al. 1994) (Fig. 4t).

## Glycoside Hydrolase Family 18

Chitinase class II group, including chitinase and chitodextrinase are enzymes belonging to this family, falling under TIM-barrel Glycoside hydrolase superfamily (CL0058) and located on endoplasmic reticulum membrane. They majorly catalyze hydrolysis of chitin oligosaccharides. This family also includes chitinase like protein that binds to chitin but does not hydrolyse it, and also varied glycoproteins (from mammals, cartilage and oviduct specific) (Fig. 4u).

## Glycoside Hydrolase Family 19

Like GH F-18, this family also has enzymes only with chitinase activity [EC 3.2.1.14], catalyzing hydrolysis of beta-1,4-N-acetyl-D-glucosamine linkages in chitin polymers (Flach et al. 1992). These enzymes also known as class IA/I and IB/II are of plant origin functioning as defense tool against fungal and insect pathogen. Catalytic domain of these enzymes consists of 220-230 residues (Henrissat 1991b) of which class IA/I has an extra N-terminal chitin domain. There are two highly conserved regions on this enzyme, of which one is located N-terminal section (containing cysteines involved in disulphide bond formation). They belong to Lysozyme like superfamily having structurally invariant core consisting of two-helices and a three stranded beta sheet that forms substrate binding and catalytic cleft (Monzingo et al. 1996) (Fig. 4v).

## Glycoside Hydrolase Family 20

Beta-hexosaminidase (EC 3.2.1.52); lacto-N-biosidase (EC 3.2.1.140) are major enzymes of this family belonging to TIM-Barrel glycosyl hydrolase superfamily (CL0058). Catalytic nucleophile/base of these enzymes are carbonyl oxygen of the C-2 acetamide group. Beta-hexosaminidase degrade GM2 gangliosides by hydrolysing the terminal non-reducing N-acetyl-D-hexoamine residues. This enzyme is found in three forms; hexosaminidase A is a trimer, with one alpha, one beta-A and one beta-B chain; hexosaminidase B is a tetramer of two beta-A and two beta-B chains; and hexosaminidase S is a homodimer of alpha chains (Fig. 4w,x).

## Glycoside Hydrolase Family 21

There is no entry of enzyme been classified under family 21 of glycoside hydrolases as per source of [cazypedia.org](http://cazypedia.org) and a detailed study of enzymes to be classified under this family is required.

## Glycoside Hydrolase Family 22

Lysozyme type C (EC 3.2.1.17) lysozyme type I (EC 3.2.1.17) and alpha-lactalbumins are enzymes classified as GH Family 22. Specifically based on activities they belong to Lysozyme like superfamilies and the catalytic nucleophile/base for the same is aspartate and/or carbonyl oxygen of the C-2 acetamido group. Five different classes of lysozymes are known; namely C (chicken) type, G (goose) type, Phage type, fungal and bacterial and all these exhibit minor sequence similarities. Though being functionally divergent, primary sequence and structure of lysozyme type C and alpha-lactalbumin are very similar, reflecting their common origin (Nitta and Sugai 1989) where approximately 40% of residues and 4 disulphide bonds are conserved. All lactalbumins and few lysozymes have the property of binding to calcium while catalysis (Nitta et al. 1987; Stuart et al. 1986) in order to obtain a stable structure. Catalytic site of these enzymes have three aspartic acid residues (Fig. 4y).

## Glycoside Hydrolase Family 23

Lysozyme type G (EC 3.2.1.17) and chitinase (EC 3.2.1.14) are activities of enzymes of glycoside hydrolase family 23. This family also include peptidoglycan lyase (EC 4.2.2.n1) also known as peptidoglycan lytic transglycosylase. Glutamic acid is the catalytic proton donor, mechanism and catalytic nucleophile/base is not known. Enhanced and elaborate studies are still under progress (Fig. 4z).

## Glycoside Hydrolase Family 24

Lysozymes (EC 3.2.1.17) are the activity reported in this group and enzymes belong to Lysozyme like superfamily. Lambda phage lysozyme and *Escherichia coli* endolysin (Weaver and Matthews 1987) are included in this family. These enzymes have inverting mechanism of action and glutamic acid acts as a catalytic proton donor. Three dimensional structure of the protein has both alpha helices and beta sheets (Fig. 4a1).

## Glycoside Hydrolase Family 25

Family 25 also comprises of enzymes with lysozyme activity (EC 3.2.1.17; cell wall lytic enzymes) (Henrissat 1991b; Croux and García 1991), with retaining mode of catalytic mechanism where aspartic acid is catalytic nucleophile or base and glutamic acid is the proton donor. DxE is a conserved active site motif, helping in catalysis by formation of oxazoline intermediate. Aspartate residue initially protonates the leaving facilitating its departure and subsequently acts as a general base to activate the hydrolytic water molecule; and the glutamic residue stabilized or deprotonated the oxazoline N- atom. Aspartic acid at sixth position might be involved in catalysis with the glutamic acid at DxE motif, provided the hydrolysis has taken place via net inversion of anomeric configuration, with the aspartic acid residue acting as general base activating the nucleophilic water molecule and glutamic acid of DxE motif acting as general acid and protonating the departing oxygen atom in a concerted fashion as the bonds cleaves (Rau et al. 2001; Korczynska et al. 2010; Hermoso et al. 2003; Al-Riyami et al. 2016). Three dimensional structure consists of  $(\beta/\alpha)_5 (\beta)_3$  TIM-like domain and thus they classified under TIM Barrel glycoside hydrolase super family. Majorly studied enzymes include lysozymes (lysine) from *Streptococcus pneumoniae* bacteriophages of the Cp family, lysozyme (endolysin) from *Lactococcus delbrueckii* phage mv1, autolytic lysozyme from *Clostridium acetobutylicum*, lysozyme M1 from *Streptomyces globisporus* and N,O-diacetylmuramidase (lysozyme ch) from the fungus *Chalaropsis* (Fig. 4b1).

## Glycoside Hydrolase Family 26

Enzymes in this group has activities of mannanase (EC 3.2.1.78) and  $\beta$ -1,3-xylanase (EC 3.2.1.32) of which main enzyme is Mannan endo-1,4-beta-mannosidase catalyzing specific hydrolysis of mannan and galactomannan and showing very little hydrolysis towards 1,4-beta-D-linkages in mannans, galacto-mannans, glucomannans and galactoglucomannans (Braithwaite et al. 1995) (Fig. 4c1).

## Glycoside Hydrolase Family 27

Alpha-galactosidases (Melibiase) (EC 3.2.1.22) (Dey and Pridham 1972)  $\alpha$ -N-acetylgalactosaminidase (EC 3.2.1.49); isomalto-dextranase (EC 3.2.1.94);  $\beta$ -L-arabinopyranosidase (EC 3.2.1.88); galactan: galactan galactosyltransferase (EC 2.4.1.-), are major activities of GH Family 27 enzymes, catalyzing hydrolysis of melibiose to glucose and galatose. Together with members of family 11 and 36, these enzymes form clan GH-D (alpha-galactosidase; alpha-N-acetylgalactosaminidases, and isomaltodextranases); belonging to TIM Barrel glycoside hydrolase superfamily. NAD and magnesium acts as cofactors for few enzymes of prokaryotic origin. Mode of action for these enzymes is retaining type,

where aspartic acid is catalytic nucleophile and base as well as proton donor. These enzymes find mechanistic commonality with family GH36 demonstrated by Comfort et al. 2007 (Fig. 4d1).

## Glycoside Hydrolase Family 28

Activities of enzymes belonging to this group includes polygalacturonase (EC 3.2.1.15);  $\alpha$ -L-rhamnosidase (EC 3.2.1.40); exo-polygalacturonase (EC 3.2.1.67); exo-polygalacturonosidase (EC 3.2.1.82); rhamnogalacturonase (EC 3.2.1.171); rhamnogalacturonan  $\alpha$ -1,2-galacturonohydrolase (EC 3.2.1.173); xylogalacturonan hydrolase (EC 3.2.1.-); all these enzymes have inverting mechanism for catalysis, with aspartic acid residue as catalytic nucleophile/base and catalytic proton donor. Three dimensional structures have prominent beta- helices. These enzymes belong to pectate-lyase like beta-helix Superfamily. Main feature of this superfamily is presence of right handed beta helix similar to first found in pectate lyase (Jenkins et al. 1998). Polygalacturonase/pectinase catalyzes hydrolysis of 1,4-alpha-D-galactosiduronic linkages in pectate/galacturonans (Ruttkowski et al. 1990). Exo-poly-alpha-D-galacturonosidase (EC 3.2.1.82) (exoPG) hydrolyzes peptic acid from the non-reducing end, releasing digalacturonate (He and Collmer 1990) (Fig. 4e1).

## Glycoside Hydrolase Family 29

Alpha-L-fucosidase (EC 3.2.1.51) and  $\alpha$ -1,3/1,4-L-fucosidase (EC 3.2.1.111) are major activities of this family, belonging to the TIM barrel glycoside hydrolase superfamily, these enzymes have retaining mechanism of action and has hexamer structure with  $(\beta/\alpha)_8$  domain observed in their three dimensional structures. Aspartic acid and glutamic acid acts as catalytic nucleophile base and proton donor, respectively (Fisher and Aronson 1989; Sulzenbacher et al. 2004). Alpha-L-fucosidase catalyze hydrolysis of alpha-1,6-linked fucose joined to the reducing-end N-acetylglucosamine (glycoproteins) (Fig. 4f1).

## Glycoside Hydrolase Family 30

Endo- $\beta$ -1,4-xylanase (EC 3.2.1.8);  $\beta$ -glucosidase (3.2.1.21);  $\beta$ -glucuronidase (EC 3.2.1.31);  $\beta$ -xylosidase (EC 3.2.1.37);  $\beta$ -fucosidase (EC 3.2.1.38); glucosylceramidase (EC 3.2.1.45);  $\beta$ -1,6-glucanase (EC 3.2.1.75); glucuronoarabinoxylan endo- $\beta$ -1,4-xylanase (EC 3.2.1.136); endo- $\beta$ 1,6-galactanase (EC:3.2.1.164); [reducing end]  $\beta$ -xylosidase (EC 3.2.1.-) are major activities of enzymes belonging to glycoside hydrolase family 30. These include enzymes of mammalian origin (Dinur et al. 1986). They follow retaining type mechanism of

action, with glutamic acid as catalytic nucleophile/base as well as proton donor. Three dimensional structure has  $(\beta/\alpha)_8$  domains (Fig. 4g1).

### Glycoside Hydrolase Family 31

Alpha-glucosidase (EC 3.2.1.20);  $\alpha$ -galactosidase (EC 3.2.1.22);  $\alpha$ -mannosidase (EC 3.2.1.24);  $\alpha$ -1,3-glucosidase (EC 3.2.1.84); sucrase-isomaltase (EC 3.2.1.48) (EC 3.2.1.10);  $\alpha$ -xylosidase (EC 3.2.1.177);  $\alpha$ -glucan lyase (EC 4.2.2.13); isomaltosyltransferase (EC 2.4.1.-); oligosaccharide  $\alpha$ -1,4-glucosyltransferase (EC 2.4.1.161); sulfoquinovosidase (EC 3.2.1.-) are activities of enzymes of family 31 of glycoside hydrolases, with retaining mechanism of action and aspartic acid residues as nucleophile/base as well proton donor, these enzymes have been reported to be of archaea, eukaryotes, prokaryotes (bacteria) origin (Kinsella et al. 1991; Naim et al. 1991; Hermans et al. 1991). These enzymes belong to TIM barrel glycoside hydrolase superfamily clan-D (Fig. 4h1).

### Glycoside Hydrolase Family 32

Invertase (EC 3.2.1.26); endo-inulinase (EC 3.2.1.7);  $\beta$ -2,6-fructan 6-levanbiohydrolase (EC 3.2.1.64); endo-levanase (EC 3.2.1.65); exo-inulinase (EC 3.2.1.80); fructan  $\beta$ -(2,1)-fructosidase/1-exohydrolase (EC 3.2.1.153); fructan  $\beta$ -(2,6)-fructosidase/6-exohydrolase (EC 3.2.1.154); sucrose:sucrose 1-fructosyltransferase (EC 2.4.1.99); fructan:fructan 1-fructosyltransferase (EC 2.4.1.100); sucrose:fructan 6-fructosyltransferase (EC 2.4.1.10); fructan:fructan 6G-fructosyltransferase (EC 2.4.1.243); levan fructosyltransferase (EC 2.4.1.-); [retaining] sucrose:sucrose 6-fructosyltransferase (6-SST) (EC 2.4.1.-); cyclinulooligosaccharide fructanotransferase (EC 2.4.1.-) forms major activities of enzymes of glycoside hydrolase family 32, with retaining mechanisms of action and fivefold  $\beta$ -propeller in their 3-D structure (Alberto et al. 2004), has aspartic acid and glutamic acid residue at their active site that serves as nucleophile/base and proton donor respectively. They belong to clan GH-J of beta-fructosidase/furanosidase superfamily (composed of glycoside hydrolase enzymes having five-bladed beta-propeller fold, built by J Mistry) (Hettwer et al. 1998; Nurizzo et al. 2002a) (Fig. 4i1, j1).

### Glycoside Hydrolase Family 33

Sialidase or neuraminidase (EC 3.2.1.18); trans-sialidase (EC 2.4.1.-); anhydrosialidase (EC 4.2.2.15); Kdo hydrolase (EC 3.2.1.-); 2-keto-3-deoxynononic acid hydrolase/KDNase (EC 3.2.1.-) are enzyme activities of family 33.ave. They catalyze through retaining mode of activity and have sixfold  $\beta$ -propeller domain as found in 3-D structure. Tyrosine and glutamic acid acts as catalytic nucleophile/base (Rothe et al. 1991; Luo et al. 1998) (Fig. 4k1).

### **Glycoside Hydrolase Family 34**

Sialidase or neuraminidase (EC 3.2.1.18) are activities of enzymes of glycoside hydrolase family 34, with retaining catalytic mechanism, tyrosine and glutamic acid serves as nucleophile/base, these enzymes have sixfold-beta propellers. They belong to Sialidase superfamily (CL0434). Neuraminidases cleave the terminal sialic acid (negatively charged sugars associated with proteins and lipids portion of the lipoproteins) residues from carbohydrate chains in glycoproteins. These enzymes have been extensively studied and have been widely reported to act as endo and exo-enzymes (Kim et al. 2013) (Fig. 411).

### **Glycoside Hydrolase Family 35**

Beta-galactosidase (EC 3.2.1.23); exo- $\beta$ -glucosaminidase (EC 3.2.1.165); exo- $\beta$ -1,4-galactanase (EC 3.2.1.-);  $\beta$ -1,3-galactosidase (EC 3.2.1.-), are major enzymes of this group, with retaining mode of action they belong to clan GH-A, where glutamic acid residue acts as catalytic nucleophile/base and proton donor and the three dimensional structure of these enzymes have  $(\beta/\alpha)_8$  barrels. They are reported to be produced from various sources including mammals, fungi, plants and bacteria (Fig. 4m1).

### **Glycoside Hydrolase Family 36**

Alpha-galactosidase (EC 3.2.1.22);  $\alpha$ -N-acetylgalactosaminidase (EC 3.2.1.49); stachyose synthase (EC 2.4.1.67); raffinose synthase (EC 2.4.1.82) are prominent activities of the family with retaining mechanism of catalysis, they belong to GH-D, containing  $(\beta/\alpha)_8$  motif. Aspartic acid residues in the conserved domain work as nucleophile/base and proton donor. They show similarity in catalytic mechanism as glycoside hydrolase family 27 (Fig. 4n1).

Hydrolysis of melibiose into galactose and glucose is done by alpha-galactosidase, prokaryotic enzyme requires NAD and magnesium as co-factor. Glycoside hydrolase family 36 have been subdivided into 11 families GH36A to GH36K (Dey and Pridham 1972; Aslanidis et al. 1989; Wang et al. 1990; Peterbauer et al. 2002; Naumoff 2011).

### **Glycoside Hydrolase Family 37**

Alpha, $\alpha$ -trehalase (EC 3.2.1.28) is the activity of the enzymes of this family, with inverting mode of catalytic mechanism and conserved glutamic and aspartic acid acting as nucleophile/base an proton donor they belong clan GH-G, of six-hairpin glycosidase superfamily. The three dimensional structure of same contains  $(\alpha/\alpha)_6$

(Kopp et al. 1993). Trehalases catalyses degradation of disaccharide  $\alpha,\alpha$ -trehalose yielding two-glucose subunits (Fig. 4o1).

### **Glycoside Hydrolase Family 38**

Alpha-mannosidase (EC 3.2.1.24); mannosyl-oligosaccharide  $\alpha$ -1,2-mannosidase (EC 3.2.1.113); mannosyl-oligosaccharide  $\alpha$ -1,3-1,6-mannosidase (EC 3.2.1.114);  $\alpha$ -2-O-mannosylglycerate hydrolase (EC 3.2.1.170); mannosyl-oligosaccharide  $\alpha$ -1,3-mannosidase (EC 3.2.1.-) are major activities of enzymes of the group with a prominent  $(\beta/\alpha)_7$  domain in their three-dimensional structure. Aspartic acid residue at the catalytic site acts as nucleophile/base and these enzymes show retaining mode of catalytic mechanism. They carry catalysis of N-linked carbohydrates released during glycoprotein turnover, catalyzing hydrolysis of terminal, non-reducing alpha-D mannose residues in  $\alpha$ -D-mannosides and are localized in plasma membrane and golgi membrane. N-terminal domain of these enzymes belongs to glycoside hydrolase/deacetylase superfamily (CL0158) and C-terminal domain is categorized in Galactose Mutarotase-like superfamily (CL0103). Both the clans were built by A Bateman. Clan CL0158 contains diverse carbohydrate catalysing enzymes including hydrolases and deacetylases (Heikinheimo et al. 2003). Clan CL0103 is group of enzymes composed of beta-sandwich acting on sugar substrate and can be observed in any location (eg., domain 5 of beta-galactosidase, central domain of copper amine oxidase; c-terminal of chondroitinase and hyaluronate lyase, N terminal of maltose phosphorylase and galactose mutarotase) (Thoden and Holden 2002) (Fig. 4p1, q1, r1).

### **Glycoside Hydrolase Family 39**

Alpha-L-iduronidase (EC 3.2.1.76);  $\beta$ -xylosidase (EC 3.2.1.37), are activities of the family. These enzymes belong to TIM-barrel glycoside hydrolase superfamily (CL0058) with prominent  $(\beta/\alpha)_8$  motif in its three dimensional structure. They follow retaining type catalytic mechanism with conserved glutamic acid residue working as nucleophile/base and proton donor (Henrissat and Bairoch 1996; Henrissat et al. 1995) (Fig. 4s1).

### **Glycoside Hydrolase Family 40**

This family does not have specified studied enzymes classified under its group. No data available through CAZyedia.

## Glycoside Hydrolase Family 41

This family does not have specified studied enzymes classified under its group. No data available through CAZypedia.

## Glycoside Hydrolase Family 42

Beta-galactosidase (EC 3.2.1.23);  $\alpha$ -L-arabinopyranosidase (EC 3.2.1.-) are activities of enzymes of this family. They undergo retaining type catalytic mechanism with conserved glutamic acid residue acting as nucleophile/base and proton donor. Three dimensional structure has  $(\beta/\alpha)_8$  barrels. They share domains with three superfamilies (beta-galactosidase, beta-galactosidase trimerisation domain and beta-galactosidase C-terminal domain belongs to TIM-barrel glycoside hydrolase, Class I glutamine amidotransferase and glycoside hydrolase domain superfamily respectively). These enzymes catalyze hydrolysis of terminal and non-reducing terminal beta-D-galactosidase residues (Shimizu et al. 1995). Class I glutamine amidotransferase superfamily contains glutaminase enzymes and also the members of DJ-1/PfpI family (peptidases, catalytic triad Cys-His-Glu). This clan was built by A Bateman. Class I glutamine amidotransferase superfamily also has Cys-His-Glu triad, but differs from that of peptidases PfpI. Glycoside hydrolase domain superfamily includes the C-terminal domain of sugar-lytic enzymes and was also developed by A Bateman (Fig. 4t1, u1, v1).

## Glycoside Hydrolase Family 43

This family consists of numerous enzymes namely with activities  $\beta$ -xylosidase (EC 3.2.1.37);  $\alpha$ -L-arabinofuranosidase (EC 3.2.1.55); xylanase (EC 3.2.1.8);  $\alpha$ -1,2-L-arabinofuranosidase (EC 3.2.1.-); exo- $\alpha$ -1,5-L-arabinofuranosidase (EC 3.2.1.-); [inverting] exo- $\alpha$ -1,5-L-arabinanase (EC 3.2.1.-);  $\beta$ -1,3-xylosidase (EC 3.2.1.-); [inverting] exo- $\alpha$ -1,5-L-arabinanase (EC 3.2.1.-); [inverting] endo- $\alpha$ -1,5-L-arabinanase (EC 3.2.1.99); exo- $\beta$ -1,3-galactanase (EC 3.2.1.145);  $\beta$ -D-galactofuranosidase (EC 3.2.1.146). They exhibit inverting mode of catalytic mechanism with aspartic acid and glutamic acid as catalytic nucleophile/base and proton donor respectively. Fivefold  $\beta$ -propellers are prominent feature of three-dimensional structure with long V-shaped groove forming a single extended substrate binding surface across the face of propeller. These enzymes belong to beta-fructosidase superfamily and have membrane localization (Nurizzo et al. 2002b) (Fig. 4w1).

## Glycoside Hydrolase Family 44

Endoglucanase (EC 3.2.1.4) and xyloglucanase (EC 3.2.1.151) forms major activities of this family with retaining mechanism of catalytic activity and glutamic acid at conserved domain acting as nucleophile/base and proton donor. They belong to TIM barrel glycoside hydrolase superfamily (CL0058) and has  $(\beta/\alpha)_8$  domain in its three-dimensional structure (Kitago et al. 2007; Ariza et al. 2011). This family was formerly known as cellulase family J (Fig. 4x1).

## Glycoside Hydrolase Family 45

Endoglucanase (EC 3.2.1.4); xyloglucan-specific endo- $\beta$ -1,4-glucanase/endo-xyloglucanase (EC 3.2.1.151); endo- $\beta$ -1,4-mannanase (EC 3.2.1.78) are prominent activities of enzymes of this group and were formerly known to classified under cellulase family K. They exhibit inverting mode of catalytic reaction, with conserved aspartic acid residues (conserved N-terminal domain with several cysteines involved in forming disulphide bonds, referred as signature sequence) working as nucleophile/base and proton donor (Davies et al. 1993). Enzymes of this family belong to Double Psi beta barrel glucanase superfamily (CL0199). This clan represents barwin like barrels and was developed by A Bateman (Mizuguchi et al. 1999; Castillo et al. 1999). Examples of enzymes of this group includes endoglucanase 5 from *Humicola insolens*, *Trichoderma reesei*, endoglucanase K from *Fusarium oxysporum* etc (Fig. 4y1).

## Glycoside Hydrolase Family 46

Chitosanases (EC 3.2.1.132) is activity of enzymes of this group, they belong to lysozyme like superfamilies (CL0037) and have inverting mode of chitinase activity, with aspartic acid and glutamic acid at probable catalytic conserved site acting as nucleophile/base and proton donor, respectively. Chitosanase catalyze the endohydrolysis of beta 1,4-linkages between N-acetyl-D-glucosamine and D-glucosamine residues in a partly acetylated chitosan (Fig. 4z1).

## Glycoside Hydrolase Family 47

Alpha-mannosidase (EC 3.2.1.113) is activity of enzymes found in this family and these protein have  $(\alpha/\alpha)_7$  domain in their three dimensional structure with conserved glutamic acid acting as catalytic proton donor. Alpha-mannosidase catalyzes maturation of Asn-linked oligosaccharides (Lal et al. 1994). Hydrolysis of terminal 1,2-linked alpha-D-mannose residues into oligo-mannose oligosaccharide man(9)(glcnac)(2) is calcium dependent process and mannose residues get trimmed to produce man(8)glcnac(2) followed by man(5)(glcnac)(2) structures. They are

prominently located in endoplasmic reticulum membrane, golgi membrane and cell membrane (Fig. 4a2).

### **Glycoside Hydrolase Family 48**

Reducing end-acting cellobiohydrolase (EC 3.2.1.176); endo- $\beta$ -1,4-glucanase (EC 3.2.1.4); chitinase (EC 3.2.1.14) are major enzymes of the group of the family (formerly known as cellulase family L). Conserved glutamic acid residues act as catalytic proton donor with the enzyme exhibiting inverting catalytic mechanism. Three dimensional structure has  $(\alpha/\alpha)_6$  barrels, these enzymes belong to six-hairpin glycosidase superfamily (CL0059) (Te'o et al. 1995) (Fig. 4b2).

### **Glycoside Hydrolase Family 49**

Dextranase (EC 3.2.1.11); isopullulanase (EC 3.2.1.57); dextran 1,6- $\alpha$ -isomaltotriosidase (EC 3.2.1.95); sulfated arabinan endo-1,4- $\beta$ -L-arabinanase (EC 3.2.1.-) and catalyze hydrolysis of alpha-1,6-glycosidic bonds in dextran polymers, through catalytic mechanism with conserved aspartic acid residues acting as catalytic nucleophile/base and proton donor. They belong to Pectate lyase-like beta helix with prominent beta helix seen in three dimensional structures (Fig. 4c2).

### **Glycoside Hydrolase Family 50**

Beta-agarase (EC 3.2.1.81) is the activity reported for this group. These enzymes are less explored and probably follow retaining mode of activity with glutamic acid residues as nucleophile/base and proton donor. They belong to clan GH-A of Tim barrel glycoside hydrolase superfamily. Few bacterial enzymes of the group are reported (Fig. 4d2).

### **Glycoside Hydrolase Family 51**

Endoglucanase (EC 3.2.1.4); endo- $\beta$ -1,4-xylanase (EC 3.2.1.8);  $\beta$ -xylosidase (EC 3.2.1.37);  $\alpha$ -L-arabinofuranosidase (EC 3.2.1.55); lichenase/endo- $\beta$ -1,3-1,4-glucanase (EC 3.2.1.73) are major enzymes of the group with retaining mode of action, and glutamic acid residues at catalytic conserved site acting as nucleophile/base and proton donor. These enzymes belong to Clan GH-A of TIM-barrel glycoside hydrolase superfamily and have  $(\beta/\alpha)_8$  motif in their three dimensional structure (Fig. 4e2).

## Glycoside Hydrolase Family 52

Beta-xylosidase (EC 3.2.1.37) is the activity of enzymes of this group, having retaining mode of catalytic mechanism, with glutamic acid and aspartic acid residues on conserved domains acting as nucleophile/base and proton donor, respectively. They belong to clan GH-O with  $(\alpha/\alpha)_6$  barrels in their three dimensional structure. These enzymes are reported to be from *Bacillus stearothermophilus*, functioning in xylan degradation through hydrolysis of 1,4- beta-D-xylans, through successive removal of D-xylose residues from non-reducing termini. Alongside it also carries out hydrolysis of xylobiose (Baba et al. 1994) (Fig. 4f2).

## Glycoside Hydrolase Family 53

Endo- $\beta$ -1,4-galactanase (EC 3.2.1.89) are major activities of family 53 enzymes. They belong to clan GH-A of TIM barrel glycoside hydrolase superfamily with  $(\beta/\alpha)_8$  barrels in their three dimensional structure. They undergo retaining type catalytic mechanism with glutamic acid as catalytic nucleophile/base and proton donor (Ryttersgaard et al. 2002) (Fig. 4g2).

## Glycoside Hydrolase Family 54

Alpha-L-arabinofuranosidase (EC 3.2.1.55);  $\beta$ -xylosidase (EC 3.2.1.37) are activities of enzymes of the group. They show retaining mode of catalytic mechanism. This group of enzymes is very less explored and needs detailed study (Fig. 4h2).

## Glycoside Hydrolase Family 55

Exo- $\beta$ -1,3-glucanase (EC 3.2.1.58); endo- $\beta$ -1,3-glucanase (EC 3.2.1.39) are activities of enzymes belonging to this family and have inverting mode of catalytic mechanism. These enzymes have prominent beta-helix in their three dimensional structure and conserved glutamic acid residue is known to be catalytic proton donor. This group of enzyme is less explored and classified is not available (Fig. 4i2).

## Glycoside Hydrolase Family 56

Hyaluronidase (EC 3.2.1.35); chondroitin hydrolase (EC 3.2.1.-) are activities of enzymes of this family (eg., venom of *Apis mellifera*; Gmachl and Kreil 1993). They belong to TIM-barrel glycoside hydrolase superfamily with  $(\beta/\alpha)_7$  motif in their three dimensional structure and are reported to be located on plasma membrane. They have retaining type of catalytic mechanism, where carbonyl oxygen of C-2

acetamido group of substrate work as nucleophile/base and conserved glutamic acid residue works as proton donor. Amino acid sequences of hyaluronidases have multiple glycosylation sites and numerous cysteines (Lathrop et al. 1990) (Fig. 4j2).

### **Glycoside Hydrolase Family 57**

Alpha-amylase (EC 3.2.1.1);  $\alpha$ -galactosidase (EC 3.2.1.22); amylopullulanase (EC 3.2.1.41); cyclomaltodextrinase (EC 3.2.1.54); branching enzyme (EC 2.4.1.18); 4- $\alpha$ -glucanotransferase (EC 2.4.1.25) are activities of enzymes of the family, they carry out retaining type catalytic mechanism and glutamic acid residues at conserved site working as nucleophile/base. Three dimensional structure has  $(\beta/\alpha)_7$  motifs. This family includes highly specific enzymes (Laderman et al. 1993) (Fig. 4k2).

### **Glycoside Hydrolase Family 58**

Endo-N-acetylneuraminidase or endo-sialidase (EC 3.2.1.129) are activities of enzymes found in this family having inverting type catalytic mechanism, water activated by carboxylate of substrate working as catalytic nucleophile/base and conserved glutamic acid working as catalytic proton donor. Three dimensional structures have sixfold  $\beta$ -propellers present. This group of enzyme is highly explored and needs extensive study and classification (Fig. 4l2).

### **Glycoside Hydrolase Family 59**

Beta-galactosidase (EC 3.2.1.23) galactocerebrosidase (EC 3.2.1.46) are activities of enzymes of this family, with  $(\beta/\alpha)_8$  motif in their three-dimensional structure, they are classified under clan GH-A of TIM barrel glycoside hydrolase superfamily (CL0058) and have conserved glutamic acid at catalytic region that acts as nucleophile/base and proton donor. These enzymes are responsible for lysosomal catabolism of galactolipids (Rafi et al. 1996; Luzi et al. 1995; Fukushima et al. 1998) (Fig. 4m2).

### **Glycoside Hydrolase Family 60**

Precise data of any particular enzyme of this family is not reported.

## Glycoside Hydrolase Family 61

Copper-dependent lytic polysaccharide monooxygenases were activities of enzymes belonging to this family, now reclassified in family AA9 (Auxiliary activity Family 9). Classification under GH Family 61 was considered incorrect.

## Glycoside Hydrolase Family 62

Alpha-L-arabinofuranosidase (EC 3.2.1.55) are activities of enzymes of this group, belonging to Clan GH-F of Beta-fructosidase/Furanosidase Superfamily. They catalyze hydrolysis of aryl alpha L arabinofuranosides by cleaving arabinosyl side chains from arabinoxylan and arabinan, but the detailed catalytic mechanism of the enzymes of this group is not known (Fig. 4n2).

## Glycoside Hydrolase Family 63

Processing  $\alpha$ -glucosidase (EC 3.2.1.106);  $\alpha$ -1,3-glucosidase (EC 3.2.1.84);  $\alpha$ -glucosidase (EC 3.2.1.20); mannosylglycerate  $\alpha$ -mannosidase/mannosylglycerate hydrolase (EC 3.2.1.170); glucosylglycerate hydrolase (EC 3.2.1.208) are major enzymes of this family belonging to clan GH-G of Six-hairpin glycosidase superfamily with  $(\alpha/\alpha)_6$  motif in their three dimensional structure. They show inverting mode of catalytic mechanism with conserved glutamic acid and aspartic acid working as catalytic nucleophile/base and proton donor respectively. These enzymes are majorly located in endoplasmic reticulum membrane and they catalyze cleavage of non-reducing terminal glucose residue from Glc(3)Man(9)GlcNAc(2). Mannosyl oligosaccharide glucosidase EC 3.2.1.106 is the first enzyme in the N-linked oligosaccharide processing pathway (Fig. 4o2).

## Glycoside Hydrolase Family 64

Beta-1,3-glucanase (EC 3.2.1.39) are activities of the group, less explored and are known to show inverting mode of catalytic mechanism, with conserved aspartic acid and glutamic acid working as catalytic nucleophile/base and proton donor respectively (Fig. 4p2).

## Glycoside Hydrolase Family 65

Alpha, $\alpha$ -trehalase (EC 3.2.1.28); maltose phosphorylase (EC 2.4.1.8); trehalose phosphorylase (EC 2.4.1.64); kojibiose phosphorylase (EC 2.4.1.230); trehalose-6-phosphate phosphorylase (EC 2.4.1.216); nigerose phosphorylase (EC 2.4.1.279); 3-O- $\alpha$ -glucopyranosyl-L-rhamnose phosphorylase (EC 2.4.1.282);

2-O- $\alpha$ -glucopyranosylglycerol: phosphate  $\beta$ -glucosyltransferase (EC 2.4.1.-);  $\alpha$ -glucosyl-1,2- $\beta$ -galactosyl-L-hydroxylysine  $\alpha$ -glucosidase (EC 3.2.1.107); are activities of enzymes of this family. They consist of three structural domains; central catalytic domain, N-terminal domain and C-terminal domain. Central catalytic domain of these families belongs to Clan GH-L of Six-hairpin glycosidase superfamily and the N-terminal domain belongs to Galactose mutarotase like superfamily. They catalyze hydrolysis through inverting mode of catalytic mechanism, through phosphate for phosphorylases; water for hydrolases acting as nucleophile/base and conserved glutamic acid residue acting as proton donor. Maltose phosphorylase (MP) (dimeric enzyme) catalyzes the conversion of maltose and inorganic phosphate into beta-D-glucose-1-phosphate and glucose (Van Tilbeurgh et al. 2001) (Fig. 4q2, r2, s2).

### **Glycoside Hydrolase Family 66**

Cycloisomaltooligosaccharide glucanotransferase (EC 2.4.1.248) and dextranase (EC 3.2.1.11) are activities of this family. They show retaining mode of catalytic mechanism with conserved aspartic acid residues acting as catalytic nucleophile/base and proton donor. They belong to TIM barrel glycoside hydrolase superfamily (CL0058) (Igarashi et al. 1995; Funane et al. 2011) (Fig. 4t2).

### **Glycoside Hydrolase Family 67**

Alpha-glucuronidase (EC 3.2.1.139) and xylan  $\alpha$ -1,2-glucuronidase (EC 3.2.1.131) are activities of enzymes of glycoside hydrolase family 67, catalyzing through inverting mode of catalytic mechanism and conserved glutamic acid residues as proton donor. They have ( $\beta/\alpha$ )<sub>8</sub> barrels in their three dimensional structure. Crystal structures of all the three domains of family are known, C-terminal, N-terminal and middle domain where the central domain contains invariant glutamic acid and aspartic residues making them catalytic centre. They catalyze removal of alpha-1,2 linked 4-O-methyl glucuronic acid from xylans. (Shoham et al. 2001 and Nurizzo et al. 2002b) (Fig. 4u2, v2, w2).

### **Glycoside Hydrolase Family 68**

Levansucrase (EC 2.4.1.10) (beta-D-fructofuranosyl transferase);  $\beta$ -fructofuranosidase (EC 3.2.1.26); inulosucrase (EC 2.4.1.9) are major activities of the family, with retaining mode of catalytic mechanism, these enzymes use conserved aspartic acid and glutamic acid as catalytic nucleophile/base and proton donor. They are classified under clan GH-J of Beta-fructosidase/Furanosidase Superfamily. L catalyze the conversion of sucrose and (2,6-beta-D-fructosyl)(N) to glucose and (2,6-beta-D-fructosyl)(N+1), where other sugars can also act as fructosyl

acceptors. Invertase, or extracellular sucrase (EC 3.2.1.26), catalyses the hydrolysis of terminal non-reducing beta-D-fructofuranoside residues in beta-D-fructofuranosides (Fig. 4x2).

### **Glycoside Hydrolase Family 69**

No enzyme is classified under this family at present. Earlier classified enzymes have been grouped as family PL16.

### **Glycoside Hydrolase Family 70**

Dextranucrase (EC 2.4.1.5); alternansucrase (EC 2.4.1.140); reuteransucrase (EC 2.4.1.-);  $\alpha$ -4,6-glucoanotransferase (EC 2.4.1.-);  $\alpha$ -1,2-branched dextranucrase (EC 2.4.1.-);  $\alpha$ -4,3-glucoanotransferase (EC 2.4.1.-) are the activities reported to be classified under glycoside hydrolase family 70. They belong to clan GH-H of TIM Barrel glycoside hydrolase superfamily with  $(\beta/\alpha)_8$  motif in their three dimensional structure. They follow retaining type catalytic mechanism with aspartic acid and glutamic acid residues working as catalytic nucleophile/base and proton donor. They catalyze transfer of D-glucopyramnosyl units from sucrose onto acceptor molecules (Croux et al. 1996) (Fig. 4y2).

### **Glycoside Hydrolase Family 71**

Alpha-1,3-glucoanase (EC 3.2.1.59) is the activity of enzyme belonging to this family and they are known to catalyze activity through inverting mechanism of reaction. This group of enzymes is not extensively studied. No known three dimensional structures is available.

### **Glycoside Hydrolase Family 72**

Beta-1,3-glucoanosyltransglycosylase (EC 2.4.1.-) is the activities of enzyme belonging to glycoside hydrolase family 72, clan GH-A of TIM Barrel glycoside hydrolase superfamily. They exhibit retaining type of catalytic mechanism with glutamic acid residues in the conserved domain acting as catalytic nucleophile/base and proton donor. These enzymes are glycolipid proteins anchored to membrane (Fig. 4z2).

### **Glycoside Hydrolase Family 73**

Lysozyme (EC 3.2.1.17); mannosyl-glycoprotein endo- $\beta$ -N-acetylglucosaminidase (EC 3.2.1.96); peptidoglycan hydrolase with endo- $\beta$ -N-acetylglucosaminidase

specificity (EC 3.2.1.-) are activities of enzymes of the group and the conserved glutamic acid acts as catalytic proton donor. They also catalyze hydrolysis of peptidoglycan (Nambu et al. 1999) (Fig. 4a3).

### **Glycoside Hydrolase Family 74**

Endoglucanase (EC 3.2.1.4); oligoxyloglucan reducing end-specific cellobiohydrolase (EC 3.2.1.150); xyloglucanase (EC 3.2.1.151) are activities of enzymes of the group. They follow inverting type catalytic mechanism with conserved aspartic acid residue as catalytic nucleophile/base and proton donor. Three dimensional structures have sevenfold  $\beta$ -propeller. This group of enzymes requires detailed studies (Fig. 4b3).

### **Glycoside Hydrolase Family 75**

Chitosanase (EC 3.2.1.132) is reported activity of enzymes of this group, with conserved aspartic acid and glutamic acid residues working as probable catalytic nucleophile/base and proton donor respectively. They catalyze through inverting mode of catalytic mechanism (Cheng et al. 2017). No known three dimensional structures are available.

### **Glycoside Hydrolase Family 76**

Alpha-1,6-mannanase (EC 3.2.1.101) and  $\alpha$ -glucosidase (EC 3.2.1.20) are activities of enzymes of this family. They belong to Six-hairpin glycosidase superfamily, and catalyses through retaining mode of action through conserved aspartic acid residues working as catalytic nucleophile/base and proton donor (Fig. 4c3).

### **Glycoside Hydrolase Family 77**

Amylomaltase or 4- $\alpha$ -glucanotransferase (EC 2.4.1.25) are activities of enzymes of this family, they belong to clan GH-H of TIM Barrel glycoside hydrolase superfamily and have  $(\beta/\alpha)_8$  motifs in their three dimensional structure. They undergo catalysis through retaining type mechanism with conserved aspartic acid and glutamic residues working as catalytic nucleophile/base and proton donor respectively. They catalyze transfer of a segment of a (1,4)- $\alpha$ -D-glucan to a new four-position in an acceptor, which may be glucose or (1,4)- $\alpha$ -D-glucan. They belong to the disproportionating family of enzymes (Okada et al. 1993) (Fig. 4d3).

## Glycoside Hydrolase Family 78

Alpha-L-rhamnosidase (EC 3.2.1.40); rhamnogalacturonan  $\alpha$ -L-rhamnohydrolase (EC 3.2.1.174); L-Rhap- $\alpha$ -1,3-D-Apif -specific  $\alpha$ -1,3-L-rhamnosidase (EC 3.2.1.-) are activities of enzymes of this family belonging to Six-hairpin glycosidase superfamily having  $(\alpha/\alpha)_6$  motif in their three dimensional structures (Zverlov et al. 2000). They catalyze hydrolysis through inverting mode of catalytic mechanism (Fig. 4e3).

## Glycoside Hydrolase Family 79

Beta-glucuronidase (EC 3.2.1.31); hyaluronoglucuronidase (EC 3.2.1.36); heparanase (EC 3.2.1.166); baicalin  $\beta$ -glucuronidase (EC 3.2.1.167);  $\beta$ -4-O-methyl-glucuronidase (EC 3.2.1.-) are activities of enzymes reported to be classified under this family. They belong to clan GH-A of TIM Barrel glycoside hydrolase superfamily with  $(\beta/\alpha)_8$  domains in its three dimensional structure. They undergo catalysis through retaining mechanism with conserved glutamic acid residues inferred to be catalytic nucleophile/base and proton donor. These enzymes play vital role in the self- assembly, insolubility and barrier properties of basement membranes and extracellular matrices and are also involved in cell migration associated with inflammation and autoimmunity (Vlodavsky et al. 2001) (Fig. 4f3).

## Glycoside Hydrolase Family 80

Chitosanase (EC 3.2.1.132) are activities of enzymes belonging to this family. They belong to clan GH-I of Pectate lyase-like beta helix superfamily and process through inverting mode of catalytic mechanism (Shimono et al. 2002). Detailed catalytic conserved domain and three dimensional structures are yet to be explored (Fig. 4g3).

## Glycoside Hydrolase Family 81

Endo- $\beta$ -1,3-glucanase (EC 3.2.1.39) are the activities of enzyme exhibiting inverting mode of catalytic mechanism. Detailed catalytic mechanism and complete analysis of conserved motifs in the three dimensional structures is yet to be explored (Fig. 4h3).

## Glycoside Hydrolase Family 82

I-carrageenase (EC 3.2.1.157) are the activities of enzymes belonging to the family with a prominent  $(\beta)$ -helix in their three dimensional structure and showing inverting

mode of catalytic mechanism through conserved aspartic acid and glutamic acid working as catalytic nucleophile/base and proton donor, respectively (Fig. 4i3).

### **Glycoside Hydrolase Family 83**

Neuraminidases (EC 3.2.1.18) are the enzyme activities reported for this family, belonging to clan GH-E of sialidase superfamily. With sixfold  $\beta$ -propeller motif in their three dimensional structure, these enzymes show retaining mode of catalytic mechanism (conserved tyrosine + glutamic acid working as catalytic nucleophile/base). Neuraminidase domain is the glycoside hydrolase domain of Hemagglutinin-neuraminidase (unit viral protein) containing both hemagglutinin and (endo) neuraminidase (Zaitsev et al. 2004 and Yuan et al. 2005) (Fig. 4j3).

### **Glycoside Hydrolase Family 84**

N-acetyl  $\beta$ -glucosaminidase (EC 3.2.1.52); hyaluronidase (EC 3.2.1.35) and [protein]-3-O-(GlcNAc)-L-Ser/Thr  $\beta$ -N-acetylglucosaminidase (EC 3.2.1.169) are prominent activities of enzymes belonging to this family with  $(\beta/\alpha)_8$  in their three dimensional structures. They exhibit retaining mode of catalytic mechanism where carbonyl oxygen of C-2 acetamido group of substrate acts as catalytic nucleophile/base and aspartic acid residues are proton donors (Macauley et al. 2005) (Fig. 4k3).

### **Glycoside Hydrolase Family 85**

Endo- $\beta$ -N-acetylglucosaminidases (EC 3.2.1.96) are activities of enzymes of GH family 85, belonging to clan, GH-A of TIM barrel glycoside hydrolase superfamily. They exhibit retaining type of catalytic mechanism with carbonyl oxygen of C-2 acetamido group of substrate acting as catalytic nucleophile/base and glutamic acid residues as proton donor. These enzymes are secretory enzymes and work on broad spectrum of substrates (Fig. 4l3).

### **Glycoside Hydrolase Family 86**

Beta-agarase (EC 3.2.1.81);  $\beta$ -porphyranase (EC 3.2.1.178) are major activities of enzymes of this group. They belong to clan GH-A of TIM Barrel glycoside hydrolase superfamily, with  $(\beta/\alpha)_8$  barrels in their three dimensional structures. Studies have reported probable retaining mode of catalytic mechanism with conserved glutamic acid residues as catalytic nucleophile/base and proton donor (Fig. 4m3).

### **Glycoside Hydrolase Family 87**

Mycodextranase (EC 3.2.1.61) and  $\alpha$ -1,3-glucanase (EC 3.2.1.59) are major enzymes of this family. These enzymes are not well explored need extensive study (Fig. 4n3).

### **Glycoside Hydrolase Family 88**

Major activities of enzymes of this group involves d-4, 5-unsaturated  $\beta$ -glucuronyl hydrolase (EC 3.2.1.-) (Hashimoto et al. 1999). They belong to six-hairpin glycosidase superfamily (CL0059) containing  $(\alpha/\alpha)_6$  motif determined in their three dimensional structure. Mechanism of catalysis by the group of enzymes has not been explored and needs detailed study (Fig. 4o3).

### **Glycoside Hydrolase Family 89**

Alpha-N-acetylglucosaminidase (EC 3.2.1.50) are activities of enzyme reported in GH Family 89, with specific  $(\beta/\alpha)_8$ , they belong to TIM-Barrel glycoside hydrolase superfamily (CL0058). They exhibit retaining mode of catalytic mechanism with conserved glutamic acid residues working as catalytic nucleophile/base and proton donor. Three structural domains of these enzymes are N-terminal domain (alpha-beta fold); central domain (TIM barrel fold) and C-Terminal domain (alpha-helical fold). Improper functioning of these enzymes leads to various disorders (Ficko-Blean et al. 2008; Li et al. 1999; Villani et al. 2002) (Fig. 4p3).

### **Glycoside Hydrolase Family 90**

Endorhamnosidases (EC 3.2.1.-) are activities of enzymes of this group, with prominent  $(\beta)$ -helix in their three-dimensional structure. They carry out catalytic mechanism through inverting mode, where conserved domain with glutamic and aspartic acid acts as nucleophile/base and aspartic acid separately acts as proton donor (Fig. 4q3).

### **Glycoside Hydrolase Family 91**

Inulin lyase [DFA-I-forming] (EC 4.2.2.17); inulin lyase [DFA-III-forming] (EC 4.2.2.18); difructofuranose 1,2':2,3' dianhydride hydrolase [DFA-IIIase] (EC 3.2.1.-) are major activities of enzymes of this family having  $\beta$ -helix domain in its three dimensional structure. They follow inverting mode of catalytic mechanism and was temporarily changed into PL19 until it was recently realized that these

“PLs” are analogous to GH23 SLTs/lysozymes (there is at least one hydrolase in the GH91 family) and therefore are better classified in the GH category (Fig. 4r3).

### **Glycoside Hydrolase Family 92**

Mannosyl-oligosaccharide  $\alpha$ -1,2-mannosidase (EC 3.2.1.113); mannosyl-oligosaccharide  $\alpha$ -1,3-mannosidase (EC 3.2.1.-); mannosyl-oligosaccharide  $\alpha$ -1,6-mannosidase (EC 3.2.1.-);  $\alpha$ -mannosidase (EC 3.2.1.24);  $\alpha$ -1,2-mannosidase (EC 3.2.1.-);  $\alpha$ -1,3-mannosidase (EC 3.2.1.-);  $\alpha$ -1,4-mannosidase (EC 3.2.1.-) and mannosyl-1-phosphodiester  $\alpha$ -1,P-mannosidase (EC 3.2.1.-) are activities of enzymes belonging to this family and pectate-lyase like beta-helix superfamily (CL0268). The conserved catalytic site consists of aspartic acid and glutamic acid residues working as nucleophile/base and proton donor for carrying out catalysis through inverting catalytic mechanism. These enzymes are reported to be critical for maturation of N- linked oligosaccharides and endoplasmic reticulum associated degradation (Liu et al. 1999) (Fig. 4s3).

### **Glycoside Hydrolase Family 93**

Exo- $\alpha$ -L-1,5-arabinanase (EC 3.2.1.-) are activities classified under this family and with six-bladed  $\beta$ -propeller motif they belong to Clan GH-E of Sialidase superfamily (CL434). They carry out catalysis through retaining mode of action with conserved Glutamic acid residues working as catalytic nucleophile/base and proton donor (Fig. 4t3).

### **Glycoside Hydrolase Family 94**

Cellobiose phosphorylase (EC 2.4.1.20); laminaribiose phosphorylase (EC 2.4.1.31); cellodextrin phosphorylase (EC 2.4.1.49); chitobiose phosphorylase (EC 2.4.1.-); cyclic  $\beta$ -1,2-glucan synthase (EC 2.4.1.-); cellobionic acid phosphorylase (EC 2.4.1.321);  $\beta$ -1,2-oligoglucan phosphorylase (EC 2.4.1.-) are activities of enzymes belonging to family and with  $(\alpha/\alpha)_6$  barrels they are classified under clan GH-Q. Catalysis involves inverting mode of mechanism and conserved phosphate and aspartic acid residues work as catalytic nucleophile/base and proton donor. These enzymes were formerly known as glycosyltransferase family GT36; and have been assigned to GH family by Hidaka et al. 2004 that reported evolutionary, structural and mechanistic relationship of these phosphorylases with glycoside hydrolases of clan GH-L (Fig. 4u3).

## Glycoside Hydrolase Family 95

$\alpha$ -L-fucosidase (EC 3.2.1.51);  $\alpha$ -1,2-L-fucosidase (EC 3.2.1.63);  $\alpha$ -L-galactosidase (EC 3.2.1.-) are major enzymes with inverting catalytic mechanism containing conserved ( $\alpha/\alpha$ )<sub>6</sub> motif in three dimensional structures. Asparagine activated by aspartic acid works as catalytic nucleophile/base and Glutamic acid as proton donor (Katayama et al. 2004) (Fig. 4v3).

## Glycoside Hydrolase Family 96

Alpha-agarase (EC 3.2.1.158) is activity classified under this family. No proper literature of three dimensional structure and catalytic mechanism for the same is reported.

## Glycoside Hydrolase Family 97

Glucoamylase (EC 3.2.1.3);  $\alpha$ -glucosidase (EC 3.2.1.20);  $\alpha$ -galactosidase (EC 3.2.1.22) are the activities of the family, with ( $\beta/\alpha$ )<sub>8</sub> motif they are classified under TIM Barrel glycoside hydrolase Superfamily (CL0058). They exhibit both inverting and retaining mode of catalytic mechanism where Glutamic acid and Aspartic acid residues acts as catalytic nucleophile/base for inverting and retaining mechanisms respectively. Catalytic proton donor in both the cases is conserved glutamic acid residues (Hughes et al. 2003). N-terminal and C-terminal of the enzyme has pre-dominant beta-strands and the non catalytic domains are involved in oligomerization and carbohydrate binding (Naumoff 2005) (Fig. 4w3).

## Glycoside Hydrolase Family 98

Blood-group endo- $\beta$ -1,4-galactosidase (EC 3.2.1.102); blood group A- and B-cleaving endo- $\beta$ -1,4-galactosidase (EC 3.2.1.-); endo- $\beta$ -1,4-xylanase (EC 3.2.1.8); endo- $\beta$ -1,4-xylanase (EC 3.2.1.8) are activities of enzymes belonging to this group. Two domains of these enzymes are known, of which putative catalytic domain is believed to be present at N-terminus of the non catalytic region (Anderson et al. 2005). They follow inverting catalytic mechanism with Aspartic acid and Glutamic acid diad as catalytic nucleophile/base and conserved glutamic acid residues as proton donor (Fig. 4x3).

## Glycoside Hydrolase Family 99

Glycoprotein endo- $\alpha$ -1,2-mannosidase (EC 3.2.1.130) and mannan endo-1,2- $\alpha$ -mannanase (3.2.1.-) are activities of the group with ( $\beta/\alpha$ )<sub>8</sub> motifs in their

three dimensional structure. They undergo retaining type catalytic mechanism with unclear, possible neighboring group participation from O<sub>2</sub> (Thompson et al. 2012) working as catalytic nucleophile/base and conserved glutamic acid residues as proton donor (Spiro et al. 1997) (Fig. 4y3).

### **Glycoside Hydrolase Family 100**

Alkaline and neutral invertase (EC 3.2.1.26) are activities of the enzymes classified under this family, with a ( $\alpha/\alpha$ )<sub>6</sub> motif in their three dimensional structure, they belong to clan GH-G of Six-hairpin glycosidase superfamily. Catalytic mechanism takes place through inverting mode with glutamic acid and aspartic acid acting as nucleophile/base and proton donor respectively (Gallagher and Pollock 1998; Lee and Sturm 1996; Sturm 1999) (Fig. 4z3).

### **Glycoside Hydrolase Family 101**

Endo- $\alpha$ -N-acetylglactosaminidases (EC 3.2.1.97) are activities reported, and contains ( $\beta/\alpha$ )<sub>8</sub> motifs, belonging to TIM barrel Glycoside hydrolase Superfamily (CL0058). They carry retaining type of catalytic mechanism with conserved Aspartic acid and glutamic acid residues working as catalytic nucleophile/base and proton donor (Fujita et al. 2005). These enzymes can be split into several subfamilies (Naumoff 2010) (Fig. 4a4).

### **Glycoside Hydrolase Family 102**

Peptidoglycan lytic transglycosylases (EC 3.2.1.-) are activities of enzymes belonging to GH family 102, exhibiting retaining type catalytic mechanism. These enzymes corresponds to family 2 of the peptidoglycan lytic transglycosylases (Blackburn and Clarke 2001) (Fig. 4b4).

### **Glycoside Hydrolase Family 103**

Peptidoglycan lytic transglycosylases (EC 3.2.1.-) are activities of enzymes belonging to GH family 102, exhibiting retaining type catalytic mechanism with carbonyl oxygen of C-2 acetamido group of substrate inferred to be catalytic nucleophile/base and conserved glutamic acid residues working as proton donor. These enzymes corresponds to family 3 of the peptidoglycan lytic transglycosylases (Blackburn and Clarke 2001) (Fig. 4C4).

## Glycoside Hydrolase Family 104

Peptidoglycan lytic transglycosylases (EC 3.2.1.-) are activities of enzymes belonging to GH family 102, exhibiting retaining type catalytic mechanism. These enzymes corresponds to family 4 of the peptidoglycan lytic transglycosylases (Blackburn and Clarke 2001) (Fig. 4d4).

## Glycoside Hydrolase Family 105

Unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172); d-4,5-unsaturated  $\beta$ -glucuronyl hydrolase (EC 3.2.1.-); d-4,5-unsaturated  $\alpha$ -galacturonidase (EC 3.2.1.-) are activities reported belonging to this family with predominant ( $\alpha/\alpha$ )<sub>6</sub> in their three dimensional structures. Catalytic mechanism for this group of enzymes is not known (Itoh et al. 2006) (Fig. 4e4).

## Glycoside Hydrolase Family 106

Alpha-L-rhamnosidase (EC 3.2.1.40); rhamnogalacturonan  $\alpha$ -L-rhamnohydrolase (EC 3.2.1.174) are activities of this family of enzymes containing ( $\beta/\alpha$ )<sub>8</sub> barrels in their three-dimensional structure. They exhibit inverting mode of catalytic mechanism (Miyata et al. 2005) (Fig. 4f4).

## Glycoside Hydrolase Family 107

Sulfated fucan endo-1,4-fucanase (EC 3.2.1.-) are activities of enzymes belonging to GH Family 107, with ( $\beta/\alpha$ )<sub>8</sub> motif in their three dimensional structure, they belong to Clan GH-R of TIM Barrel Glycoside Hydrolase Superfamily. They exhibit retaining mode of catalytic mechanism with conserved Aspartic acid residues and Histidine acting as catalytic nucleophile/base and proton donor (Colin et al. 2006 and Vickers et al. 2018) (Fig. 4g4).

## Glycoside Hydrolase Family 108

N-acetylmuramidase (EC 3.2.1.17) are the activities of enzymes belonging to this family and belong to Lysozyme like superfamily (CL0037). Conserved glutamic acid acts as catalytic proton donor. One of their probable activities is activation of secretion of large proteins via the breaking and rearrangement of bacterial peptidoglycan layer during secretion (Stojković and Rothman-Denes 2007; Pei and Grishin 2005; Kondo et al. 1994; Emina and Lucia 2007) (Fig. 4h4).

### **Glycoside Hydrolase Family 109**

Alpha-N-acetylgalactosaminidase (EC 3.2.1.49) are the activities of enzymes related to this family, with their catalytic mechanism unusually involving NAD<sup>+</sup>, catalyzing through retaining mode (Liu et al. 2007) (Fig. 4i4).

### **Glycoside Hydrolase Family 110**

Alpha-galactosidase (EC 3.2.1.22);  $\alpha$ -1,3-galactosidase (EC 3.2.1.-) are activities of enzymes reported for this family and they carry out catalysis through inverting mechanism (Liu et al. 2007). No structure is reported till date for enzymes of this family.

### **Glycoside Hydrolase Family 111**

keratan sulfate hydrolase (endo- $\beta$ -N-acetylglucosaminidase) (EC 3.2.1.-) are the activities reported with no further details available.

### **Glycoside Hydrolase Family 112**

Lacto-N-biose phosphorylase or galacto-N-biose phosphorylase (EC 2.4.1.211); D-galactosyl- $\beta$ -1,4-L-rhamnose phosphorylase (EC 2.4.1.247) and galacto-N-biose/lacto-N-biose phosphorylase (EC 2.4.1.-) are activities reported for this family and studies show that they undergo inverting mode of catalytic mechanism with Aspartic acid residues (through site directed mutagenesis) acting as proton donor (Fig. 4j4).

### **Glycoside Hydrolase Family 113**

Beta-mannanase (EC 3.2.1.78) are activities of enzymes belonging to this family and with specific ( $\beta/\alpha$ )<sub>8</sub> barrels in their three dimensional structure they are classified under clan GH-A of TIM Barrel Glycoside hydrolase Superfamily. They exhibit retaining mode of catalytic mechanism with conserved glutamic acid residues working as catalytic nucleophile/base and proton donor (Zhang et al. 2008) (Fig. 4k4).

### **Glycoside Hydrolase Family 114**

Endo- $\alpha$ -1,4-polygalactosaminidase (EC 3.2.1.109) are activities of enzymes belonging to this group, exhibiting retaining mode of catalytic mechanism (Tamura et al. 1995) (Fig. 4I4).

### **Glycoside Hydrolase Family 115**

Xylan  $\alpha$ -1,2-glucuronidase (3.2.1.131) and  $\alpha$ -(4-O-methyl)-glucuronidase (3.2.1.-) are activities of enzymes belonging to this family and they catalyze reaction through inverting mechanism (Ryabova et al. 2009) (Fig. 4m4).

### **Glycoside Hydrolase Family 116**

Beta-glucosidase (EC 3.2.1.21);  $\beta$ -xylosidase (EC 3.2.1.37); acid  $\beta$ -glucosidase/ $\beta$ -glucosylceramidase (EC 3.2.1.45) and  $\beta$ -N-acetylglucosaminidase (EC 3.2.1.52) are activities of enzymes classified under this family, with  $(\alpha/\alpha)_6$  motif in their three dimensional structure, they belong to clan GH-O of glycoside hydrolases superfamily. They exhibit retaining mode of catalytic mechanism through glutamic acid and aspartic acid residues working as catalytic nucleophile/base and proton donor (Ferrara et al. 2013) (Fig. 4n4).

### **Glycoside Hydrolase Family 117**

Alpha-1,3-L-neoagarooligosaccharide hydrolase (EC 3.2.1.-) and  $\alpha$ -1,3-L-neoagarobiase/neoagarobiose hydrolase (EC 3.2.1.-) are major activities of the enzymes classified under this family. These enzymes are distantly related to GH Family 43 (Fig. 4o4).

### **Glycoside Hydrolase Family 118**

This family contains enzymes with activities of  $\beta$ -agarase (EC 3.2.1.81), found to exhibit inverting mode of catalytic mechanism.

### **Glycoside Hydrolase Family 119**

Alpha-amylases (EC 3.2.1.1) are enzymes classified under GH Family 119. They are distantly related to GH family 57 and process through inverting catalytic mechanism (Watanabe et al. 2006)

### **Glycoside Hydrolase Family 120**

Beta-xylosidase (EC 3.2.1.37) are activities of enzymes exhibiting retaining type catalytic mechanism with conserved aspartic acid and glutamic acid residues working as catalytic nucleophile/base and proton donor (Shao et al. 2010) (Fig. 4p4).

### **Glycoside Hydrolase Family 121**

Beta-L-arabinobiosidases (EC 3.2.1.-) are activities belonging to this group and enzymes catalyze through retaining mechanism (Fujita et al. 2011).

### **Glycoside Hydrolase Family 122**

Alpha-glucosidase (EC 3.2.1.20) is activity of enzymes belonging to this family (Comfort et al. 2008).

### **Glycoside Hydrolase Family 123**

$\beta$ -N-acetylgalactosaminidase (EC 3.2.1.53) and glycosphingolipid  $\beta$ -N-acetylgalactosaminidase (EC 3.2.1.-) are the activities for the family, exhibiting retaining mode of catalytic mechanism through carbonyl oxygen of C-2 acetamido group of substrate acting as catalytic nucleophile/base and conserved glutamic acid residues as proton donor (Sumida et al. 2011) (Fig. 4q4).

### **Glycoside Hydrolase Family 124**

Endoglucanase (EC 3.2.1.4) is activity for the enzymes belonging to this family and they carry catalysis through inverting mechanism (Bras et al. 2011) (Fig. 4r4).

### **Glycoside Hydrolase Family 125**

Exo- $\alpha$ -1,6-mannosidase (EC 3.2.1.-) is the activity reported for this group of enzymes belonging to clan GH-G with specific  $(\alpha/\alpha)_6$  of Six-hairpin glycosidase superfamily. They carry out catalysis through inverting mechanism with glutamic acid and aspartic acid residues working as catalytic nucleophile/base and proton donor (Gregg et al. 2011) (Fig. 4s4).

## Glycoside Hydrolase Family 126

Alpha-amylase (EC 3.2.1.-), with  $(\alpha/\alpha)_6$  are classified under this family (Ficko-blean et al. 2011) (Fig. 4t4).

## Glycoside Hydrolase Family 127

Beta-L-arabinofuranosidase (EC 3.2.1.185) and 3-C-carboxy-5-deoxy-L-xylose (aceric acid) hydrolase (EC 3.2.1.-) are activities of the enzymes reported under this family and with specific  $(\alpha/\alpha)_6$  motif, they belong to clan GH-P of Glycoside hydrolase superfamilies exhibiting retaining mode of catalytic mechanism (Fujita et al. 2011) (Fig. 4u4).

## Glycoside Hydrolase Family 128

Beta-1,3-glucanase (EC 3.2.1.39) are activities of enzymes belonging to GH Family 128, with  $(\beta/\alpha)_8$  motif in their three dimensional structures, they are classified under Clan GH-A of TIM barrel glycoside hydrolase superfamily (Sakamoto et al. 2011). They exhibit retaining mode of catalysis with conserved glutamic acid residues working as catalytic nucleophile/base and proton donor.

## Glycoside Hydrolase Family 129

Enzymes of this family exhibit  $\alpha$ -N-acetylgalactosaminidase (EC 3.2.1.49) with retaining mode of action and Aspartic acid residues as catalytic nucleophile/base (Kiyohara et al. 2012) (Fig. 4v4).

## Glycoside Hydrolase Family 130

Activities of enzymes of this family includes  $\beta$ -1,4-mannosylglucose phosphorylase (EC 2.4.1.281);  $\beta$ -1,4-mannooligosaccharide phosphorylase (EC 2.4.1.319);  $\beta$ -1,4-mannosyl-N-acetyl-glucosamine phosphorylase (EC 2.4.1.320);  $\beta$ -1,2-mannobiose phosphorylase (EC 2.4.1.-);  $\beta$ -1,2-oligomannan phosphorylase (EC 2.4.1.-) and  $\beta$ -1,2-mannosidase (EC 3.2.1.-). These enzymes have fivefold  $\beta$ -propeller in their three dimensional structure and exhibit inverting mode of catalytic mechanism (Senoura et al. 2011) (Fig. 4w4).

### **Glycoside hydrolase Family 131**

Enzymes with broad specificity exo- $\beta$ -1,3/1,6-glucanase with endo- $\beta$ -1,4-glucanase activity (EC 3.2.1.-) belong to this family (Lafond et al. 2012) (Fig. 4x4).

### **Glycoside Hydrolase Family 132**

Activity on  $\beta$ -1,3-glucan (curdlan) shown for the *Aspergillus fumigatus* Sun4 protein; activity on laminarioligosaccharides shown for *Aspergillus fumigatus* Sun4 protein and *Candida albicans* Sun41 protein; transglycosylation activity are activities of enzymes belonging to this family and they show retaining type of catalytic mechanism (Gastebois et al. 2013).

### **Glycoside Hydrolase Family 133**

Enzymes of this exhibit amylo- $\alpha$ -1,6-glucosidase (EC 3.2.1.33) activity through inverting mechanism with conserved aspartic acid residues working as catalytic nucleophile/base and proton donor (Nakayama et al. 2001) (Fig. 4y4).

### **Glycoside Hydrolase Family 134**

These are enzymes with endo- $\beta$ -1,4-mannanase (EC 3.2.1.78) with inverting mode of action (a unique feature compared to all other beta-mannanses) (Shimizu et al. 2015; Jin et al. 2016) (Fig. 4z4).

### **Glycoside Hydrolase Family 135**

Alpha-1,4-galactosaminogalactan hydrolase (EC 3.2.1.-) are activities of enzymes of this family, showing retaining type catalytic mechanism (Bamford et al. 2015) (Fig. 4a5).

### **Glycoside Hydrolase Family 136**

Lacto-N-biosidase (EC 3.2.1.140) is the activity reported for the group, with prominent ( $\beta$ )-helix in their 3-D structure. They catalyze through retaining mode of catalytic mechanism with conserved aspartic acid residues as catalytic nucleophile base and proton donor (Yamada et al. (2017) (Fig. 4b5).

### **Glycoside hydrolase Family 137**

Beta-L-arabinofuranosidase (EC 3.2.1.185) are major activities of the enzymes belonging to GH Family 137, having fivefold  $\beta$ -propeller in their three dimensional structure and conserved glutamic acid residues acting as catalytic nucleophile/base and proton donor (Ndeh et al. 2017) (Fig. 4c5).

### **Glycoside Hydrolase Family 138**

This family of glycoside hydrolase have activities of rhamnogalacturonan  $\alpha$ -1,2-galacturonohydrolase (EC 3.2.1.173); with enzymes having  $(\beta/\alpha)_8$  barrels in their three dimensional structure, exhibiting retaining mode of catalytic mechanism with conserved glutamic acid residues acting as catalytic nucleophile/base and proton donor (Ndeh et al. 2017; Labourel et al. 2019) (Fig. 4d5).

### **Glycoside Hydrolase Family 139**

Alpha-2-O-Me-L-fucosidase (EC 3.2.1.-) is the activity of enzymes reported for this group (Ndeh et al. 2017).

### **Glycoside Hydrolase Family 140**

Beta-1,2-apiosidase (EC 3.2.1.-) are the activities of enzymes reported in this group, having  $(\beta/\alpha)_8$  barrels in three dimensional structures and exhibiting retaining mode of catalytic mechanism (Ndeh et al. 2017) (Fig. 4e5).

### **Glycoside Hydrolase Family 141**

Alpha-L-fucosidase (EC 3.2.1.51) and xylanases (EC 3.2.1.8) are activities of enzymes of this group, specifically containing  $(\beta)$ -helix in their three dimensional structures; and conserved aspartic acid acts as catalytic nucleophile/base and proton donor (Ndeh et al. 2017 and Heinz et al.) (Fig. 4f5).

### **Glycoside Hydrolase Family 142**

Beta-L-arabinofuranosidase (EC 3.2.1.185) is the reported activity for the enzymes of this family with prominent  $(\alpha/\alpha)_6$  barrel in their three-dimensional structure. Conserved aspartic acid residues acts as catalytic nucleophile/base (Ndeh et al. 2017) (Fig. 4g5).

### **Glycoside Hydrolase Family 143**

Enzymes with activity of 2-keto-3-deoxy-D-lyxo-heptulosaric acid hydrolase (EC 3.2.1.-) belong to this family and have prominent fivefold  $\beta$ -propeller in their three dimensional structures. They catalyze through retaining mode of reaction with conserved tyrosine and glutamic acid residues acting as nucleophile/base and individual glutamic acid acting as catalytic proton donor (Fig. 4h5).

### **Glycoside Hydrolase Family 144**

Endo- $\beta$ -1,2-glucanase (EC 3.2.1.71) and  $\beta$ -1,2-glucooligosaccharide sophorohydrolase (EC 3.2.1.-) are activities of enzymes belonging to this group with specific  $(\alpha/\alpha)_6$  motif in their three dimensional structures catalyzing enzymatic reaction through inverting mode (Abe et al. 2017) (Fig. 4i5).

### **Glycoside Hydrolase Family 145**

L-Rh $\alpha$ - $\alpha$ -1,4-GlcA  $\alpha$ -L-rhamnohydrolase (EC 3.2.1.-) are activities reported for this family, having seven-bladed  $\beta$ -propeller in their three dimensional structures. These enzymes are reported to exhibit retaining mode of catalytic mechanism with conserved Histidine residues working as catalytic nucleophile/base. They carry out hydrolysis of alpha-L-rhamnose residues linked to position 4 of a glucuronic acid. One of the sites of these enzymes which is still under study shows string resemblance to family PL25 suggesting polysaccharide lyase activity (Fig. 4j5).

### **Glycoside Hydrolase Family 146**

Beta-L-arabinofuranosidases (EC 3.2.1.185) are activities of the enzymes having  $(\alpha/\alpha)_6$  barrels in their three dimensional structure, they are classified under clan GH-P with retaining type of catalytic mechanism (Luis et al. 2018) (Fig. 4k5).

### **Glycoside Hydrolase Family 147**

Beta-galactosidase (EC 3.2.1.23) is activity reported for this group, with specific  $(\beta/\alpha)_8$  (inferred) present in their three dimensional structures, they have been classified under Clan GH-A of TIM Barrel glycoside hydrolase superfamily. Catalysis is carried out through retaining mode with conserved glutamic acid residues acting as catalytic nucleophile/base and proton donor (Luis et al. 2018).

### **Glycoside Hydrolase Family 148**

Enzymes of this family are reported to have  $\beta$ -1,3-glucanase (EC 3.2.1.-) activities, and with specific  $(\beta/\alpha)_8$  (inferred) present in their three dimensional structures, they have been classified under Clan GH-A of TIM Barrel glycoside hydrolase superfamily. Conserved glutamic acid residues act as catalytic nucleophile/base and proton donor (Angelov et al. 2017).

### **Glycoside Hydrolase Family 149**

$\beta$ -1,3-glucan phosphorylases (EC 2.4.1.97) are activities reported for enzymes belonging to family GH F-149, having  $(\alpha/\alpha)_6$  barrels in their three dimensional structures, they are classified under clan GH-Q of glycoside hydrolase superfamily sharing this family GH F-94. They carry out inverting mode of catalytic mechanism, with phosphate ion and conserved aspartic acid residues acting as catalytic nucleophile/base and proton donor (Kuhadomlarp et al. 2018) (Fig. 415).

### **Glycoside Hydrolase Family 150**

l-carrageenase (EC 3.2.1.-) are activities of enzymes belonging to this family, and have been reported to carry out catalysis through inverting mechanism (PMID:16926183).

### **Glycoside Hydrolase Family 151**

Alpha-L-fucosidase (EC 3.2.1.51) are activities of enzymes belonging to this family (Sela et al. 2012), and have been reported to carry out catalysis through retaining mechanism (inferred through transglycosylation activity) (Benesová et al. 2013).

### **Glycoside Hydrolase Family 152**

Beta-1,3-glucanase (EC 3.2.1.39) are activities reported of this group of enzymes with only few structural details available for the same (Sakamoto et al. 2006) (Fig. 4m5).

### **Glycoside Hydrolase Family 153**

Poly- $\beta$ -1,6-D-glucosamine hydrolase (EC 3.2.1.-) are activities of enzyme belong to this family. They have specific  $(\beta/\alpha)_8$  barrels in their three dimensional structures (Little et al. 2018) (Fig. 4n5).

### **Glycoside Hydrolase Family GH F-154**

Beta-glucuronidase (3.2.1.31) is the activity of enzymes reported for this group (Cartmell et al. 2018).

### **Glycoside Hydrolase Family GH F-155**

This family was reported by Cartmell et al. (2018) with retaining type catalytic mechanism, but presently this family has been deleted.

### **Glycoside Hydrolase Family 156**

Exo- $\alpha$ -sialidase (EC 3.2.1.18) is the reported activity for the enzymes belonging to this family, catalyzing reaction through inverting mode and have been found through functional metagenomics study of DNA from thermal springs (Chuzel et al. 2018) (Fig. 4o5).

### **Glycoside Hydrolase Family 157**

Endo- $\beta$ -1,3-glucanase (EC 3.2.1.39) and endo- $\beta$ -1,3-glucanase/laminarinase (EC 3.2.1.39) are activities reported for this family, they carry out enzymatic reaction through retaining mode and conserved glutamic acid residues in their active site acts as catalytic nucleophile/base and proton donor. They belong to clan GH-A of TIM Barrel glycoside hydrolase superfamily (Helbert et al. 2019).

### **Glycoside Hydrolase Family 158**

Like family 158, endo- $\beta$ -1,3-glucanase (EC 3.2.1.39) are also activities of enzymes of family 158, belong to clan GH-A of Barrel glycoside hydrolase superfamily, carrying out catalytic mechanism through retaining mode with conserved glutamic acid residues working as catalytic nucleophile/base and proton donor (Helbert et al. 2019).

### **Glycoside Hydrolase Family 159**

$\beta$ -D-galactofuranosidases (EC 3.2.1.146) are the activities reported for this group of enzymes (Helbert et al. 2019).

## **Glycoside Hydrolase Family 160**

Endo- $\beta$ -1,4-galactosidase (EC 3.2.1.-) are the activities reported for enzymes belonging to this family and are known to have probable folding similarities with several PL families (Helbert et al. 2019).

## **Glycoside Hydrolase Family 161**

Beta-1,3-glucan phosphorylase (EC 2.4.1.-) are the activities of enzymes reported for this family of enzymes, with  $(\alpha/\alpha)_6$  (inferred) motif in their three dimensional structure they are classified under clan GH-Q. They carry out catalysis through inverting mechanism with phosphate ion and conserved aspartic acid acting as catalytic nucleophile/base and proton donor respectively (Kuhadomlarp et al. 2019).

## **Glycoside Hydrolase Family 162**

Endo- $\beta$ -1,2-glucanase (EC 3.2.1.71) is the activity of enzyme of this family, having  $(\alpha/\alpha)_6$  motif in their three dimensional structure, exhibiting inverting mode of catalytic mechanism, with conserved aspartic acid and glutamic acid residues working as catalytic nucleophile/base and proton donor (Tanaka et al. 2019) (Fig. 4p5).

## **Glycoside Hydrolase Family 163**

Endo- $\beta$ -N-acetylglucosaminidase cleaving GlcNAc- $\beta$ -1,2-Man (EC 3.2.1.-) are the activities of enzymes reported to belong to this family (Briliute et al. 2019).

## **Glycoside Hydrolase Family 164**

Beta-mannosidase (EC 3.2.1.25) are activities of enzymes of this family (Helbert et al. 2019) (Note: it appears alpha-mannosidase in reference due to uncorrected typo in Table 2)

## **Glycoside Hydrolase Family 165**

Beta-galactosidase (EC 3.2.1.23) is activity of enzymes determined through metagenomics study (Cheng et al. 2017)

**Table 2** Active Site pattern and PDB Data and crystal structure availability of Glycoside hydrolase Family 1-166 and non classified group

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue   | Prosite number   | Taxonomic range                                      | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---|------------------|--|--|-------------------|
| 1     | Family 1                   | [LIVMFSTC]-[LIVFYSL]-[LIV]-<br>[LIVMST]-E-N-G-[LIVMFAR]-<br>[CSAGN]<br>E is the active site residue   | PS00572          | Archaea,<br>eukaryotes,<br>prokaryotes<br>(bacteria) | 95   | 0                 |
| 2     | Family 2                   | N-x-[LIVMFYWD]-R-[STACN](2)-H-<br>Y-P-x(4)-[LIVMFYWS](2)-x(3)-<br>[DN]-x(2)-G-[LIVMFYW](4)  | PS00719          | Eukaryotes,<br>prokaryotes<br>(bacteria)             | 63   | 2                 |
| 3     | Family 3                   | [LIVM](2)-[KR]-x-[EQKRD]-x(4)-G-<br>[LIVMFSTC]-[LIVT]-[LIVMF]-[ST]-D-<br>x(2)-[SGADNIT]   | PS00775          | Eukaryotes,<br>prokaryotes<br>(bacteria)             | 27   | 4                 |
| 4     | Family 4                   | [PS]-x-[SAC]-x-[LIVMFY](2)-[QN]-x<br>(2)-N-P-x(4)-[TA]-x(9,11)-[KRD]-x-<br>[LIV]-[IGN]-x-C  | PS01324          | Prokaryotes<br>(bacteria)                            | 4  | 0                 |
| 5     | Family 5                   | [LIV]-[LIVMFYWGA](2)-[DNEQG]-<br>[LIVMGST]-[SENR]-N-E-<br>[PV]-[RHDNSTLIVFY]  | PS0659           | Eukaryotes,<br>prokaryotes<br>(bacteria)             | 55   | 0                 |
| 6     | Family 6                   | -   | PS00655, PS00656 | Eukaryotes,<br>prokaryotes<br>(bacteria)             | 76   | 0                 |
| 7     | Family 7                   |   |                  | Eukaryotic   | 86   | 0                 |
| 8     | Family 8                   | A-[ST]-D-[AG]-D-x(2)-[IM]-A-x-<br>[SA]-[LIVM]-[LIVMG]-x-A-x(3)-[FW]   | PS00812          | Prokaryotes<br>(bacteria)                            | 5  | 1                 |
| 9     | Family 9                   | [HLV]-[AILMV]-[FIL]-G-x-[NSTW]-x<br>(2,4)-[SCTV]-[FY]-[LIVMFY]-[SITV]-<br>G-x(1,5)-[GSY]-x(2)-[AFPSTY]-<br>[FLPSV]-x(2)-[AILPQVM]-<br>[HV]-[DHLS]-[KRS] | PS00592          | Eukaryotes,<br>prokaryotes<br>(bacteria)             | 13   | 0                 |

|    |           |  |   |  |                |   |
|----|-----------|--|---|--|----------------|---|
| 10 | Family 10 | -<br>[GTA]-{QNAG}-{GSV}-[LIVN]-x-<br>[IVMF]-[ST]-E-[LIY]-[DN]-[LIVMF]  | PS51760 (Domain profile)<br>PS00591   | Eukaryotes,<br>prokaryotes<br>(bacteria) | 82<br>67       | 2 |
| 11 | Family 11 | -<br>[PSA]-[LQ]-x-E-[YF]-Y-[LIVM](2)-<br>[DE]-x-[FYWHN]<br>[LIVMF]-x(2)-E-[AG]-[YWG]-<br>[QRFGS]-[SG]-[STAN]-G-x-[SAF] | PS51761 (Domain profile)<br>PS00776 (Active Site<br>Signature I)<br>PS00777 (Active Site<br>Signature II) | Eukaryotes,<br>prokaryotes<br>(bacteria) | 82<br>85<br>64 | 2 |
| 12 | Family 12 |  |   | Eukaryotes,<br>prokaryotes<br>(bacteria) | 43             | 1 |
| 13 | Family 13 |  | $\alpha$ -amylase, N-terminal ig-G<br>like domains  |  | 34             | 2 |
| 14 | Family 14 |  | $\alpha$ -amylase catalytic domain<br>PF01373   | Eukaryotes,<br>prokaryotes<br>(bacteria) | 419<br>43      | 0 |
| 15 | Family 15 |  | PF00723   | Eukaryotes,<br>prokaryotes<br>(bacteria) | 25             | 1 |
| 16 | Family 16 |  | PS51762 GH F-16 Domain<br>Profile)<br>PS01034 (GH F-16 Active<br>Site)<br>PS00587                         | Eukaryotes,<br>prokaryotes<br>(bacteria) | 35<br>21       | 1 |
| 17 | Family 17 | [LIVMKS]-x-[LIVMFYWA](3)-<br>[STAG]-E-[STACVI]-G-[WY]-P-<br>[STN]-x-[SAGQ]   |   | Eukaryotes                               | 4              | 1 |
| 18 | Family 18 |  | PF00704   | Eukaryotes,<br>prokaryotes<br>(bacteria) | 281            | 3 |

(continued)

Table 2 (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue   | Prosite number  | Taxonomic range   | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---|---|---|--|-------------------|
| 19    | Family 19                  | C-x(4,5)-F-Y-[ST]-x(3)-[FY]-[LIVMF]-<br>x-A-x(3)-[YF]-x(2)-F-[GSA]<br>[LIVM]-[GSA]-F-x-[STAG](2)-<br>[LIVMFY]-W-[FY]-W-[LIVM] | PS00773 (Signature 1)<br>PS00774 (Signature 2)  | Eukaryotes<br>Eukaryotes                                    | 11   | 0                 |
| 20    | Family 20                  |   | PF02838 (Domain 2)<br>PF00728 (Catalytic domain)  | Eukaryotes,<br>prokaryotes<br>(bacteria)                    | 85<br>78   | 0                 |
| 21    | Family 21                  |   |   |   |  | 0                 |
| 22    | Family 22                  | C-x(3)-C-x(2)-[LMF]-x(3)-[DEN]-<br>[LI]-x(5)-C  | PS51348 (Domain Profile)<br>PS00128 (Domain Signature)  | Eukaryotes  | 530<br>1091  | 0                 |
| 23    | Family 23                  |   |   | Eukaryotes and<br>prokaryotes                               | 76   | 0                 |
| 24    | Family 24                  |   | PF00959 (pfam domain)   | Prokaryotes (phage<br>lysozymes)                            | 666  | 1                 |
| 25    | Family 25                  | D-[LIVM]-x(3)-[NQ]-[PGE]-x(9,15)-<br>[GR]-x(4)-[LIVMFY](2)-K-x-[ST]-E-<br>[GS]-x(2)-[FY]-x-[DN]                               | PS51904 (Glycosyl hydrolase<br>family 25 (GH25) domain<br>profile)<br>PS00953 (Glycosyl<br>hydrolases family 25 active<br>site signature) | Bacteriophages,<br>eukaryotes,<br>prokaryotes<br>(bacteria) | 7<br>7   | 0                 |
| 26    | Family 26                  |   | PF02156   | Eukaryotes,<br>prokaryotes<br>(bacteria)                    | 42   | 2                 |

|    |           |  |  |   |          |   |
|----|-----------|--|--|---|----------|---|
| 27 | Family 27 | G-[LIVMFY]-x(2)-[LIVMFY]-x-[LIVM]-D-[DF]-x(1,2)-W-x(3,7)-[RV]-[DNSF]   | PS00512  | Eukaryotes and prokaryotes (bacteria)       | 40       | 0 |
| 28 | Family 28 | [GSDENKRH]-x(2)-[VMFC]-x(2)-[GS]-H-G-[LIVMAG]-x(1,2)-[LIVM]-G-S  | PS00502 (Polygalacturonase catalytic site)   | Eukaryotes, prokaryotes (bacteria)          | 6        | 3 |
| 29 | Family 29 | P-x(2)-L-x(3)-K-W-E-x-C  | PS00385 (alpha-1-fucosidase putative active site)  | Eukaryotes                                  | 6        | 0 |
| 30 | Family 30 |  | PF02055  | Eukaryotes                                  | 45       | 1 |
| 31 | Family 31 | [GFY]-[LIVMF]-W-x-D-M-[NSA]-E<br>G-[AVP]-[DT]-[LIVMTAS]-[CG]-G-[FY]-x(3)-[STP]-x(3)-L-[CL]-x-R-W-x(2)-[LVM]-[GSA]-[SA]-[FY]-x-P-[FY]-x-R-[DNA] | PS00129 (active site)<br>PS00707 (Signature 2)   | Archaea, eukaryotes, prokaryotes (bacteria) | 31<br>27 | 0 |
| 32 | Family 32 | H-x(2)-[PV]-x(4)-[LIVMA]-N-D-P-N-[GA]  | PS00609 (active site)  | Eukaryotes, prokaryotes (bacteria)          | 13       | 2 |
| 33 | Family 33 |  | PF02973  | Eukaryotes and prokaryotes                  | 60       | 0 |
| 34 | Family 34 |  | PF00064  | Viruses                                     | 149      | 0 |
| 35 | Family 35 | G-G-P-[LIVM](2)-x(2)-Q-x-E-N-E-[FY]  | PS01182 (putative active site)   | Eukaryotes, prokaryotes (bacteria)          | 17       | 0 |
| 36 | Family 36 |  | PF02065 (Melibiase)<br>PF05691 (Raffinose)   | Eukaryotes, prokaryotes (bacteria)          | 48<br>0  | 0 |
| 37 | Family 37 | P-G-G-R-F-x-E-x-Y-x-W-D-x-Y<br>Q-W-D-x-P-x-[GAV]-W-[PAS]-P   | PS00927 (Trehalase signature I; located centrally)<br>PS00928 (Trehalase signature II) (C-terminal region) | Eukaryotes, prokaryotes (bacteria)          | 8<br>8   | 0 |

(continued)

**Table 2** (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue | Prosites number                                   | Taxonomic range                       | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---------------------------------------|---|---------------------------------------|--|-------------------|
| 38    | Family 38                  |                                       | PF01074 (N-terminal Domain)                       | Eukaryotes, prokaryotes (bacteria)    | 63   | 0                 |
|       |                            |                                       | PF09261 (alpha-mannosidase middle domain)         |                                       | 63   |                   |
|       |                            |                                       | PF01074 (C-terminal Domain)                       |                                       | 63   |                   |
| 39    | Family 39                  | W-x-F-E-x-W-N-E-P-[DN]                | PS01027 (Active Site)                             | Eukaryotes, prokaryotes (bacteria)    | 15   | 0                 |
| 40    | Family 40                  | Not defined                           |   |                                       |  | 0                 |
| 41    | Family 41                  | Not defined                           |   |                                       |  | 0                 |
| 42    | Family 42                  |                                       | PF02449 (beta-galactosidase)                      | Prokaryotes                           | 15   | 1                 |
|       |                            |                                       | PF08532 (Beta-galactosidase trimerisation domain) |                                       | 9  |                   |
|       |                            |                                       | PF08533 (Beta-galactosidase C terminal domain)    |                                       | 7  |                   |
| 43    | Family 43                  |                                       | PF04616   | Eukaryotes and prokaryotes (bacteria) | 75   | 2                 |
| 44    | Family 44                  |                                       | PF12891   | Prokaryotes                           | 18   | 0                 |
| 45    | Family 45                  | [STA]-T-R-Y-[FYW]-D-x(5)-[CA]         | PS01140 (Active Site)                             | Eukaryotes and prokaryotes (bacteria) | 3  | 0                 |
| 46    | Family 46                  |                                       | PF01374   | Prokaryotes                           | 7  | 0                 |

|    |           |  |         |                                       |    |   |
|----|-----------|--|---------|---------------------------------------|----|---|
| 47 | Family 47 |  | PF01532 | Eukaryotes and prokaryotes (bacteria) | 19 | 0 |
| 48 | Family 48 |  | PF02011 | Prokaryotes                           | 28 | 0 |
| 49 | Family 49 |  | PF03718 | Eukaryotes and prokaryotes (bacteria) | 6  | 0 |
| 50 | Family 50 |  |         | Prokaryotes                           | 6  | 0 |
| 51 | Family 51 |  |         | Eukaryotes and prokaryotes (bacteria) | 19 |   |
| 52 | Family 52 |  | PF0312  | Prokaryotes                           | 1  | 0 |
| 53 | Family 53 |  | PF07745 | Eukaryotes and prokaryotes (bacteria) | 14 | 0 |
| 54 | Family 54 |  |         | Eukaryotes and prokaryotes (bacteria) | 4  | 0 |
| 55 | Family 55 |  |         | Eukaryotes and prokaryotes (bacteria) | 13 | 0 |
| 56 | Family 56 |  | PF01630 | Eukaryotes and prokaryotes            | 6  | 0 |
| 57 | Family 57 |  | PF03065 | Eukaryotes and prokaryotes (bacteria) | 9  | 0 |
| 58 | Family 58 |  |         | Prokaryotes                           | 7  | 0 |
| 59 | Family 59 |  |         | Eukaryotes, prokaryotes (bacteria)    | 13 | 0 |

(continued)

**Table 2** (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue | Prosite number                     | Taxonomic range                    | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---------------------------------------|------------------------------------|------------------------------------|--|-------------------|
| 60    | Family 60                  |                                       |                                    |                                    |  | 0                 |
| 61    | Family 61                  |                                       |                                    |                                    |  | 0                 |
| 62    | Family 62                  |                                       | PF03664                            | Eukaryotes, prokaryotes (bacteria) | 12   | 1                 |
| 63    | Family 63                  |                                       | PF03200                            | Eukaryotes and prokaryotes         | 1  | 0                 |
| 64    | Family 64                  |                                       |                                    | Eukaryotes and prokaryotes         | 5  | 0                 |
| 65    | Family 65                  |                                       | PF03633 (C-terminal domain)        | Eukaryotes and prokaryotes         | 5  | 1                 |
|       |                            |                                       | PF03632 (Central catalytic domain) |                                    |  |                   |
|       |                            |                                       | PF003636 (N-terminal domain)       |                                    |  |                   |
| 66    | Family 66                  |                                       | PF13199                            | Prokaryotes                        | 11   | 0                 |
| 67    | Family 67                  |                                       | PF07477 (C_terminus domain)        | Eukaryotes and prokaryotes         | 12   | 0                 |
|       |                            |                                       | PF03648 (N-terminus domain)        |                                    |  |                   |
|       |                            |                                       | PF07488 (middle domain)            |                                    |  |                   |
| 68    | Family 68                  |                                       | PF02435 (Levansucrase/invertase)   | Prokaryotes                        | 22   | 0                 |
| 69    | Family 69                  |                                       |                                    |                                    |  | 0                 |
| 70    | Family 70                  |                                       | PF02324                            | Prokaryotes                        | 16   | 0                 |

|    |           |  |   |                                       |    |   |
|----|-----------|--|---|---------------------------------------|----|---|
| 71 | Family 71 |  | PF03659   | Eukaryotes and prokaryotes (bacteria) | 0  | 0 |
| 72 | Family 72 |  | PF03198   | Eukaryotes and prokaryotes (bacteria) | 4  | 0 |
| 73 | Family 73 |  | PF01832—Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase | Prokaryotes                           | 17 | 0 |
| 74 | Family 74 |  |   | Eukaryotes and prokaryotes (bacteria) | 18 | 1 |
| 75 | Family 75 |  | PF07335   | Eukaryotes and prokaryotes (bacteria) | 0  | 0 |
| 76 | Family 76 |  | PF03663   | Eukaryotes and prokaryotes            | 12 | 0 |
| 77 | Family 77 |  | PF02446—4-alpha-glucanotransferase                              | Eukaryotes and prokaryotes            | 20 | 0 |
| 78 | Family 78 |  | PF05592—Bacterial alpha-L-rhamnosidas                           | Eukaryotes and prokaryotes            | 4  | 0 |
| 79 | Family 79 |  | PF03662—Glycosyl hydrolase family 79, N-terminal domain         | Eukaryotes and prokaryotes            | 8  | 0 |
| 80 | Family 80 |  | PF13647 (Chitosanase A)   | Prokaryotes                           | 1  | 0 |
| 81 | Family 81 |  | PF03639   | Eukaryotes and prokaryotes            | 3  | 0 |
| 82 | Family 82 |  |   | Prokaryotes                           | 3  | 0 |
| 83 | Family 83 |  | PF00423—Haemagglutinin-neuraminidase                            | Prokaryotes (viruses)                 | 42 | 0 |

(continued)

**Table 2** (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue | Prosite number  | Taxonomic range                       | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---------------------------------------|---|---------------------------------------|--|-------------------|
| 84    | Family 84                  |                                       |   | Eukaryotes and prokaryotes            | 70   | 0                 |
| 85    | Family 85                  |                                       | PF03644   | Eukaryotes and prokaryotes (bacteria) | 6  | 0                 |
| 86    | Family 86                  |                                       |   | Prokaryotes                           | 5  | 0                 |
| 87    | Family 87                  |                                       |   | Prokaryotes                           | 8  | 0                 |
| 88    | Family 88                  |                                       | PF07470   | Eukaryotes and prokaryotes            | 21   | 0                 |
| 89    | Family 89                  |                                       | PF12972—Alpha-N-acetylglucosaminidase (NAGLU) C-terminal domain | Eukaryotes and prokaryotes            | 7  | 0                 |
|       |                            |                                       | PF12971—Alpha-N-acetylglucosaminidase (NAGLU) N-terminal domain |                                       |  |                   |
|       |                            |                                       | PF05089—Alpha-N-acetylglucosaminidase (NAGLU) tim-barrel domain |                                       |  |                   |
| 90    | Family 90                  |                                       |   | Prokaryotes                           | 18   | 0                 |
| 91    | Family 91                  |                                       |   | Prokaryotes                           | 9  | 0                 |
| 92    | Family 92                  |                                       | PF07971   | Eukaryotes and prokaryotes (bacteria) | 8  | 0                 |
| 93    | Family 93                  |                                       |   | Eukaryotes and prokaryotes (bacteria) | 7  | 0                 |

|     |            |  |  |                                       |    |   |
|-----|------------|--|--|---------------------------------------|----|---|
| 94  | Family 94  |  |  | Eukaryotes and prokaryotes            | 31 | 0 |
| 95  | Family 95  |  |  | Eukaryotes and prokaryotes            | 6  | 0 |
| 96  | Family 96  |  |  | Prokaryotes                           | 0  | 0 |
| 97  | Family 97  |  | PF10566  | Eukaryotes and prokaryotes            | 8  | 0 |
| 98  | Family 98  |  | PF08306  | Prokaryotes                           | 5  | 0 |
|     |            |  | PF08307—Glycosyl hydrolase family 98 C-terminal domain |                                       |    |   |
| 99  | Family 99  |  |  | Eukaryotes and prokaryotes            | 30 | 0 |
| 100 | Family 100 |  | PF12899—Alkaline and neutral invertase                 | Eukaryotes and prokaryotes (bacteria) | 4  | 0 |
| 101 | Family 101 |  | PF12905—Endo-alpha-N-acetylgalactosaminidase           | Prokaryotes                           | 8  | 0 |
|     |            |  | PF11308  |                                       | 0  |   |
| 102 | Family 102 |  |  | Prokaryotes                           | 10 | 0 |
| 103 | Family 103 |  |  | Eukaryotes and prokaryotes (bacteria) | 13 | 0 |
| 104 | Family 104 |  |  | Prokaryotes                           | 3  | 0 |
| 105 | Family 105 |  |  | Eukaryotes and prokaryotes            | 11 | 0 |

(continued)

**Table 2** (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue | Prosite number | Taxonomic range            | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---------------------------------------|----------------|----------------------------|--|-------------------|
| 106   | Family 106                 |                                       |                | Eukaryotes and prokaryotes | 4  | 0                 |
| 107   | Family 107                 |                                       |                | Prokaryotes                | 4  | 0                 |
| 108   | Family 108                 |                                       | PF05838        | Prokaryotes                | 3  | 0                 |
| 109   | Family 109                 |                                       |                | Prokaryotes                | 2  | 0                 |
| 110   | Family 110                 |                                       |                | Prokaryotes                | 0  | 0                 |
| 111   | Family 111                 |                                       |                | Prokaryotes                | 0  | 0                 |
| 112   | Family 112                 |                                       |                | Prokaryotes                | 6  | 0                 |
| 113   | Family 113                 |                                       |                | Eukaryotes and prokaryotes | 9  | 0                 |
| 114   | Family 114                 |                                       |                | Eukaryotes and prokaryotes | 2  | 0                 |
| 115   | Family 115                 |                                       |                | Eukaryotes and prokaryotes | 4  | 1                 |
| 116   | Family 116                 |                                       |                | Eukaryotes and prokaryotes | 10   | 0                 |
| 117   | Family 117                 |                                       |                | Prokaryotes                | 11   | 0                 |
| 118   | Family 118                 |                                       |                | Prokaryotes                | 0  | 1                 |

|     |            |  |  |                                       |    |   |
|-----|------------|--|--|---------------------------------------|----|---|
| 119 | Family 119 |  |  | Prokaryotes                           | 0  | 0 |
| 120 | Family 120 |  |  | Eukaryotes and prokaryotes (bacteria) | 3  | 0 |
| 121 | Family 121 |  |  | Prokaryotes                           | 0  | 0 |
| 122 | Family 122 |  |  | Prokaryotes                           | 0  | 0 |
| 123 | Family 123 |  |  | Eukaryotes and prokaryotes (bacteria) | 8  | 0 |
| 124 | Family 124 |  |  | Prokaryotes                           | 3  | 0 |
| 125 | Family 125 |  |  | Eukaryotes and prokaryotes            | 12 | 0 |
| 126 | Family 126 |  |  | Prokaryotes                           | 2  | 0 |
| 127 | Family 127 |  |  | Eukaryotes and prokaryotes            | 9  | 0 |
| 128 | Family 128 |  |  | Eukaryotes and prokaryotes            | 0  | 0 |
| 129 | Family 129 |  |  | Prokaryotes                           | 5  | 0 |
| 130 | Family 130 |  |  | Eukaryotes and prokaryotes            | 22 | 0 |
| 131 | Family 131 |  |  | Eukaryotes                            | 3  | 0 |
| 132 | Family 132 |  |  | Eukaryotes                            | 0  | 0 |

(continued)

**Table 2** (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue | Prosite number | Taxonomic range                       | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---------------------------------------|----------------|---------------------------------------|--|-------------------|
| 133   | Family 133                 |                                       |                | Eukaryotes and prokaryotes            | 2  | 0                 |
| 134   | Family 134                 |                                       |                | Eukaryotes and prokaryotes (bacteria) | 9  | 0                 |
| 135   | Family 135                 |                                       |                | Eukaryotes and prokaryotes            | 2  | 0                 |
| 136   | Family 136                 |                                       |                | Eukaryotes and prokaryotes (bacteria) | 3  | 0                 |
| 137   | Family 137                 |                                       |                | Prokaryotes                           | 3  | 0                 |
| 138   | Family 138                 |                                       |                | Prokaryotes                           | 4  | 0                 |
| 139   | Family 139                 |                                       |                | Eukaryotes and prokaryotes (bacteria) | 0  | 0                 |
| 140   | Family 140                 |                                       |                | Prokaryotes                           | 1  | 0                 |
| 141   | Family 141                 |                                       |                | Eukaryotes and prokaryotes            | 1  | 0                 |
| 142   | Family 142                 |                                       |                | Eukaryotes and prokaryotes (bacteria) | 2  | 0                 |
| 143   | Family 143                 |                                       |                | Prokaryotes                           | 2  | 0                 |

|     |               |         |  |   |    |   |
|-----|---------------|---------|--|---|----|---|
| 144 | Family<br>144 |         |  | Prokaryotes                                 | 5  | 0 |
| 145 | Family<br>145 |         |  | Eukaryotes and<br>prokaryotes               | 5  | 0 |
| 146 | Family<br>146 |         |  | Eukaryotes and<br>prokaryotes<br>(bacteria) | 1  | 0 |
| 147 | Family<br>147 |         |  | Prokaryotes                                 | 0  | 0 |
| 148 | Family<br>148 |         |  | Prokaryotes                                 | 0  | 0 |
| 149 | Family<br>149 |         |  | Eukaryotes and<br>prokaryotes<br>(bacteria) | 3  | 0 |
| 150 | Family<br>150 |         |  | Prokaryotes<br>(bacteria)                   | 0  | 0 |
| 151 | Family<br>151 |         |  | Prokaryotes<br>(bacteria)                   | 0  | 0 |
| 152 | Family<br>152 |         |  | Eukaryotes and<br>prokaryotes               | 11 | 0 |
| 153 | Family<br>153 |         |  | Prokaryotes<br>(bacteria)                   | 7  | 0 |
| 154 | Family<br>154 |         |  | Eukaryotes and<br>prokaryotes               | 0  | 0 |
| 155 | Family<br>155 | Deleted |  | –   | 0  | 0 |
| 156 | Family<br>156 |         |  | Prokaryotes<br>(bacteria)                   | 5  | 0 |
| 157 | Family<br>157 |         |  | Prokaryotes                                 | 0  | 0 |

(continued)

**Table 2** (continued)

| SI no | Glycoside hydrolase family | Consensus pattern/active site residue | Prosite number | Taxonomic range                       | PDB/Pfam domain/<br>PDB accessions<br>DATA entries | Crystal structure |
|-------|----------------------------|---------------------------------------|----------------|---------------------------------------|--|-------------------|
| 158   | Family 158                 |                                       |                | Prokaryotes                           | 0  | 0                 |
| 159   | Family 159                 |                                       |                | Prokaryotes                           | 0  | 0                 |
| 160   | Family 160                 |                                       |                | Prokaryotes                           | 0  | 0                 |
| 161   | Family 161                 |                                       |                | Eukaryotes and prokaryotes            | 0  | 0                 |
| 162   | Family 162                 |                                       |                | Eukaryotes and prokaryotes (bacteria) | 3  | 0                 |
| 163   | Family 163                 |                                       |                | Eukaryotes and prokaryotes (bacteria) | 0  | 0                 |
| 164   | Family 164                 |                                       |                | Prokaryotes                           | 0  | 0                 |
| 165   | Family 165                 |                                       |                | Prokaryotes (bacteria)                | 0  | 0                 |
| 166   | Family 166                 |                                       |                | Prokaryotes (bacteria)                | 1  | 0                 |
| 167   | Non classified             |                                       |                | Eukaryotes and prokaryotes            | 55   | 0                 |

Data as per PROSITE and EMBL-EBI (Pfam Domain)

<https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/>

<https://prosite.expasy.org/>

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## Glycoside Hydrolase Family 166

Alpha-1,4-galactosaminogalactan hydrolase (EC 3.2.1.-) is the activities of enzymes belonging to this family, exhibiting retaining type catalytic mechanism (Le Mauff et al. 2019) (Fig. 4q5).

## Glycoside Hydrolase Family Non Classified

Group of glycoside hydrolase enzymes to which family has yet not been designated are reported here (Fig. 4r5).

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## Conclusions

Glycoside hydrolases, as stated earlier in this chapter, finds varied applications and this feature is much supported by the presence of diverse catalytic sites on these enzymes. They have been classified under 166 families which are grouped in 16 different superfamilies and three added clans (not specified yet under any superfamily). Being broadly studied for its action on numerous substrates, glycoside hydrolases like amylases, cellulases, chitinases, xylanases, glucanases, glucuronidases are few in humungous list finding usage in numerous technologies involved in production of various value added components. Specific region of catalytic domain, mode of action (retaining/inverting) and three dimensional structures for majority are available with added data on crystal structures of few specifically studied (like family 2, 3, 10, 11 etc.) and all figures have been reported in this chapter. In the present book few extensively studied reports on glycoside hydrolases have been brought forward, which broadly includes, structure, mechanism and applications of glycoside hydrolases, followed by a note on directed evolution of glycoside hdyrolases, its applications in degradation of lignocellulosic biomass, bioethanol production, food industries, paper and pulp industries, marine glycoside hydrolases as industrial tool and role of metagenomics in discovering industrially significant cellulases. With this minute initiative we bring to you an insight on industrial applications of glycoside hydrolases, an initial approach.

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